PART 1 – Diabetes and The Heart
Diabetic Gluco-Lipotoxic Cardiomyopathy: Amendable by Metabolic Manipulation?
Chapter 1

Introduction

Type 2 diabetes (T2DM) has grown to epidemic proportions and is estimated to affect 4.4% of people world-wide in 2030. Patients with T2DM are at increased risk of cardiovascular disease, in particular coronary artery disease (CAD) and heart failure, and consequently, heart disease is the most common cause of death in T2DM. In asymptomatic patients, cardiac structural and functional abnormalities exist, even in the absence of CAD or hypertension, due to diabetic cardiomyopathy (DCM). Left ventricular (LV) diastolic dysfunction is a common and early finding, which, particularly in the presence of cardiac ischemia, may readily develop into overt heart failure. Although DCM is a multi-factorial condition, diabetes-related metabolic derangements seem to be key contributors to the observed cardiac abnormalities. This review focuses on the potential role of myocardial metabolic changes in cardiac dysfunction in human diabetes. Furthermore, current therapeutic options that may impact on cardiac metabolism and their clinical consequences are summarized.

Background and Epidemiology

Large population-based studies in people with diabetes using echocardiography and more recently cardiac magnetic resonance (CMR) have identified myocardial structural and functional abnormalities, including increased LV mass and relative wall thickness, a reduced endocardial and mid-wall fractional shortening and, most importantly, an increased prevalence of LV diastolic dysfunction, collectively providing evidence for the existence of DCM. In fact, LV diastolic filling abnormalities can already be found in obese and insulin resistant individuals and in those with the metabolic syndrome. In T2DM, even before structural abnormalities become manifest, 60% of patients without CAD or diabetes-related complications already show LV filling abnormalities. The progressive nature of LV dysfunction in diabetes is illustrated by the 2-8 fold increase in congestive heart failure (CHF) in this population, with risk ratios twice as large in women as compared to men. Conversely, approximately 19% of CHF patients have diabetes, and CHF is strongly associated with the presence of insulin resistance. In the Uppsala Longitudinal Study of Adult Men (ULSAM), insulin resistance predicted CHF incidence independently of diabetes and other established risk factors.

Pathophysiology of Diabetic Cardiomyopathy

DCM was first described by Rubler et al. in 1972 as a separate disease entity, based on post-mortem findings in 4 diabetic patients with nephropathy and heart failure, who appeared to have normal coronary arteries at autopsy. These authors already then suggested that the metabolic abnormalities directly related to diabetes might be implicated in the development of DCM. Ever since, various mechanisms have been proposed to underlie DCM, in addition to the acknowledged metabolic hallmarks of the T2DM phenotype such as insulin resistance, hyperlipidemia and hyperglycemia, all of which are currently regarded as contributors to altered myocardial substrate handling and subsequent oxidative stress and mitochondrial dysfunction (Figure 1). These additional mechanisms include microangiopathy, vascular endothelial function, activation of the renin-angiotensin system (RAS), inflammation, formation of glycation-induced collagen cross-links, alterations in structural and contractile proteins, interstitial fibrosis and abnormalities in calcium (Ca++) homeostasis. The diabetes-related metabolic derangements are believed to negatively influence myocardial energy metabolism and ultimately contribute to the observed derangements in energy demanding functions, including LV diastolic relaxation and contractile function. Formation of advanced glyca-
tion end products (AGEs), fibrosis and microangiopathy will further aggravate myocardial stiffness resulting in decreased LV compliance and LV filling abnormalities. Disturbances in myocardial Ca\(^{2+}\) homeostasis, most likely occurring secondary to the metabolic changes and oxidative stress,\(^{20}\) have been associated with the LV functional abnormalities in DCM.\(^{21}\) Finally, cardiac autonomic neuropathy was shown to further aggravate LV structural and functional changes.\(^{22}\) Due to the versatile beneficial actions of insulin on the myocardium, impaired cardiac insulin signaling is regarded among the key defects underlying the development of DCM (Figure 1).\(^{23}\)

**Clinical Presentation and Diagnostic Procedures in Human DCM**

Several stages in DCM have been identified.\(^{24,25}\) In these early stages patients rarely develop clinical symptoms, although early DCM was associated with a reduced exercise capacity.\(^{26}\) Over time, especially in the presence of co-morbidities such as hypertension, microangiopathy, ischemia and cardiac autonomic dysfunction, DCM may readily proceed to overt CHF.\(^{27}\) Echocardiography is widely used in the evaluation of LV function, since it is a non-invasive, readily available and inexpensive method.\(^{28,29}\) LV diastolic functional estimates are derived from Doppler measurements of trans mitral inflow velocities, and include the early diastolic LV filling velocity (E-wave), the atrial filling velocity (A-wave), the E/A ratio and deceleration time. The earliest stage in DCM is typified by subclinical diastolic functional changes (E/A ratio < 1), with a preserved ejection fraction and normal LV wall and ventricle sizes. The next stage is characterized by further impairment of diastolic filling due to increased LV pressure and a somewhat increased LV mass and wall thickness. To meet sufficient LV filling, left atrial (LA) pressure will gradually increase over time, resulting in a echocardiographical-inflow pattern that is indistinct from normal (pseudonormal) and is regarded as an intermediate phase. A further increase in LA pressure leads to a restrictive filling pattern (E/A ratio > 2), leading to a reduction in ejection fraction and the development of clinical symptoms of CHF.

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**Figure 1.** The interrelationship of potential mechanisms underlying diabetic cardiomyopathy.
Conventional echocardiography might, however, be insensitive to detect subtle functional alterations, especially to discriminate between normal and pseudonormal diastolic function, thereby potentially underestimating the prevalence of DCM. The Valsalva maneuver and assessment of pulmonary venous flow can be used to uncover the otherwise undetected diastolic functional abnormalities. Tissue Doppler Imaging (TDI) is relatively insensitive to the effects of pre-load compensation and can therefore overcome the limitations of conventional echocardiography. Novel methods including computed tomography and CMR are increasingly being employed to quantify LV systolic and diastolic function, as these methods are operator independent and therefore highly reproducible. The B-type natriuretic peptide (BNP) (or NT-proBNP) level is a useful marker in the evaluation of heart failure, hypertrophy and CAD. Screening for LV dysfunction in diabetes using BNP might have potential in high-risk symptomatic patients, but not in asymptomatic individuals without overt vascular disease. Previously, in a cohort of high-risk T2DM patients, but without structural heart disease and normal ejection fraction, BNP levels were similar in patients with and without LV diastolic function. At present, TDI and CMR are regarded as the most reliable tools for detection of subclinical LV dysfunction.

Methods for In Vivo Assessment of Myocardial Metabolism in Humans

Pioneering studies, measuring arterial-coronary sinus differences in myocardial substrate concentrations to evaluate myocardial metabolism in humans, stem from the middle of the last century. This technique was later expanded by the additional use of labeled substrates like \(^{13}\text{C}\)-palmitate and \(^{13}\text{C}\)-oleic acid. The development of dedicated tracers for single photon emission computed tomography (SPECT) and positron emission tomography (PET) has further increased our insight into human myocardial substrate handling in health and disease. Only PET allows quantification of metabolic processes using tracers to assess myocardial glucose, lactate and fatty acid (FA) and oxidative metabolism (Figure 2). The uptake and processing of tracers in the heart depend on tracer specific properties. Thus, the glucose analogue 2-deoxy-2-[\(^{18}\text{F}\)]-fluoro-D-glucose ([\(^{18}\text{F}\)-FDG]) is trapped following uptake and phosphorylation to FDG-6-phosphate, thereby representing the transmembrane uptake and phosphorylation of exogenous glucose. The fatty acid analog 14\text{R,S}-[\(^{18}\text{F}\)]fluoro-6-thia-heptadecanoic acid ([\(^{18}\text{F}\)-FTHA]) is used to estimate \(\beta\)-oxidation as it is partially oxidized with the majority of its metabolites trapped in mitochondria. \(^{13}\text{C}\)-glucose, \(^{13}\text{C}\)-lactate and \(^{13}\text{C}\)-palmitate are fully metabolized, and as such provide information about both uptake and oxidation. \(^{13}\text{C}\)-palmitate use allows to quantify FA esterification as well. \(^{13}\text{C}\)-acetate, which is almost exclusively metabolized in the tricarboxylic acid (TCA) cycle, but also \(^{13}\text{C}\)-O\(_2\) allow the quantification of overall myocardial oxidative metabolism by PET (Figure 2). Combining CMR or echocardiography with PET-derived parameters enable the calculation of cardiac efficiency, i.e. the ratio of cardiac work to myocardial oxygen consumption. Although the relative distribution of \(^{18}\text{F}\)-FDG already yields information about cardiac metabolism, the myocardial metabolic rate of glucose uptake (MMRglu) can be measured in absolute units. To measure MMRglu, both dynamic data acquisition and graphical plot or compartmental model analysis is required. Compartment modeling is a mathematical approach also used in the analysis of \(^{11}\text{C}\)-tracers, which describes the actual rate of tracer processing through several pre-defined physiological compartments and requires the determination of radioactive metabolites such as \(^{11}\text{CO}_2\) and/or \(^{11}\text{C}\)-lactate for additional correction, in order to obtain reliable kinetic results. Exponential curve fitting is an alternative, though less accurate, method which yields an useful index of tracer oxidation.

The more recent development of cardiovascular molecular imaging enables the imaging and quantification of molecular and cellular targets in humans in vivo. Current MR-spectroscopy (MRS) techniques, such as \(^{1}\text{H}\)-MRS and \(^{1}\text{H}\)-MRS are used for the respective assessment of myocardial...
high-energy phosphate metabolism, expressed as the phosphocreatine-to-adenosine triphosphate (PCr/ATP) ratio, thus representing myocardial mitochondrial function, and myocardial lipid content. Novel developments include molecular imaging with 18F and 13C. The high potential of this metabolic imaging technique was previously shown in rats and pigs using polarized 13C-pyruvate nuclear-MRS, which allowed quantification of the distribution of pyruvate and mapping of its major metabolites lactate and alanine. Moreover, multiparametric MRI by the combined use of 1H, 18F and 13C will have great potential to monitor and quantify biological processes and localize them in space and time.

| Figure 2. | Schematic illustration of cardiomyocyte metabolism, including substrate tracers for non-invasive quantification of cellular metabolic processes. |

Cardiac substrate uptake, including non-esterified fatty acids (NEFA), glucose and lactate, is largely receptor mediated, however NEFA may enter the cell by diffusion. Following uptake, NEFA are converted to fatty acyl-CoA, which are then transported into the mitochondria through carnitine palmitoyltransferase (CPT) 1 and 2. There fatty acyl-CoAs undergo β-oxidation (β-ox) generating acetyl-CoA and the reducing equivalents nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH). Acetyl-CoA can also be esterified into triglycerides. Intracellular glucose is degraded to pyruvate via glycolysis, generating ATP and NADH. Anaerobic degradation of glucose can also lead to generation of lactate. In the presence of oxygen, pyruvate is transported into the mitochondria through the multienzyme complex pyruvate dehydrogenase (PDH). Pyruvate is converted to acetyl-CoA, with the formation of NADH, and fatty acyl-CoA are converted to acetyl-CoA with formation of NADH and FADH. Oxidation of acetyl-CoAs in the citric acid or tricarboxylic acid (TCA) cycle generates CO2 and guanosine triphosphate (GTP) as well as NADH and FADH. Electrons (e-) derived from NADH/FADH are transferred via electron-transport complexes I to IV from the electron transport chain (ETC). Here, electrons are transferred to oxygen which is then reduced to water and consequently a proton (H+) gradient is formed. As protons re-enter the mitochondria through ATP-synthase, adenosine-triphosphate (ATP) is generated from adenosine-diphosphate (ADP). Cardiac substrate and oxidative metabolism in humans can be assessed non-invasively by positron emission tomography (PET) or single-photon emission computed tomography (SPECT) using dedicated tracers (displayed in rectangles below their natural substrates). Cardiac molecular imaging is used to assess several metabolic process (displayed in ovals): myocardial triglyceride content (1H-MRS), mitochondrial high-energy metabolism (13C-MRS), and pyruvate metabolism (18F-MRS). For description tracers see text.
Chapter 1
Substrate Metabolism and the Role of Insulin Signaling in the Normal Heart

Under physiological conditions, the normal heart utilizes primarily non-esterified fatty acids (NEFA), but also glucose, and to a lesser extent lactate, ketones, amino acids and pyruvate, in order to produce sufficient adenosine triphosphate (ATP) to sustain contractile function (Figure 2). The major part of ATP is produced by mitochondrial oxidative metabolism which consumes large amounts of oxygen. In the well-oxygenated heart, FA β-oxidation provides approximately 60-90% of the required ATP, whereas carbohydrate metabolism provides most of the remaining 10-40%. Of note, when NEFA are the substrate, oxidation of 1 mole of carbon yields 29% more ATP, as compared to glucose, however, one mole of oxygen produces 12% more ATP when glucose is the substrate as compared to NEFA. During daily physiological activities but even more so under stress conditions, including ischemia, the heart can readily switch to the most advantageous substrate according to the circumstances, and as such may be regarded a metabolic omnivore. NEFA utilized by the heart may be either circulating NEFA, bound to albumin, derived from adipose tissue via lipolysis, or NEFA released from triglyceride-rich lipoproteins by hydrolysis via lipoprotein lipase. NEFA are taken up by cardiomyocytes by diffusion and via transport through plasma membrane associated proteins, including the main transporter, FA translocase (FAT)/CD36, as well as FA binding protein (FABP) and FA transport proteins (FATP1 and FATP6). Cytosolic NEFA bind to FABP and are subsequently esterified to acyl-CoA by fatty acyl-CoA synthase. The main part of acyl-CoA is transported into mitochondria via a carnitine-dependent transport system, to undergo β-oxidation to acetyl-CoA, which then enters the TCA cycle (Figure 2). A small portion is converted to triglycerides or phospholipids. Carnitine palmitoyl transferase (CPT)-1, the key enzyme involved in FA oxidation that is located on the outer mitochondrial membrane, is inhibited by malonyl-CoA, which in turn is regulated by AMP-activated protein kinase (AMPK).

Glucose is supplied to the heart either via the circulation or by release of glucose from intracellular glycogen stores. Exogenous glucose is taken up via facilitated transport, in proportion to ambient glucose levels, through the glucose transporters GLUT1, which is insulin independent and the predominant GLUT4, which is regulated by insulin.

Intracellular glucose is phosphorylated to glucose-6-phosphate by a hexokinase, and may subsequently be converted to glycogen, enter the glycolysis pathway or the pentose phosphate pathway. Under aerobic conditions, glycolysis, which is under control of the rate-limiting enzyme phosphofructokinase (PFK)-1, accounts for approximately 10% of ATP formation, ultimately yielding 2 molecules of pyruvate and 2 NADH per molecule glucose. Pyruvate and NADH are shuttled into the mitochondria, where the pyruvate dehydrogenase (PDH) complex synthesizes acetyl-CoA from the pyruvate and this acetyl-CoA then enters the TCA cycle. Regulation of glucose metabolism occurs at the level of uptake, as AMPK stimulates translocation of cytosolic GLUT4 to the sarcolemma, as well as at the level of metabolism, where the rate-limiting enzyme of the glycolytic pathway PFK-1 can be inhibited by ATP, low pH and fructose-1,6-phosphate and activated by ADP, AMP and free phosphate. Additional regulation occurs at the level of PDH that can become inactivated by pyruvate dehydrogenase kinase (PDK), or inhibited by acetyl-CoA, NADH and ATP.

Insulin regulates myocardial substrate uptake and metabolism both indirectly, by acting on its target organs therefore regulating substrate availability, and by directly acting on the myocardium. Thus, impaired insulin signaling will lead to elevated circulating NEFA and glucose levels due to unsuppressed lipolysis from adipocytes, increased hepatic output of very-low density lipoprotein (VLDL)-triglycerides and elevated hepatic glucose production. At the level of the heart, insulin regulates NEFA and glucose uptake by stimulating the translocation of GLUT4 and CD36 to the sarcolemma. Following insulin stimulation, glucose is mainly oxidized or stored as glycogen, while
NEFA are diverted towards esterification into triglycerides. Finally, adipokines such as leptin and adiponectin exert significant metabolic actions on the heart, among others by activation of AMPK, however, at present their role in human DCM is unknown.

Myocardial Lipotoxicity and Insulin Resistance
In animal models of insulin resistance and diabetes, myocardial insulin resistance is associated with reduced cardiac glucose and increased FA metabolism. In a rat model of diet-induced insulin resistance, decreased glucose uptake was associated with impaired insulin signaling and enhanced rates of NEFA uptake were associated with the sustained sarcolemmal presence of CD36. When NEFA uptake surpasses mitochondrial oxidative capacity, formation of toxic intermediates ensues, as well as generation of reactive oxygen species (ROS), mitochondrial dysfunction and activation of pro-apoptotic pathways, paralleled by increased esterification of NEFA into triglycerides. Increased NEFA utilization is additionally associated with mitochondrial uncoupling, which leads to decreased ATP production and consequently to reduced cardiac efficiency. Also, NEFA serve as natural ligands for peroxisome proliferator-activated receptor (PPAR)-α, which is an important regulator of fat metabolism by inducing the expression of target genes involved in NEFA utilization, including enzymes involved in mitochondrial and peroxisomal β-oxidation pathways.

In human obesity-related insulin resistance and diabetes, several invasive and non-invasive approaches (outlined above) have been used in search of evidence for the existence of cardiac lipotoxicity. Although the number of human studies is limited, increased myocardial lipid content was found in myocardial biopsy samples from obese individuals and patients with CHF using oil-red O staining. In addition, using 1H-MRS, increased myocardial lipid content was reported in obesity and subjects with impaired glucose tolerance (IGT) and diabetes (Table 1). Evaluation of myocardial FA metabolism with FA-tracers using SPECT and PET in various populations with different glucometabolic abnormalities and insulin resistance, however, reported unaltered, increased, or decreased FA uptake as well as unaltered, increased, or decreased FA oxidation, thus leaving the question regarding the occurrence of myocardial lipotoxicity in humans unresolved (Table 1).

For long, the existence of myocardial insulin resistance was unsettled, since traditionally, the heart was neglected as a target organ for insulin signaling. Using PET technology, several studies have assessed insulin-stimulated 18F-FDG uptake in the myocardium in various (pre)diabetic populations, however, these studies have yielded conflicting results (Table 1), due to differences in subject characteristics, including the presence of co-morbidities, the use of medications and the severity and duration of metabolic deregulation, but also methodological issues, such as the use of different insulin concentrations when assessing insulin-stimulated glucose uptake. Finally, the inclusion of both sexes in these studies might also have influenced results, since glucose extraction fraction and utilization, but not fatty acid metabolism, are lower in women. By performing 18F-FDG PET under standardized hyperinsulinemic-euglycemic clamp conditions in well-characterized patient groups, Iozzo et al. have convincingly shown that insulin-stimulated 18F-FDG uptake was reduced in patients with T2DM with, as well as in those without CAD, but not in T1DM patients (Table 1).
Glucosetoxicity and Oxidative Stress
The mechanism whereby chronic hyperglycemia mediates tissue injury through the generation of ROS has been elucidated largely through the work of Michael Brownlee and colleagues. Hypoglycemia leads to increased glucose oxidation and mitochondrial generation of superoxide. In turn, excess superoxide leads to DNA damage and activation of poly (ADP ribose) polymerase (PARP) as a reparative enzyme. However, PARP also mediates the ribosylation and inhibition of glyceraldehyde phosphate dehydrogenase (GAPDH), diverting glucose from its glycolytic pathway and into alternative biochemical pathways that are regarded as mediators of hyperglycemia-induced cellular injury. Among these are increases in AGEs, increased hexosamine and polyol flux, and activation of classical isoforms of protein kinase C. In addition to hyperglycemia-associated ROS formation, also the elevated NEFA flux through the β-oxidation cascade will result in an increased supply of reducing equivalents to the mitochondrial electron transport chain which will ultimately lead to increased ROS production.

It may not be easy to obtain direct evidence for these mechanisms to occur in human DCM. However, high glycated haemoglobin (HbA1c) indicating longstanding hyperglycemia was found to be related to impaired LV diastolic as well as systolic function in T1DM and T2DM patients. Further, increased serum AGE levels were associated with LV stiffness in T1DM patients, whereas in T2DM patients serum AGE levels were increased and even higher when CAD was present. Finally, in ischemic CHF patients, cardiac biopsy analysis showed increased myocardial AGEs deposition in diabetic CHF patients.

Mitochondrial Dysfunction
Mitochondrial dysfunction plays a role in DCM according to various lines of evidence. Accordingly, structural and functional mitochondrial changes have been demonstrated in several rodent models of diabetes. A reduction in mitochondrial oxidative capacity has been documented in animal models of T1DM. Decreased protein expression of the oxidative phosphorylation components, i.e. creatine phosphate activity, ATP synthase activity, and creatine-stimulated respiration were previously described. Moreover, increased myocardial oxygen consumption and decreased cardiac efficiency in obesity and diabetes may contribute to the development of cardiac dysfunction, by increased mitochondrial uncoupling. Recent studies in humans have provided support for a role of mitochondrial dysfunction in DCM. In permeabilized human atrial muscle fibers from diabetic and non-diabetic males undergoing routine cardiac surgery, total oxidative phosphorylation and respiratory capacity were decreased and, paradoxically, H2O2 generation in diabetic patients was increased when fibers were exposed to both carbohydrate- and NEFA-based substrates in vitro. A reduction in the PCR/ATP ratio was described in patients with T1DM and T2DM, who had no evidence of CAD, and was found to associate with LV diastolic dysfunction (Table 1). In addition, in young obese women, an increase in PET-measured cardiac NEFA metabolism and a decrease in efficiency was reported (Table 1). Taken together, these results implicate a substantial role for mitochondrial dysfunction in the development of DCM. Further studies are needed to provide data on myocardial oxygen consumption and myocardial efficiency in patients with diabetes.
### Table 1. Non-invasive assessment methods in human diabetic cardiomyopathy.

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<td>123I-HDA</td>
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<td>PET</td>
<td>13C-palmitate, 13C-acetate</td>
<td>= MFAU / = MFAO / ↑ MVCO₂</td>
<td>↑ LVM, ↑ CO</td>
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<td>↓ EPRR in obese, IGT, T2DM MTG no ↔ EF or EPRR</td>
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T1DM/T2DM, type 1/ type 2 diabetes mellitus; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; CAD, coronary artery disease; PET, positron emission tomography; SPET, single photon emission tomography; $^{31}$P-MRS, phosphorus magnetic resonance spectroscopy; $^{1}$H-MRS, proton magnetic resonance spectroscopy; MGU, myocardial glucose uptake; NEFA, non-esterified fatty acids; MFAU, myocardial fatty acid uptake; MFAO, myocardial fatty acid oxidation; MVO2, myocardial oxygen consumption; PCr/ATP, phosphocreatine-to-adenosine-tri-phosphate ratio; DP, diastolic function; LVMI, left ventricular mass (index); EF, ejection fraction; CO, cardiac output; MTG, myocardial triglyceride content; EPRR, early peak flow rate; E dec Peak, E deceleration Peak; ↑, increased; ↓, decreased; =, no difference; ↔, correlation.
Diabetic Gluco-Lipotoxic Cardiomyopathy: Amendable by Metabolic Manipulation

Calcium metabolism
Intracellular Ca\(^{2+}\) metabolism in cardiac myocytes is impaired in experimental DCM.\(^{111}\) These abnormalities include reduced activity of ATPases, including the sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\) ATPase2a (SERCA2a),\(^{112}\) decreased ability of the sarcoplasmic reticulum to take up Ca\(^{2+}\), and reduced activities of other exchangers such as Na\(^+\)-Ca\(^{2+}\) and the sarcolemmal Ca\(^{2+}\)-ATPase.\(^{113-115}\) Currently, there are few studies reporting the role of disturbed Ca\(^{2+}\) metabolism in human DCM. Biopsy studies in CHF patients have reported evidence for deregulated Ca\(^{2+}\) handling.\(^{116-118}\) In non-ischemic CHF patients with or without diabetes versus controls, gene expression of SERCA2a was significantly depressed in patients with diabetes compared with non-diabetic controls.\(^{116}\) In patients undergoing coronary artery bypass surgery, cardiac myofilament responsiveness to Ca\(^{2+}\) was decreased by 29% in T2DM relative with non-diabetic patients, and a near significant reduction in maximum Ca\(^{2+}\)-saturated force generation was found.\(^{119}\) Thus, more studies are needed to establish the role of disturbed myocardial Ca\(^{2+}\) metabolism in human DCM.

Linking Abnormal Metabolism to Myocardial Dysfunction
Animal studies of DCM show concurrent impairments of cardiac metabolism and function, however, a causal relationship remains difficult to establish. The supraphysiological, relatively short-lived conditions, even in non-genetically manipulated models, such as severe hyperglycemia, exposure to extreme, in part deficient diets as well as methodological limitations of cardiac metabolic and functional measurements in rodents, may not represent the human situation in which relatively mild but chronic abnormalities are at play. Although in humans there are data showing the association of systemic metabolic abnormalities and cardiac function, direct evidence supporting the existence of myocardial dysmetabolic changes as contributor to myocardial dysfunction are relatively scarce and inconsistent (Table 1). An inter-relationship between metabolism and myocardial function in humans is suggested by the reported reversible association between changes in glycemia and myocardial diastolic function in some,\(^{125-127}\) but not all studies.\(^{124}\)

A large number of studies measured myocardial substrate metabolism in human DCM using SPECT and PET, but only a few concomitantly assessed cardiac function (Table 1). Lozzo et al, reported a weak correlation between insulin-stimulated myocardial \(^{125}\)F-FDG uptake and the ejection fraction in a pooled analysis of controls, CAD and T2DM patients.\(^{126}\) Furthermore, an inverse association between the PCR/ATP ratio and diastolic functional parameters was reported in pooled data from T2DM patients and controls.\(^{127}\) McGavock et al, found an increase in myocardial triglyceride content in obese patients with IGT and T2DM relative to lean controls, but no relation was established with diastolic or systolic function.\(^{128}\) Szczepaniak et al, reported an elevated myocardial triglyceride content, that was accompanied by increased LV mass and a subtle reduction of septal wall thickening, a measure of regional systolic function, in clinically healthy subjects with a wide range of BMIs.\(^{129}\) However, in that study, LV ejection fraction was unrelated to myocardial triglyceride content. We found an independent association between decreased LV diastolic functional parameters and myocardial triglyceride content as measured by MRI and \(^{12}H\)-MRS in well-controlled T2DM patients relative to age- and BMI-matched controls.\(^{130}\) Thus, in human (pre)diabetes only a few studies have performed combined measurements and analysis of cardiac metabolism and function of which some, but not all (depending on the population studied and the methods used) found evidence for the existence of a link between cardiac metabolism and function.
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Therapeutic Options to Improve Myocardial Metabolism in DCM

Since impaired insulin signaling is the key to altered myocardial substrate handling and energy metabolism in T2DM, it is tempting to propose that the use of insulin or insulin sensitizing therapies will have beneficial effects on cardiac function. Table 2 lists regular blood glucose lowering agents and drugs interfering with specific metabolic pathways, their mode of action, non-cardiac metabolic effects and their reported effects on human myocardial metabolism.

In the UK Prospective Diabetes Study (UKPDS) only the use of the biguanide metformin was associated with a 36% reduction of cardiovascular disease outcomes, particularly all-cause mortality.144 Accordingly, metformin, in addition to lifestyle recommendations, is currently regarded as first-line therapy in patients with T2DM according to the combined statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD).145 Although metformin has been reported to activate AMPK, using 18F-FDG-PET, Hallsten et al found no effect of metformin, on insulin stimulated myocardial 18F-FDG uptake.146 Moreover, we found metformin to decrease insulin stimulated myocardial 18F-FDG uptake significantly.147 In addition, experimental data indicate that metformin inhibits mitochondrial complex I activity leading to the impairment of mitochondrial function.148-149 Thus, although metformin showed to be beneficial in the UKPDS, the reported effects on myocardial glucose uptake and mitochondrial function are not unequivocal and therefore warrant further research. Chronic treatment with sulfonylurea increases myocardial glucose uptake, independent of glycemic control in T2DM patients.150 Insulin may have direct inotropic effects,151 but may also indirectly increase LV ejection fraction by stimulating myocardial glucose uptake. Acute administration of insulin to healthy controls and to a lesser extend in T2DM patients increased LV ejection fraction.152 Whole body insulin sensitivity was positively associated with the LV ejection fraction.153

Rosiglitazone increased myocardial glucose uptake in T2DM patients with and without CAD.154, 155 Pioglitazone improved LV diastolic function with a concomitant increase in insulin-mediated myocardial glucose uptake in men with uncomplicated T2DM and no CAD, but interestingly, these two phenomena were unrelated.156 Moreover, pioglitazone did not alter myocardial triglyceride content but decreased liver fat content. Pioglitazone combined with insulin for 6 months, but not insulin alone, reduced myocardial triglyceride content in a small group of patients with longstanding T2DM, but blood pressure and heart function remained unchanged.157 Furthermore, the association of oxidative stress and cardiac function in human DCM was suggested by the reported association of rosiglitazone induced reduction of the circulating oxidative stress marker malondialdehyde and therapy-related improvement of LV diastolic function in T2DM patients without CAD.158 The use of thiazolidinediones has recently been scrutinized, because of the elevated risk of CHF. Moreover, rosiglitazone use was associated with cardiac ischemic events.159 However, this was not observed for pioglitazone.160 Additionally, metformin should be used carefully in those with CHF and renal dysfunction due to the possible increased risk of severe lactic acidosis.161

Novel agents such as the injectable glucagon-like-peptide 1 receptor agonists (GLP-1RA) exenatide and liraglutide and the oral dipeptidyl-peptidase (DPP)-4 inhibitors sitagliptin and vildagliptin are prescribed to lower blood glucose in T2DM patients. GLP-1RA therapy results in a sustained glycemic improvement and progressive reduction in bodyweight, which support a shift toward a more favorable cardiovascular risk profile.162 GLP-1RA act through G-protein coupled receptors, which are also present on cardiomyocytes, and raise cyclic AMP.163-164 Their effect on LV function and metabolism requires further study, however, infusion of GLP-1 improved cardiac function in animals165-166 and patients with CHF.167-168 Recently, the cardioprotective effects of GLP-1 and its metabolite GLP-1(9-36), which is generated by DPP-4 degradation of GLP-1, were demonstrated in a GLP1 -/- mouse model.169 Thus, the inotropic effects of GLP-1 and its stimulating actions on glucose uptake, ischemic preconditioning and vasodilation were shown to be GLP-1 receptor-mediated, whereas the beneficial effects of GLP-1(9-36) on postischemic recovery of cardiac function are compatible with a GLP-
To date, there is no data to show effects of DPP-4 inhibitors on the human heart. Since GLP-1RA and DPP-4 inhibitors do not cause fluid retention, hypoglycemia or lactic acidosis these drugs may be an important option in the treatment of T2DM, especially in vulnerable patients with ischemia or CHF. Large prospective intervention trials in humans applying this novel drug class are eagerly awaited.

Table 2. Current bloodglucose lowering agents and experimental metabolic modifiers and their effects on systemic and cardiac metabolism

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mode of action</th>
<th>Cardiac metabolic effects in humans*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bloodglucose lowering agents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biguanides</td>
<td>Partially unknown, activation of AMPK</td>
<td>=/↑ MGU 3, 14, 180</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ myocardial NEFA oxidation 140</td>
</tr>
<tr>
<td></td>
<td></td>
<td>= PCr/ATP 180</td>
</tr>
<tr>
<td>Sulfonylurea derivatives</td>
<td>Blocking of ATP-dependent K⁺-channels</td>
<td>=/↑ MGU 3, 14, 180</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ NEFA utilization and oxidation 175</td>
</tr>
<tr>
<td>Insulin</td>
<td>Activation of insulin receptors</td>
<td>=/↑ MGU 3, 14, 180</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ NEFA utilization and oxidation 175</td>
</tr>
<tr>
<td></td>
<td>↓ glucose uptake in target organs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ glycogen synthesis in liver</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ lipolysis</td>
<td></td>
</tr>
<tr>
<td>Thiazolidinediones</td>
<td>Activation of PPAR-γ</td>
<td>=/↑ MGU 3, 14, 180</td>
</tr>
<tr>
<td></td>
<td></td>
<td>=/↑ MTG 3, 140</td>
</tr>
<tr>
<td></td>
<td>= PCr/ATP 140</td>
<td></td>
</tr>
<tr>
<td>GLP-1 receptor agonists</td>
<td>Activation of GLP-1 receptors</td>
<td>=/↑ MGU 3, 14, 180</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ NEFA utilization and oxidation 175</td>
</tr>
<tr>
<td></td>
<td>↓ glucose uptake / -production</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ glucagon secretion</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ insulin sensitivity secondary to weight loss</td>
<td></td>
</tr>
<tr>
<td>DPP-4 inhibitors</td>
<td>Inhibition of DPP-4, preventing degradation of endogenous GLP-1 and GIP</td>
<td>=/↑ insulin secretion / production</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ glucagon secretion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not reported</td>
</tr>
<tr>
<td><strong>Metabolic modifiers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perhexilene</td>
<td>Inhibition of CPT-1 and 2</td>
<td>↓ NEFA ,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ glucose metabolism</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not reported</td>
</tr>
<tr>
<td>Trimetazidine / Ranolazine</td>
<td>Possibly weak inhibition of CPT-1 or inhibition of LC 3-KAT</td>
<td>↓ NEFA ,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ glucose metabolism</td>
</tr>
<tr>
<td></td>
<td></td>
<td>= NEFA uptake,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ β-oxidation rate constant = efficiency 134</td>
</tr>
<tr>
<td>Etomoxir</td>
<td>Inhibition of CPT-1</td>
<td>↓ NEFA ,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ glucose metabolism</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not reported</td>
</tr>
</tbody>
</table>

*GLP- receptor agonist-mediated increases in MGU were reported in dogs and rats only. MGU, myocardial glucose uptake; AMPK, AMP-activated protein kinase; NEFA, non-esterified fatty acids; MTG, myocardial triglyceride content; ↑ = increased; ↓ = decreased; = = no difference; PCr/ATP, phosphocreatinine-to-adenosine-tri-phosphate ratio; PPAR-γ, peroxisome proliferator-activated gamma; DPP-4, dipeptidyl peptidase-4; GLP-1, glucagon-like-peptide-1; GIP = glucose-dependent insulinotropic polypeptide; CPT = carnitine-palmitoyl-transferase; LC 3-KAT = long-chain 3-ketoacyl coenzyme A thiolase.
Chapter 1

Metabolic modifiers, such as perhexiline, trimetazidine, ranozoline and etomoxir decrease myocardial FA metabolism and increase glucose metabolism by various different mechanisms (Table 2). The antianginal effect of these agents might be directly due to a rise in myocardial efficiency. Recently, three-months treatment with trimetazidine was compared with placebo in T2DM patients with CAD, and improved LV systolic function and functional capacity despite no change in myocardial perfusion. In CHF patients, three months therapy with etomoxir improved LV function, cardiac output at peak exercise and clinical status. However, some concerns exist about the long-lasting safety profile of these metabolic modifiers, which might induce neurotoxicity and/or lipotoxicity (perhexiline) or phospholipodosis (etomoxir).

Conclusion

In experimental DCM, insulin resistance and altered myocardial substrate metabolism lead to glucose-lipotoxicity, mitochondrial dysfunction, oxidative stress and altered Ca²⁺ handling, which adversely affects myocardial contractility. Evidence for myocardial insulin resistance and altered substrate handling to be causal for the observed cardiac functional abnormalities in human DCM, however, remains limited. In selected populations, therapies aimed at improving insulin sensitivity and/or interfering with substrate metabolism have shown to beneficially affect myocardial function. Further studies in the various stages of human DCM are needed to determine the cardiac metabolic changes and their association to functional alterations over time, in order to establish an evidence based rationale for therapies that target insulin resistance and cardiac metabolism, as well as their appropriate timing in the course of the disease.
Reference List


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ber;51(10):3020-4.


Ref Type: Abstract.


Chapter 1


173. Tuunanen H, Engblom E, Naum A et al. Trimetazidine, a metabolic modulator, has cardiac and extracardiac ben-

37
Myocardial steatosis is an independent predictor of diastolic dysfunction in type 2 diabetes mellitus
Abstract

OBJECTIVES: The purpose of this study was to compare myocardial triglyceride content and function between patients with uncomplicated Type 2 diabetes mellitus (T2DM) and healthy subjects within the same range of age and body mass index (BMI), and to study the associations between myocardial triglyceride content and function.

BACKGROUND: T2DM is a major risk factor for cardiovascular disease. Increasing evidence is emerging indicating that lipid oversupply to cardiomyocytes plays a role in the development of diabetic cardiomyopathy, by causing lipotoxic injury and myocardial steatosis.

METHODS: Myocardial triglyceride content and myocardial function were measured in 38 T2DM patients and 28 healthy volunteers in the same range of age and BMI by proton magnetic resonance (MR) spectroscopy and MR imaging, respectively. Myocardial triglyceride content was calculated as a percentage relative to the signal of myocardial water.

RESULTS: Myocardial triglyceride content was significantly higher in T2DM patients compared with healthy volunteers (0.96±0.07 vs. 0.65±0.05%, p<0.05). Systolic function did not significantly differ between both groups. Indexes of diastolic function, including the ratio of maximal left ventricular early peak filling rate and the maximal left ventricular atrial peak filling rate (E/A) and E peak deceleration, were significantly impaired in T2DM compared with those in healthy subjects (1.08±0.04 ml/s'/× 10⁴ vs. 1.24±0.06 ml/s'/× 10⁴ and 3.6±0.2 ml/s'/× 10⁴ vs. 4.4±0.3 ml/s'/× 10⁴ respectively, p<0.05). Multivariable analysis indicated that myocardial triglyceride content was associated with E/A and E peak deceleration, independently of diabetic state, age, BMI, heart rate, visceral fat and diastolic blood pressure.

CONCLUSIONS: Myocardial triglyceride content is increased in uncomplicated T2DM and associated with impaired left ventricular diastolic function, independently of age, BMI, heart rate, visceral fat, and diastolic blood pressure.
Chapter 2

Introduction

Type 2 diabetes mellitus (T2DM) is a major risk factor for cardiovascular disease and early death (1,2). The pathophysiology of non-ischemic diabetic cardiomyopathy is complex and the exact mechanisms of disease remain partly unknown (2). Increasing evidence is emerging indicating that lipid oversupply to cardiomyocytes, which may lead to lipotoxic injury, plays a role in the development of diabetic cardiomyopathy (3,4,5). Increased fatty acid (FA) fluxes arising from the disproportionate amount of insulin resistant (visceral) adipose tissue lead to excessive FA delivery and uptake in the heart. This FA uptake exceeds the oxidizing requirements of the organ, giving rise to fatty acyl-CoA esters, diacylglycerol and ceramide as intermediates (5). Increasing evidence exists that accumulation of these FA intermediates causes mitochondrial dysfunction and reactive oxygen species, giving rise either directly to cell-damage and apoptosis or indirectly through the induction of inflammatory cascades, which leads to myocardial dysfunction (6,7,8,9). In animal models, anti-steatotic treatment with thiazolidinediones reduced myocardial triglyceride accumulation and ceramide content, and prevented myocardial dysfunction (7). Recently, it has been demonstrated in a heterogeneous group of T2DM patients that myocardial triglyceride content was increased compared with that of young, lean, healthy control subjects (10). However, direct correlations between myocardial triglyceride accumulation and heart function could not be established. To investigate the net contribution of T2DM to myocardial triglyceride accumulation and the associated functional consequences, it is essential to select controls within the same range of age and body mass index (BMI). In addition, to investigate the association between myocardial triglyceride accumulation and heart function, underlying ischemic heart disease should be excluded. Left ventricular (LV) diastolic function can be assessed using flow velocity encoded magnetic resonance imaging (MRI). Using this technique, myocardial diastolic functional parameters have been shown to associate with myocardial triglyceride accumulation in healthy volunteers (11). Furthermore, in explanted hearts of obese and T2DM patients with end-stage heart failure, lipid staining was a common finding (4).

In addition to myocardial accumulation of triglycerides, T2DM is associated with increased visceral adipose tissue, which associates with elevations of circulating plasma triglycerides and non-esterified fatty acid (NEFA) levels, and thus contributes to lipid overexposure to non-adipose tissue compartments.

Therefore, the purpose of the present study was to compare myocardial and hepatic triglyceride content and myocardial function between patients with uncomplicated T2DM and healthy subjects within the same range of age and BMI and to study the associations between myocardial triglyceride accumulation and heart function.

Methods

Subjects

Forty-one male T2DM patients were included in this study, which was approved by the local ethics committee. Hormonal status or use of contraceptives may affect lipid metabolism in women. Plasma estrogen levels influence lipid metabolism (including plasma lipid levels, adipose tissue), and gender differences in expression of certain cell surface receptors/transporters of fatty acids have been reported (12,13). Therefore, we decided to exclude women to avoid possible confounding influences of fluctuation in lipid metabolism in women on hepatic and myocardial triglyceride accumulation.
Myocardial steatosis is an independent predictor of diastolic dysfunction in type 2 diabetes mellitus

accumulation. All participants signed informed consent. Patients were recruited by advertisements in the local newspapers according to the following inclusion criteria: 1) age: 45 to 65 years; 2) T2DM diagnosed according to World Health Organization criteria (14) and treated with sulfonylurea derivatives in stable doses; 3) glycated hemoglobin below 8.5%; and 4) sitting blood pressure <150/85 mmHg, with or without antihypertensive drugs. Exclusion criteria included impaired hepatic function or a history of liver disease, substance abuse, known cardiovascular disease or diabetes-related complications, including proliferative retinopathy, autonomic neuropathy, as excluded by Ewing’s tests (15); microalbuminuria, as excluded by measurements of albumin/creatinine ratio in a urine sample; and all contra-indications for MRI. Furthermore, to avoid interference with the main study parameters, patients on lipid lowering therapy (such as statins, fibrates) were excluded. Most importantly, however, myocardial ischemia was excluded by means of high-dose dobutamine stress echocardiography.

Thirty healthy male control subjects within the same range of age (45-65 years) and BMI (25-32 kg/ m²) as the patient group were recruited by advertisements in the local newspapers. Subjects were included when they fulfilled the following criteria: no known acute or chronic disease based on history and physical examination and standard laboratory tests (blood counts, fasting blood glucose, lipids, serum creatinine, liver enzymes, and electrocardiogram). Exclusion criteria included chronic use of any drug, substance abuse, hypertension and impaired glucose tolerance (as excluded by a 75-g oral glucose tolerance test) (16).

Magnetic resonance (MR) spectroscopy
All subjects underwent MR scanning in the morning for the assessment of ectopic triglyceride content and heart function after an overnight fast and after blood sampling. All MR studies were performed with the use of a 1.5-T whole-body MR scanner (Gyroscan ACS/NT15, Philips, Best, the Netherlands) with subjects in supine position at rest. Myocardial ¹H-MR spectra were obtained as described previously (17). Briefly, the body coil was used for radiofrequency transmission and a 17 cm diameter circular surface coil was used for signal reception. An 8-ml voxel was positioned in the interventricular septum. Spectroscopic data acquisition was double triggered using electrocardiographic triggering and respiratory navigator echoes to minimize breathing influences (17). Water suppressed spectra were acquired for the detection of the triglyceride signals at end systole, with an echo time of 26 ms and a repetition time of at least 3000 ms. 1024 data points were collected using a 1000 Hz spectral width and averaged over 128 acquisitions. Without changing any parameter, spectra without water suppression with a repetition time of 10 s and 4 averages were obtained from the same voxel, to be used as an internal standard.

¹H-MR spectroscopy of the liver was performed with an 8 ml voxel positioned in the liver, avoiding gross vascular structures and adipose tissue deposits. Spectra were obtained using the same parameters as described for myocardial ¹H-MR spectroscopy. Only 64 averages were collected with water suppression.
All ¹H-MR spectroscopic data were fitted using Java-based MR user interface software (JMRUI version 2.2; developed by A. van den Boogaart, Katholieke Universiteit Leuven, Leuven, Belgium) (18) as described before (17). Myocardial and hepatic triglyceride content was calculated as a percentage relative to water as (signal amplitude of triglyceride) /(signal amplitude of water) × 100.
MR imaging
All images were analyzed quantitatively using dedicated software (FLOW® or MASS®, Medis, Leiden, the Netherlands). The entire heart was imaged in short-axis orientation using electrocardiographically gated breath-holds with a sensitivity encoding balanced turbo-field echo sequence. Imaging parameters included the following: echo time = 1.7 ms, repetition time = 3.4 ms, flip-angle = 35 degrees, slice thickness = 10 mm with a gap of 0 mm, field of view = 400 mm², reconstructed matrix size = 256 x 256. LV ejection fraction was assessed for the determination of LV systolic function. In addition, LV mass/ (end diastolic) volume ratio was calculated.
An electrocardiographically gated gradient-echo sequence with velocity encoding was performed to measure blood flow across the mitral valve for the determination of LV diastolic function. Imaging parameters included the following: echo time = 5 ms, repetition time = 14 ms, flip-angle = 20 degrees, slice thickness = 8 mm, field of view = 350 mm², matrix size = 256 x 256, Velocity encoding gradient = 100 cm/s, scan percentage = 80%. The resulting biphasic diastolic inflow pattern consists of two peaks, representing the early filling phase and the atrial contraction. Analysis of the early filling phase and the atrial contraction was performed by calculation of their peak filling rates and ratio of the peak filling rates (E/A). Furthermore, the peak deceleration gradient of the early filling phase (E deceleration peak) was calculated automatically. In addition, an estimation of LV filling pressures (E/Ea) was assessed as described before (19). During MR imaging, blood pressure and heart-rate were measured.
Abdominal visceral fat depots were quantified by magnetic resonance imaging (20). A turbo spin echo imaging protocol was used and imaging parameters included the following: echo time = 11ms, repetition time = 168ms, flip-angle = 90 degrees, slice thickness = 10mm. Three consecutive transverse images were obtained during 1 breath hold with the middle image at a level just above the fifth lumbar vertebra. The volumes of the visceral fat depots of all slices were calculated by converting the number of pixels to square centimeters multiplied by the slice thickness. The total volume of the fat depots was calculated by summing the volumes of all three slices.

Assays
All samples were analyzed at one certified central laboratory (Amsterdam). Plasma glucose was measured using a hexokinase-based technique (Roche diagnostics, Mannheim, Germany), glycated hemoglobin by high-performance liquid chromatography (Menarini Diagnostics, Florence, Italy; reference values: 4.3-6.1%). Plasma total cholesterol, high density lipoprotein cholesterol, and triglycerides were determined using enzymatic colorimetric methods (Modular, Hitachi, Japan). Low density lipoprotein cholesterol was calculated using Friedewald’s formula. Insulin was measured by an immunoradiometric assay (Bayer Diagnostics, Mijdrecht, The Netherlands). NEFA were assessed using ELISA (WAKO chemicals, Neuss, Germany).

Statistical analysis
Statistical analysis was performed using SPSS for windows version 12.0. Data are expressed as mean ± standard error when normally distributed; nonnormally distributed data are expressed as the median (interquartile range). Nonnormally distributed data were log-transformed, and unpaired t tests (or, when appropriate, non-parametric tests) were used for comparisons. To detect determinants of myocardial triglyceride content and LV function, univariate and multivariable linear regression analyses were performed. P < 0.05 was considered statistically significant.
Myocardial steatosis is an independent predictor of diastolic dysfunction in type 2 diabetes mellitus

Results

Baseline characteristics of patients and healthy subjects are displayed in Table 1. The MR scan protocol could not successfully be completed due to technical constraints in 3 patients and in 2 healthy subjects. Therefore, data obtained from 38 patients and 28 healthy subjects was used for analysis. Mean systolic blood pressure in both groups was well within the normal range, although it was higher in patients than in controls (127 ± 2 vs. 116 ± 2 mmHg, p < 0.05). Although autonomic neuropathy was absent, documented by Ewing’s test in the patients, their resting heart rate was increased relative to controls (median (interquartile range): 65 [62 - 72] vs. 59 [52 - 63] bpm, p < 0.05).

Table 1. Clinical and biochemical characteristics

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects</th>
<th>Patients with T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>28</td>
<td>38</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54 ± 1</td>
<td>57 ± 1</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.9 ± 0.3</td>
<td>28.1 ± 0.3</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>116 ± 2</td>
<td>127 ± 2*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>71 ± 2</td>
<td>71 ± 1</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>59 (52 – 63)</td>
<td>65 (62 – 72)*</td>
</tr>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td>5.3 ± 0.1</td>
<td>9.1 ± 0.3*</td>
</tr>
<tr>
<td>Plasma insulin (pmol/l)</td>
<td>35 (24 – 51)</td>
<td>55 (35 – 100)*</td>
</tr>
<tr>
<td>Glycated Hemoglobin (%)</td>
<td>5.3 ± 0.05</td>
<td>7.21 ± 0.17*</td>
</tr>
<tr>
<td>Plasma triglycerides (mmol/l)</td>
<td>1.0 (0.8 – 1.3)</td>
<td>1.6 (1.1 – 2.8) *</td>
</tr>
<tr>
<td>Non-esterified fatty acids (mmol/l)</td>
<td>0.45 ± 0.04</td>
<td>0.49 ± 0.03</td>
</tr>
<tr>
<td>High density lipoprotein cholesterol (mmol/l)</td>
<td>1.39 ± 0.06</td>
<td>1.09 ± 0.04*</td>
</tr>
<tr>
<td>Low density lipoprotein cholesterol (mmol/l)</td>
<td>3.3 (3.0 – 3.7)</td>
<td>2.9 (2.6 – 3.4)*</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.29 ± 0.14</td>
<td>5.00 ± 0.13</td>
</tr>
</tbody>
</table>

Data are mean ± standard error or median (interquartile range). * p < 0.05 compared to healthy subjects.

Myocardial and hepatic triglyceride content

Myocardial triglyceride content was significantly higher in patients with T2DM compared to healthy volunteers (0.96 ± 0.07% vs. 0.65 ± 0.05%) as was hepatic triglyceride content (8.6% [2.7% – 24.3%] vs. 2.2% [1.2% – 3.8%], p < 0.05, Table 2 and Figure 1). Myocardial triglyceride content showed significant univariate correlations with age, visceral adipose tissue volume, plasma triglycerides, plasma high density lipoprotein cholesterol, plasma glucose, plasma insulin concentrations and hepatic triglyceride content (Pearson r = 0.28, 0.36, 0.37, -0.39, 0.45, 0.30, and 0.37 respectively, p < 0.05), but not with BMI, in all study subjects pooled. Visceral adipose tissue volume and plasma high density lipoprotein cholesterol and glucose concentrations as well as hepatic triglyceride content levels remained significantly correlated to myocardial triglyceride content after adjustment for diabetic state, whereas the association between plasma triglyceride levels and myocardial triglyceride content showed borderline significance when adjusted for diabetic state (p = 0.051).
Bar graphs show increased myocardial and hepatic triglyceride content in diabetic patients as compared to healthy control subjects. Bars represent mean + standard error for myocardial triglycerides and median + interquartile range for hepatic triglycerides. * p < 0.05

**Association between myocardial steatosis and myocardial function**

Differences in cardiovascular function between T2DM patients and healthy volunteers are displayed in Table 2. LV diastolic parameters were significantly impaired in T2DM patients. LV systolic function was not significantly different between healthy subjects and T2DM patients (ejection fraction = 58 ± 1 vs. 60 ± 1%, p > 0.05).

E/A and E peak deceleration were used as parameters of LV diastolic function for further analysis. Table 3 lists Pearson correlations of E/A and the E deceleration peak with several parameters in all study subjects and shows that both parameters were significantly inversely correlated to age, heart rate, blood pressure, plasma glucose levels and myocardial triglyceride content (p < 0.05, Figure 2). Multivariable analysis was performed in all subjects to study the association between diastolic function and myocardial triglyceride content. To this purpose, E/A was entered as a dependent variable, and subsequently, myocardial triglyceride content, the presence of type 2 diabetes mellitus, age, heart rate, and diastolic blood pressure were entered as independent variables into the model (Table 4). The diabetic state had no effect on the association between E/A and myocardial triglyceride content. Furthermore, adjustment for age, heart rate, and diastolic blood pressure, which were all significantly correlated to E/A, had no effect on the association between E/A and myocardial triglyceride content. Identical analyses were performed with E peak deceleration as independent variable. This analysis indicated myocardial triglyceride content associated with E peak deceleration, independently of diabetic state, age, heart rate, and diastolic blood pressure.
Increased myocardial triglyceride content is significantly associated with decreased myocardial function.

\( E = \) early diastolic filling phase, \( E/A = \) ratio of maximal left ventricular early peak filling rate and the maximal left ventricular atrial peak filling rate

**Table 2.** Magnetic resonance study parameters

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects</th>
<th>Patients with T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial triglyceride content (%)</td>
<td>0.65 ± 0.05</td>
<td>0.96 ± 0.07*</td>
</tr>
<tr>
<td>Hepatic triglyceride content (%)</td>
<td>2.2 (1.2 – 3.8)</td>
<td>8.6 (2.7 – 24.3)*</td>
</tr>
<tr>
<td>Visceral fat (ml)</td>
<td>284 ± 24</td>
<td>420 ± 31*</td>
</tr>
<tr>
<td>Left ventricular mass (g)</td>
<td>108 ± 5</td>
<td>105 ± 3</td>
</tr>
<tr>
<td>LV mass/volume ratio (g/ml)</td>
<td>1.23 ± 0.02</td>
<td>0.11 ± 0.02*</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%)</td>
<td>58 ± 1</td>
<td>60 ± 1</td>
</tr>
<tr>
<td>E peak filling rate (ml/s)</td>
<td>490 ± 21</td>
<td>426 ± 14*</td>
</tr>
<tr>
<td>E peak deceleration (ml/s × 10^-1)</td>
<td>4.4 ± 0.1</td>
<td>3.6 ± 0.2*</td>
</tr>
<tr>
<td>A peak filling rate (ml/s)</td>
<td>402 ± 9</td>
<td>401 ± 10</td>
</tr>
<tr>
<td>E/A</td>
<td>1.24 ± 0.06</td>
<td>1.08 ± 0.04*</td>
</tr>
<tr>
<td>E/Ea</td>
<td>9.1 ± 0.6</td>
<td>10.0 ± 0.7</td>
</tr>
</tbody>
</table>

Data are mean ± standard error or median (interquartile range). * p<0.05 compared to healthy subjects. A= atrial contraction; \( E = \) early diastolic filling phase; \( E/A = \) ratio of maximal left ventricular early peak filling rate and the maximal left ventricular atrial peak filling rate; \( E/Ea = \) estimation of left ventricular filling pressures; \( LV = \) left ventricular; LV mass/volume ratio = left ventricular mass/left ventricular end-diastolic volume; T2DM = type 2 diabetes mellitus.
Table 3. Univariate correlations between diastolic function and anthropometric and biochemical markers and fat compartments.

<table>
<thead>
<tr>
<th></th>
<th>E/A</th>
<th>E peak deceleration (ml/s² × 10⁻⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>-0.58*</td>
<td>-0.44*</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>-0.25*</td>
<td>-0.18</td>
</tr>
<tr>
<td>Log heart rate (bpm)</td>
<td>-0.36*</td>
<td>-0.36*</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>-0.26*</td>
<td>-0.24</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>-0.41*</td>
<td>-0.45*</td>
</tr>
<tr>
<td>LV mass/volume ratio (g/ml)</td>
<td>-0.35*</td>
<td>-0.46*</td>
</tr>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td>-0.32*</td>
<td>-0.34*</td>
</tr>
<tr>
<td>Log plasma triglyceride (mmol/l)</td>
<td>0.01</td>
<td>-0.05</td>
</tr>
<tr>
<td>Non-esterified fatty acids (mmol/l)</td>
<td>0.01</td>
<td>0.09</td>
</tr>
<tr>
<td>High density lipoprotein cholesterol (mmol/l)</td>
<td>0.21</td>
<td>0.28*</td>
</tr>
<tr>
<td>Visceral fat (ml)</td>
<td>-0.31*</td>
<td>-0.20</td>
</tr>
<tr>
<td>Hepatic triglyceride content (%)</td>
<td>-0.30*</td>
<td>-0.21</td>
</tr>
<tr>
<td>Myocardial triglyceride content (%)</td>
<td>-0.42*</td>
<td>-0.40*</td>
</tr>
</tbody>
</table>

Values are Pearson r. * p < 0.05. Abbreviations as in Table 2.

Discussion

In this study, we showed that accumulation of myocardial triglycerides in T2DM patients is associated with LV diastolic dysfunction, independently of age, BMI, heart rate, visceral fat and diastolic blood pressure.

We were able to extend the findings in previous studies showing myocardial steatosis in T2DM patients (4,10). McGavock et al. demonstrated that excessive triglyceride accumulation in human cardiomyocytes occurs early in the natural history of T2DM. In their study, a heterogeneous group of T2DM patients showed increased myocardial triglyceride content compared to healthy subjects.

The use of insulin (a well-known lipogenic agent, which might have increased myocardial triglyceride levels) by the T2DM patients and differences in age and BMI between the two groups could have influenced their observations. In addition, the occurrence of silent ischemia, which can be present in up to 22% of asymptomatic T2DM patients with a normal electrocardiogram (21), could influence accumulation of triglycerides in the reversibly injured myocardium during reperfusion (22,23). Therefore, in the present study, we used controls of the same gender and within the same range of age and BMI as patients, and included only T2DM patients with normal dobutamine stress echocardiography to control for reversible ischemia. By using these well-defined groups we could confirm that myocardial triglyceride content in T2DM patients is significantly elevated in comparison to healthy volunteers, independently of age or BMI.

In our study, diabetic patients had higher plasma triglyceride levels and showed significantly lower levels of high density lipoprotein cholesterol compared to control subjects. Interestingly, plasma
triglyceride concentrations were positively associated with myocardial triglyceride content. We also found an inverse correlation between myocardial triglyceride content and high density lipoprotein cholesterol levels, also after adjustment for diabetic state. These data are in line with prior observations demonstrating that increased serum triglyceride and decreased serum high density lipoprotein cholesterol concentrations correlate with lipid content in skeletal muscle in patients with HIV-lipodystrophy (24). High density lipoprotein cholesterol is considered as an important mediator of reverse cholesterol transport, a process that involves the transfer and uptake of free cholesterol from the peripheral tissues, with subsequent delivery to the liver where it can be eliminated. Artificial elevation of high density lipoprotein in cholesterol-fed rabbits induced the regression of early aortic fatty streaks (25). Based on our results, we hypothesize that reverse cholesterol transport might also play a role in protecting the heart from accumulating lipids.

In the present study, we are the first to demonstrate that myocardial triglyceride accumulation in patients with uncomplicated T2DM is associated with left ventricular diastolic dysfunction, independently of age, BMI, blood pressure and heart-rate. Neutral triglycerides are probably inert and harmless to cells and may initially even provide a protective buffer by diverting NEFA from deleterious pathways (26). Eventually, however, excessive triglyceride stores enter a continuous cycle between hydrolysis and fatty acid re-esterification, yielding cardiotoxic intermediates, such as ceramide and diacylglycerol, which seem to be an important route leading to myocardial dysfunction, at least in animal models (7,27).

In the present study, left ventricular ejection fraction was not different between patients and healthy control subjects and was dissociated from myocardial triglyceride content. We excluded patients with myocardial ischaemia and contractile abnormalities, using dobutamine stress echocardiography, however, microvascular disease cannot be ruled out. Prior experimental studies (28) have shown decreased contractility and diastolic function contemporary with hypertrophy and concentric remodeling. In our patient population with uncomplicated diabetes mellitus, cardiac mass was not different from healthy volunteers. LV mass/volume ratio, was increased in T2DM patients, indicating mild concentric remodeling (29). Therefore, LV mass/volume ratio was included in the multivariable analysis, but had no influence on the independent relationship between myocardial triglyceride content and diastolic function.

Based on these findings, we hypothesize that there may be a disease course starting with lipid accumulation in the myocardium leading to diastolic dysfunction. In a later stage of disease, global systolic function may be impaired, as diastolic abnormalities are observed most commonly earlier than systolic abnormalities (30).

Furthermore, in uncomplicated T2DM, visceral fat volume and hepatic triglyceride content were increased and correlated to myocardial triglyceride content after adjustment for diabetic state. Our findings support the evidence that lipotoxic processes in cardiomyocytes constitute an important mechanism underlying the epidemiological association between visceral adiposity, ectopic steatosis and cardiovascular disease (10,31,32).

In the present study design, we cannot establish a causal relationship between increased myocardial triglyceride content and reduced left ventricular function. We cannot discriminate if myocardial triglyceride accumulation per se hampers myocardial function due to a mechanical effect, the formation of cardiotoxic intermediates, or by intervening in other mechanisms such as calcium handling. In addition, excluding women from the study limits the generalizability of the present study. Further studies need to be initiated to make these distinctions and to extend the present findings to female subjects to identify the role of myocardial triglyceride accumulation in the human diabetic heart, because in animal models, therapeutic interventions aiming at reduction of
myocardial triglyceride accumulation due to disturbed fatty acid metabolism have been shown to have beneficial effects on myocardial function (7). Furthermore, pioglitazone, a peroxisome proliferator-activated receptor γ agonist, which has the capacity to divert fat from non-adipose tissue to subcutaneous tissue, has been shown to lower myocardial triglyceride content in T2DM patients on an insulin–based treatment regimen (33). Therefore, myocardial triglyceride content might be a useful indicator of myocardial steatosis for predicting the severity of diabetic cardiomyopathy and for evaluating the effects of antisteatotic therapy.

Conclusion

Myocardial triglyceride content is increased in uncomplicated T2DM and associated with impaired LV diastolic function, independently of age, BMI, heart rate, visceral fat, and diastolic blood pressure. Therefore, myocardial steatosis might be a useful indicator for predicting the severity of diabetic cardiomyopathy and for evaluating the effects of antisteatotic therapy.
Myocardial steatosis is an independent predictor of diastolic dysfunction in type 2 diabetes mellitus

Reference List

Altered Myocardial Substrate Metabolism and Decreased Diastolic Function in Non-Ischemic Human Diabetic Cardiomyopathy: Studies with Cardiac PET and MRI

Chapter 3

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Robert J Heine
Adriaan A Lammertsma
Johannes WA Smit
Michaela Diamant

J Am Coll Cardiol. 2009;54:1524-32
Abstract

OBJECTIVES: This study was designed to evaluate myocardial substrate and high-energy phosphate (HEP) metabolism in asymptomatic men with well-controlled, uncomplicated type 2 diabetes (T2DM) with verified absence of cardiac ischemia, and age-matched control subjects, and to assess the association with myocardial function.

BACKGROUND: Metabolic abnormalities, particularly an excessive exposure of the heart to circulating non-esterified fatty acids (NEFA) and myocardial insulin resistance are considered important contributors to diabetic cardiomyopathy in animal models of diabetes. The existence of myocardial metabolic derangements in uncomplicated human T2DM and their possible contribution to myocardial dysfunction still remain undetermined.

METHODS: In 78 insulin-naive T2DM men (age 56.5 ± 5.6 years; Body mass index 28.7 ± 3.5 kg/m²; glycated hemoglobin A1c 7.1 ± 1.0% expressed as mean ± SD) without cardiac ischemia and 24 normoglycemic control subjects (age 54.5 ± 7.1 years; Body mass index 27.0 ± 2.5 kg/m²; glycated hemoglobin A1c 5.3 ± 0.2%), we assessed myocardial left ventricular (LV) function by magnetic resonance imaging, and myocardial perfusion and substrate metabolism by positron-emission tomography using H215O, [11C]palmitate and [18F]-2-fluoro-2-deoxy-D-glucose. Cardiac HEP metabolism was assessed by 31P-MR spectroscopy.

RESULTS: In patients, compared with controls, LV diastolic function (E/A ratio 1.04±0.25 versus 1.26±0.36, p=0.003) and myocardial glucose uptake (260±128 versus 348±154 nmol/ml/min, p=0.015) were decreased, whereas myocardial NEFA uptake (88±31 versus 68±18 nmol/ml/min, p=0.021) and oxidation (85±30 versus 63±19 nmol/ml/min, p=0.007) were increased. There were no differences in myocardial HEP metabolism or perfusion. No association was found between LV diastolic function and cardiac substrate-or HEP metabolism.

CONCLUSIONS: Patients versus control subjects showed impaired LV diastolic function, altered myocardial substrate metabolism, but unchanged HEP metabolism. We found no direct relation between cardiac diastolic function and parameters of myocardial metabolism.
Introduction

Altered cardiac function, structure and dimensions are common findings in asymptomatic patients with type 2 diabetes mellitus (T2DM), even in the absence of hypertension and coronary artery disease (CAD). These alterations are attributed to diabetic cardiomyopathy (DCM) (1). DCM is a multifactorial disease entity that is clinically characterized by an initial increase in left ventricular (LV) stiffness (2) and subclinical diastolic dysfunction. However, DCM may advance to compromised LV systolic function, with a high propensity to progress into overt congestive heart failure (3).

In animal models of diabetes, cardiac dysfunction coexists with increased myocardial NEFA utilization, triglyceride accumulation and subsequent increased production of toxic intermediates, which, in the presence of hyperglycemia, contribute to increased formation of reactive oxygen species (ROS), mitochondrial uncoupling, decreased ATP synthesis, mitochondrial dysfunction and finally apoptosis (1, 4). These deleterious processes are commonly referred to as gluco-lipotoxicity.

In patients with T2DM, it is technically challenging to demonstrate the existence of these abnormalities as causal mechanisms underlying DCM. Only a few studies have investigated myocardial glucose and NEFA metabolism in the human (pre)diabetic heart, yielding conflicting results (1, 5-14). Even fewer studies related altered cardiac substrate metabolism to function (8, 9), however; these results are not unequivocal. Previously, we and others showed decreased cardiac PCr/ATP ratios in patients with T2DM (15, 16), which was associated with diastolic dysfunction only in our study. Consequently, the interrelation between myocardial substrate and energy metabolism and LV function is currently unclear in human T2DM.

The present study aimed to investigate the influence of T2DM on myocardial substrate and HEP metabolism and their relation to myocardial function using cardiac magnetic resonance (CMR), phosphorus-31 magnetic resonance spectroscopy (31P-MRS) and positron-emission tomography (PET) in asymptomatic patients with recently diagnosed, uncomplicated T2DM. As coronary artery disease (CAD) and ischemia impact on myocardial substrate preference (9, 17), only T2DM patients with verified absence of inducible ischemia as assessed by dobutamine stress echocardiography were included.

Methods

Patients

Patients and healthy control subjects were recruited by advertisements in local newspapers. Men with uncomplicated T2DM, aged 45-65 years, were eligible. Inclusion criteria were a glycated hemoglobin A1c (HbA1c) level of 6.5-8.5 % at screening, BMI of 25 to 32 kg/m² and blood pressure not exceeding 150/85 mm Hg (with or without the use of anti-hypertensives). Patients were excluded when any history or complaints related to cardiovascular disease, diabetes or any clinically noticeable disorder was present. Patients were also excluded if they used thiazolidinediones, fibrates, insulin or other hormonal replacement therapy. Patients were screened at two separate occasions. A general screening was performed, consisting of a medical history, physical examination, echocardiogram, fasting tests to exclude cardiac autonomic neuropathy and fasting blood- and urine analysis. When patients fulfilled the initial criteria, they underwent a dobutamine-stress echocardiography (DSE) to exclude the presence of cardiac ischemia or arrhythmias at a separate visit. Following successful screening, participants entered a 10-week run-in period during which their regular blood-glucose lowering agents were transferred to glimepiride monotherapy, and titrated until a stable
Dose was reached during 8 weeks prior to assessments, to exclude possible confounding effects on myocardial metabolism of differential agents. Mean HbA1c levels at screening and at the end of the run-in period were comparable (data not shown). Healthy males, aged 45 to 65 years, with normal glucose metabolism, as assessed by a 75-g oral glucose tolerance test, were eligible as controls. Inclusion criteria were a BMI of 25 to 32 kg/m² and blood pressure less than 150/85 mm Hg. Exclusion criteria were a history of or current cardiovascular disease, dyslipidemia and the use of any prescribed medication. Controls underwent the same first screening visit as patients. CAD in controls was considered to be absent when there were no cardiac complaints at rest and during exercise and when a normal electrocardiogram was present. The study was performed at two sites (Amsterdam and Leiden, The Netherlands) and the protocol was approved by the Medical Ethics Committee of both centers and performed in full compliance with the Declaration of Helsinki.

**Biochemical Analyses**

HbA1c was determined by high-performance liquid chromatography (HPLC; Menarini Diagnostics, Florence, Italy) with reference values of 4.3-6.1%. NT-pro-BNP was measured using an electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany). The intra-assay coefficient of variation (CV) was 1.5%, the inter-assay CV was 1.9%. The lower detection limit was 5 ng/L. The plasma concentration of malondialdehyde (MDA) was determined in duplo by HPLC after alkaline hydrolysis and reaction with thiobarbituric acid (18). The intra-assay CV was 5.7%. Ultra-sensitive C-reactive protein (us-CRP) was determined by ELISA (DSL, Webster, Texas, USA).

**Imaging**

The study protocol was performed on two different occasions separated by two days and consisted of CMR and 31P-MRS on the first and PET on the second occasion or vice versa. All participants underwent CMR measurements. Due to the demanding protocol 31P-MRS was offered as an optional test. The PET measurements were performed in the first 60 patients entering the study. The patients did not take glucose lowering medication in the morning prior to assessments.

**Cardiac magnetic resonance imaging protocol**

CMR assessments were performed after an overnight fast at one single center (Leiden), using a 1.5-T whole-body MR scanner (Gyrosan ACS/NT15; Philips, Best, the Netherlands). During CMR examinations, blood pressure and heart rate were monitored and blood samples were collected to determine substrates. Rate pressure product (RPP) was calculated as the product of systolic blood pressure and heart rate. The entire heart was imaged in the short-axis orientation using electrocardiographically gated breath-hold balanced steady state free precession imaging (19).

Measures of systolic function were LV ejection fraction (EF), cardiac work and cardiac index (CI = Cardiac output/body surface area). LV end-diastolic volume (index) (LVEDV(i)), LV end-systolic volume (LVESV), LV stroke volume (LVSV) and LV mass (LVM) index (I) were cardiac dimensions. An electrocardiographically gated gradient-echo sequence with velocity encoding was performed to measure blood-flow across the mitral valve for the determination of LV diastolic function parameters, including peak filling rates of the early filling phase (E) and the atrial contraction (A) and their ratio (E/A) were calculated. Additionally, the peak deceleration of E (E-dec) was calculated (19). The LV filling pressures (E/Ea) were estimated (20). Images were analyzed quantitatively using dedicated software (MASS® and FLOW®, Medis, Leiden, the Netherlands).
Phosphorus magnetic resonance spectroscopy

A 100-mm-diameter surface coil was used to acquire ECG-triggered 31P-MR spectra of the LV anter-
rior wall with subjects in the supine position. Volumes of interest were selected by image-guided
spectroscopy with 3D-ISIS. Shimming was performed automatically and tuning and matching of the
31P surface coil was performed manually. Technical details of data acquisition and spectral quanti-
 fica tion were similar as described elsewhere (21). Briefly, spectroscopic volume size was typically
7 × 7 × 7 cm. Acquisitions were based on 192 averaged free induction decays, and total acquisition
time was 10 min. The 31P-MR spectra were quantified automatically in the time domain using prior
spectroscopic knowledge and were corrected for partial saturation effects and for the adenosine-
triphosphate (ATP) contribution from blood in the cardiac chambers. The phosphocreatine (PCr)/
ATP ratios of the spectra were calculated and used as a parameter representing myocardial HEP
metabolism (22).

Positron emission tomography imaging protocol

PET examinations were performed after an overnight fast at one single center (Amsterdam) using
an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, TN, USA). Patients received two venous cat-
 heters, one in an antecubital vein and the other in the vein of the opposite hand, the latter being
 wrapped into a heated blanket to obtain arterialized blood. During procedures patients were moni-
tored by telemetry and blood pressure was measured at 5-minute intervals. PET was used to meas-
ure myocardial blood flow (MBF) with H215O, myocardial NEFA uptake (MFAU), β-oxidation (MFAO)
and esterification (MFAE) with [11C]palmitate, and myocardial metabolic rate of glucose uptake
(MMRglu) with [11F]-2-fluoro-2-deoxy-D-glucose ([11FDG]. Perfusion and NEFA metabolism were as-
 sessed in the fasting state, whereas MMRglu was measured during a euglycemic-hyperinsulinemic
clamp (see below). Following a 10-min transmission scan for attenuation correction, H215O was
 injected (t=10 min) and a 10-min dynamic emission scan (40 frames with increasing frame length)
was acquired. Subsequently, a 30-min dynamic emission scan (34 frames) was performed follow-
ing [11C]palmitate injection (t=35 min). Hereafter, the clamp was started (t=65 min), as described
elsewhere (23), to approximate an isometabolic steady state (plasma glucose level 5 mmol/l) and
measure whole-body insulin sensitivity. At steady state (around t=155 min), following a new trans-
mission scan, [11FDG] was injected and a 60-min dynamic emission scan (40 frames) was acquired.
Blood samples were collected during [11C]palmitate and [11FDG] scans at predefined time points to
 measure glucose, NEFA, lactate, lipids and insulin levels. In addition, 11CO2 was measured during
the [11C]palmitate scan (14). Total radiation exposure was 4.87 mSv.

PET image analysis

The PET data were quantitatively reconstructed using filtered backprojection applying all appro-
 priate corrections. To generate myocardial time-activity curves, region of interest (ROIs) were defined
onto the dynamic images. The ROIs were drawn as previously described and grouped for further
analysis (24). Myocardial segments exposed to liver spill-in were omitted from the analysis of [11C]
palmitate data. Additional ROIs were defined in left and right ventricular chambers for [11C]palmit-
 ate and H215O image-derived input functions (IDIF). A separate aorta ascendance ROI was defined
for [11FDG] IDIF. MBF was determined using the standard single-tissue compartment model (25).
[11C]palmitate time-activity curves were analyzed using a three-tissue plasma input kinetic model,
which, together with plasma NEFA concentrations, enabled calculation of MFAU, MFAO and MFAE
(26). The [11C]palmitate IDIF was corrected for 11CO2 metabolites and difference between plasma
Altered Myocardial Substrate Metabolism and Decreased Diastolic Function

and whole blood concentrations as described elsewhere (14). This model is similar to that described by Bergmann et al. (27), but with a reduced number of free parameters, thereby increasing precision of derived estimates (details online). MMRglu was calculated by multiplying the net influx constant for 18FDG, K, by the mean plasma glucose concentration. For determination of K, Patlak graphical analysis was used (28).

Statistical analysis
Values are expressed as mean ± (standard deviation; SD) or median (interquartile range; IQR). Non-normally distributed data were log transformed. Comparisons between patients and control subjects were made using the independent-samples t test. Linear regression was used to adjust for BMI differences between groups. Univariate and multiple analyses with a forward selection procedure were performed.

The goal of these analyses were to determine which factors were responsible for the difference in LV diastolic function (E/A and E-dec) between groups. We employed a two step strategy for the selection of variables. The first step was that a variable had to be significantly different between groups. If so, in univariate analysis there had to be an association between this variable and the dependent variable with a P < 0.1. The variables fulfilling these criteria were then entered in a forward multivariable regression analysis and those with a P < 0.05 were considered independently related to the dependent variable. Analyses were performed with SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA). A two-tailed probability value <0.05 was considered significant.

Results

Subject characteristics, hemodynamics and LV dimensions and function
A total of 173 patients underwent the general screening of whom 96 patients fulfilled all inclusion criteria. Therefore, 96 patients underwent DSE. Sixteen patients were excluded (17.7%) as result of a positive test and 2 patients withdrew before measurements, leaving a total of 78 patients entered the study.

Patients and controls were similar with respect to age, but patients had a slightly higher BMI and waist circumference (Table 1). The lipids profiles were different between patients and controls (Table 1). Table 2 shows hemodynamic and cardiac parameters. Blood pressures and heart rates were within the normal range, but slightly higher in patients compared to controls. Patients had a significantly lower LVEDV(I) and LVSV than controls in the presence of comparable LVM(I), LVEF, CI and cardiac work. Patients had significantly decreased diastolic functional parameters. Mean E/Ea and NT-proBNP, both estimates of LV filling pressure, were not different between both groups. In T2DM patients the relation between LVEDVI and E/Ea was shifted towards decreased compliance (Figure 1).
Relation between LV end-diastolic volume index (LVEDVI) and the estimate of LV filling pressure E/Ea in controls (□, n=24) and T2DM patients (■, n=78). Values are means ± SD. The LV end-diastolic volume index (LVEDVI) was significantly lower in T2DM patients (P<0.001) at similar estimates of LV filling pressure (E/Ea, P=0.228) compatible with decreased LV compliance in T2DM patients.

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control subjects N=24</th>
<th>Type 2 diabetic patients N=78</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>54.5±7.1</td>
<td>56.5±5.6</td>
</tr>
<tr>
<td>Time since diagnosis of diabetes (yrs)</td>
<td>NA</td>
<td>4 (2-6)</td>
</tr>
<tr>
<td>Current smoker – n/N</td>
<td>0/24</td>
<td>17/78*</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.0±2.5</td>
<td>28.7±3.5*</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>99±19</td>
<td>104±20*</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>5.3±0.2</td>
<td>7.1±1.0*</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.3±0.7</td>
<td>4.7±1.0</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.4±0.65</td>
<td>2.7±0.7*</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.4 (1.3-1.6)</td>
<td>1.1 (0.9-1.3)*</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.9 (0.7-1.1)</td>
<td>1.5 (1.0-2.2)*</td>
</tr>
<tr>
<td>Medications – % (n/n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>NA</td>
<td>49 (38/78)</td>
</tr>
<tr>
<td>Any antihypertensive medication</td>
<td>NA</td>
<td>44 (34/78)</td>
</tr>
<tr>
<td>β-blockers</td>
<td>NA</td>
<td>9 (7/78)</td>
</tr>
<tr>
<td>Diuretics</td>
<td>NA</td>
<td>15 (12/78)</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>NA</td>
<td>23 (18/78)</td>
</tr>
<tr>
<td>Angiotensin II blocker</td>
<td>NA</td>
<td>12 (9/78)</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>NA</td>
<td>5 (4/78)</td>
</tr>
</tbody>
</table>

Data are mean±SD, median (IQR). ACE, angiotensin-converting enzyme. N.A., not applicable.
* p<0.001, † p<0.01, ‡ p<0.05.
Table 2. Hemodynamic parameters, cardiac dimensions and function in the study population.

<table>
<thead>
<tr>
<th></th>
<th>Control subjects N=24</th>
<th>Type 2 diabetic patients N=78</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Haemodynamics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>118±11</td>
<td>128±12*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>72±8</td>
<td>76±7†</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>56 (51-62)</td>
<td>64 (60-70)*</td>
</tr>
<tr>
<td>Rate pressure product (beats/min mm Hg)</td>
<td>6684±1441</td>
<td>8345±1457*</td>
</tr>
<tr>
<td><strong>Systolic function and dimensions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV mass (g)</td>
<td>111±24</td>
<td>107±17</td>
</tr>
<tr>
<td>LV mass index (g·m⁻²)</td>
<td>51 (47-60)</td>
<td>50 (46-55)</td>
</tr>
<tr>
<td>LV end-systolic volume (ml)</td>
<td>72 (63-82)</td>
<td>59 (52-71)†</td>
</tr>
<tr>
<td>LV Stroke volume (ml)</td>
<td>107±23</td>
<td>94±16†</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>59±4</td>
<td>60±5</td>
</tr>
<tr>
<td>Cardiac index (l/min·m⁻²)</td>
<td>2.9 (2.6-2.9)</td>
<td>2.8 (2.6-3.2)</td>
</tr>
<tr>
<td>Cardiac work (mm Hg·ml⁻¹·min⁻¹)</td>
<td>51.7±10.9</td>
<td>56.5±11.8</td>
</tr>
<tr>
<td><strong>Diastolic function and dimensions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV end-diastolic volume (ml)</td>
<td>181±36</td>
<td>156±25*</td>
</tr>
<tr>
<td>LV end-diastolic volume index (ml·m⁻²)</td>
<td>87±14</td>
<td>74±11*</td>
</tr>
<tr>
<td>E peak filling rate (ml/s)</td>
<td>503±112</td>
<td>417±84†</td>
</tr>
<tr>
<td>E deceleration peak (ml/s²·10⁻⁶)</td>
<td>4.73 (3.11-5.19)</td>
<td>3.40 (2.89-3.99)†</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.26±0.36</td>
<td>1.04±0.25†</td>
</tr>
<tr>
<td>E/Ea</td>
<td>8.8±4.0</td>
<td>11.0±4.3</td>
</tr>
</tbody>
</table>

Data are means±SD or median (IQR). LV = left ventricular. E = early diastolic filling phase. A = diastolic atrial contraction. E/Ea = estimate of the left ventricular filling pressure. * p<0.001, † p<0.01, ‡ p<0.05.

Myocardial substrate and HEP metabolism

All "¹⁸F-DG and all but one H₂¹⁷O scans were appropriate for analysis. Due to technical difficulties [¹³C]palmitate images from 8 patients and 4 controls were not available for analysis. Due to the demanding protocol or insufficient spectral quality (CRSD >20%) [21], [¹³C]-MRS data were available for analysis in 35 patients and 19 controls.

Table 3 lists the fasting biochemical variables obtained prior to the onset of H₂¹⁷O and [¹³C]palmitate PET and during euglycemic hyperinsulinemia, that is during "¹⁸F-DG-PET. Myocardial blood flow was similar between patients and controls (0.88±0.213 versus 0.89±0.215 gram/ml/min, p=0.804). In patients, MFAU (88±31 versus 68±18 mmol/ml/min, p=0.021) and MFAO (85±30 versus 63±19 mmol/ml/min, p=0.007) were higher than in control subjects, whereas the negligible MFAE was lower in patients versus controls (2.3±4.5 versus 5.3±5.4 mmol/ml/min, p=0.028) (Figure 2A). MMRglu was lower in patients than in control subjects (260±128 versus 348±154 mmol/ml/min, p=0.015; Figure 2B). Following correction for BMI differences between groups, only the difference in MFAE was no longer statistically significant. Analysis of T2DM patients and controls in whom the PCR/ATP ratio was available did, however, not reach significance: MFAU (76±24 versus 68±19 mmol/ml/min, p=0.303), MFAO (74±23 versus 63±19 mmol/ml/min, p=0.127) and MMRglu (284±149 versus 363±161 mmol/ml/min, p=0.116). Moreover, the PCR/ATP ratio was comparable in the two groups (2.09±0.33 versus 2.12±0.32, p=0.817, Figure 2C).
Myocardial fatty acid uptake (MFAU), oxidation (MFAO) and esterification (MFAE) (A), the metabolic rate of glucose uptake (MMR\textsubscript{g\textsubscript{lu}}) (B), Phosphocreatine (PCr)/ATP ratio (C) in controls (□) and T2DM patients (■). * p<0.001, † p<0.01, ‡ p<0.05.

**Table 3.** Biochemical and metabolic characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Control subjects N=24</th>
<th>Type 2 Diabetic patients N=78</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasting</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td>5.3 (5.0-5.6)</td>
<td>8.3 (7.1-10.0)*</td>
</tr>
<tr>
<td>Plasma non-esterified fatty acids (mmol/l)</td>
<td>0.46 (0.37-0.52)</td>
<td>0.50 (0.40-0.62)</td>
</tr>
<tr>
<td>Plasma insulin (pmol/l)</td>
<td>28 (19-33)</td>
<td>64 (36-92)*</td>
</tr>
<tr>
<td>Plasma lactate (mmol/l)</td>
<td>0.8 (0.7-0.9)</td>
<td>1.2 (1.0-1.5)*</td>
</tr>
<tr>
<td>NT-proBNP (ng/l)</td>
<td>28 (21-76)</td>
<td>27 (20-42)*</td>
</tr>
<tr>
<td>usCRP (mg/l)</td>
<td>3.0 (1.7-6.4)</td>
<td>3.8 (2.4-6.1)</td>
</tr>
<tr>
<td>Malondialdehyde (μmol/l)</td>
<td>6.1 ± 0.7</td>
<td>9.8 ± 2.3*</td>
</tr>
<tr>
<td><strong>During hyperinsulinemia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma non-esterified fatty acids (mmol/l)</td>
<td>0.04 (0.02-0.05)</td>
<td>0.08 (0.05-0.16)*</td>
</tr>
<tr>
<td>Plasma insulin (pmol/l)</td>
<td>513±64</td>
<td>583±143</td>
</tr>
<tr>
<td>Plasma lactate (mmol/l)</td>
<td>1.1 (0.9-1.3)</td>
<td>1.1 (1.0-1.3)</td>
</tr>
<tr>
<td>M/I value (mg/kg-min)/(pmol/l)</td>
<td>0.99 (0.72-1.65)</td>
<td>0.46 (0.24-0.74)*</td>
</tr>
</tbody>
</table>

Data are mean\textpm SD or median (IQR). usCRP = ultrasensitive C-reactive protein. NT-proBNP = N-terminal probrain natriuretic peptide. M/I value = whole body insulin sensitivity adjusted during the steady state. * p<0.001.
Correlations and determinants of LV diastolic function

Whole-body insulin sensitivity and cardiac substrate or HEP metabolism were not directly associated to LV function. In pooled analysis M/I value was positively correlated with MMRglu (r=0.452, p=0.001), whereas an inverse correlation was found with NEFA (r=0.361, p=0.001), MFAU (r=0.255, p=0.042) and MFAO (r=0.288, p=0.021). NEFA was negatively associated with MMRglu (r=-0.562, p<0.001). Forward multiple regression analysis revealed heart rate, HbA1c and diastolic blood pressure as independent determinants of E/A ratio (R²=0.29, p<0.001) and E-dec_peak (R²=0.27, p<0.001) (Table 4).

Table 4. Univariable and multivariable linear regression analysis of diastolic function measurements E/A ratio and E deceleration Peak

<table>
<thead>
<tr>
<th></th>
<th>Univariable</th>
<th>Multivariable</th>
<th>Univariable</th>
<th>Multivariable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>p Value</td>
<td>β</td>
<td>p Value</td>
</tr>
<tr>
<td>Systolic bloodpressure (mm Hg)</td>
<td>-0.018</td>
<td>0.001</td>
<td>-0.028</td>
<td>0.003</td>
</tr>
<tr>
<td>Diastolic bloodpressure (mm Hg)</td>
<td>-0.015</td>
<td>&lt;0.001</td>
<td>-0.009</td>
<td>0.017</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>-0.012</td>
<td>&lt;0.001</td>
<td>-0.009</td>
<td>0.003</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>-0.091</td>
<td>&lt;0.001</td>
<td>-0.051</td>
<td>0.007</td>
</tr>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td>-0.340</td>
<td>0.007</td>
<td>-0.150</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Plasma lactate (mmol/l)</td>
<td>-0.095</td>
<td>0.057</td>
<td>-0.429</td>
<td>&lt;0.016</td>
</tr>
<tr>
<td>Plasma non-esterified fatty acids (mmol/l)</td>
<td>-0.277</td>
<td>0.059</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diabetic status</td>
<td>-0.211</td>
<td>0.002</td>
<td>-0.971</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Discussion**

This study is novel in several ways. To our knowledge, this is the (i) largest group of well-characterized male T2DM patients with (ii) verified absence of (inducible) cardiac ischemia who had extensive evaluation of (iii) combined cardiac substrate and HEP metabolism and function with CMR and PET technology under standardized conditions. In addition, (iv) this study is the first to show convincingly an increase in myocardial fatty-acid metabolism in T2DM patients, whereas (v) no direct relation was found between cardiac diastolic function and parameters of myocardial substrate or energy metabolism.
Myocardial function
The findings of the present study are in line with previous reports, showing LV diastolic dysfunction in asymptomatic patients with T2DM (15). The strength of the present study is the a priori exclusion of participants with inducible ischemia, as ischemia is a critical determinant of both cardiac function and substrate and energy metabolism (9, 17). Previous studies evaluating LV function and dimensions in human diabetes have mainly used echocardiography, which is hampered by several well-known limitations, in particular in overweight/obese populations (29). We used CMR, which represents the gold standard for reproducible, observer-independent quantitative assessment of LV volume and mass and has shown to be an important tool for the assessment of diastolic and systolic function together with excellent accuracy and reproducibility (29). The observed LV diastolic dysfunction and decreased LV volumes, in the presence of normal LVM, systolic function and cardiac work, unveils early abnormalities in LV function and compliance in the course of DCM. Thus, the functional changes cannot be explained by increased LVM, even after adjustment for BMI, or by raised LV pressure since the estimates of LV filling pressure measured were similar in both groups.

Myocardial substrate metabolism
Myocardial substrate metabolism was previously studied in humans with various degrees of glucometabolic abnormalities and co-morbidities (5-14). These small-sized studies used PET or single photon emission computed tomography (SPECT) to assess glucose and NEFA metabolism applying several tracers with different isotopes and metabolic fates, but also used various approaches to data analysis. Due to these differences in populations and methodology, different, often conflicting results were obtained (5-8, 14). In the present study, T2DM patients had increased NEFA uptake and oxidation, compatible with the data derived from animal models of DCM (1, 30). Increased myocyte NEFA metabolism can be caused by elevated plasma NEFA supply due to unsuppressed lipolysis from insulin resistant adipocytes (31, 32). Interestingly, similar to findings by others in comparable populations (9-11), but in contrast to most animal studies (30), fasting plasma NEFA levels in our T2DM patients were similar to control subjects. In addition, myocardial perfusion was comparable between both study groups and hence these factors do not explain the observed increases in cardiac NEFA metabolism. Increased NEFA metabolism can, however, be explained by alterations at a cellular level. In rats fed a high-fat diet the fatty acid transporter CD36 was relocated to the sarcolemma, and basal phosphorylation of a mediator of CD36, that is protein kinase B (PKB/AKT), was increased (33). In addition, in Zucker Diabetic Fatty (ZDF) rats, increased myocardial NEFA utilization was found and this was shown to be related to increased peroxisome proliferator-activated receptor α (PPARα) and/or its co-activator PGC-1 activation (34, 35). Finally, in ZDF rats increased mRNA levels of the mitochondrial NEFA shuttling enzyme carnitine palmitoyltransferase (CPT-1) was found, which subsequently may lead to enhanced NEFA oxidation (35). Furthermore, NEFA levels under hyperinsulinemia were significantly higher in patients than in control subjects and were inversely associated with whole-body insulin sensitivity and the myocardial metabolic rate of glucose uptake, confirming previous findings (9, 12, 13). Theoretically, increased cardiac NEFA metabolism should be paralleled by a decrease in myocardial glucose uptake, measured as insulin-stimulated \[^{18}F\]DG uptake. However, studies in T2DM subjects without CAD have reported both decreased (9, 11-13) and unaltered (10) cardiac glucose uptake. Again, differences in population characteristics and methodological issues preclude comparison of these studies. We found decreased glucose uptake, which is compatible with NEFA substrate competition, decreased myocardial insulin signaling (32) and/or reduced membrane bound GLUT-4 (35). Altogether, these findings implicate that the nonischemic myocardium in uncomplicated T2DM is resistant to insulin-mediated glucose uptake and that cardiac NEFA metabolism is augmented.
Myocardial substrate and HEP metabolism in relation to function

Studies aimed at linking cardiac substrate and HEP metabolism and function in humans with glucose metabolic disorders are largely lacking. Using 131I-heptadecanoic acid (HDA) SPECT, Turpeinen et al., reported decreased myocardial NEFA uptake and kinetics in subjects with impaired glucose tolerance and T2DM, relative to T1DM patients and controls, which was associated with LVM measured by ultrasound (8). There were no functional differences between the groups and no correlation was reported between functional measurements and HDA metabolism (8). Lozzo et al., reported a weak correlation (r=0.38, p<0.02) between insulin-stimulated myocardial glucose uptake and ejection fraction in a pooled analysis of control subjects, patients with T1DM, CAD patients without T2DM and patients with T2DM without CAD. These authors suggested that the impairment of myocardial glucose uptake as such might compromise myocardial function. In their combined group of subjects of various age groups, the ejection fraction varied substantially, with approximately 50% of participants having an ejection fraction <40% with extreme values as low as 10% (9). In our study, the ejection fraction in T2DM subjects and controls was similar, with small standard deviations in both groups, which may have precluded the detection of an association of LV function and myocardial glucose uptake. The interrelation of altered myocardial substrate handling, HEP metabolism and LV function is complex and depends on many factors, including additional co-morbidities, such as hypertension and cardiac autonomic neuropathy, and the presence or absence of stress or ischemia. Previously, we and others reported decreased myocardial PCr/ATP ratio in T2DM patients relative to control subjects (15, 16). In the present study we could not confirm these previous findings. Several differences in patient characteristics between the present and the two other studies, such as age range (36), BMI differences (37), inclusion of only males or both genders (38), glycemic control and fasting NEFA levels may have led to this discrepant findings. Since altered myocardial substrate metabolism may affect cardiac PCr/ATP (39), we argue that the insufficient contrast between the present subgroups regarding cardiac substrate metabolism may have potentially contributed to the finding of comparable myocardial PCr/ATP values in both groups.

In our previous study (15) a relation was found between myocardial LV diastolic function and cardiac PCr/ATP. This relation was not found by Scheuermann-Freestone et al (16) who found a similar LV diastolic function in T2DM patients and control subjects. Likewise, we did not find a relation between LV diastolic function and cardiac PCr/ATP in the present study. Again, the lack of contrasts between T2DM patients and controls, with respect to LV diastolic function (16) or cardiac PCr/ATP (this study), may have precluded the finding of the previously observed association. Interestingly, a recent study in ZDF rats showed that not myocardial energy metabolism per se, but rather cardiac remodeling affected the observed cardiac dysfunction (40).

Although we did not find a direct association between altered myocardial substrate metabolism and LV functional changes in T2DM, an indirect effect of cardiac metabolic derangements may influence LV function. Accordingly, recent data indicate that diabetes-related metabolic changes might indirectly impair LV diastolic function by inducing tissue oxidative stress, thereby promoting apoptosis and oxidative modification of contractile proteins, and/or by stimulating cardiac macrophage infiltration leading to local inflammation and fibrosis (41).
Chapter 3

Study limitations
Due to the intra-individual diurnal fluctuations of substrate plasma levels in T2DM, real-life cardiac substrate metabolism may be difficult to establish. The euglycemic hyperinsulinemic clamp guarantees an isometabolic state in experimental groups, allowing comparisons of cardiac glucose metabolism between groups. Inasmuch as hyperinsulinemia will lead to direct uptake of plasma NEFA into non-adipose tissues other than the myocardium, NEFA metabolism was assessed in the fasting state and therefore does not allow establishing a direct association of cardiac NEFA and glucose metabolism. Furthermore, the present study does not address myocardial efficiency as the used tracers do not measure myocardial oxygen consumption. Further studies are needed to settle this issue in T2DM patients. Finally, the assessment of only men limits generalization of the findings.

Conclusion
In the absence of myocardial ischemia, patients with uncomplicated T2DM showed impaired LV diastolic function and decreased compliance, in addition to whole-body and myocardial insulin resistance, collectively leading to altered myocardial substrate metabolism, favoring NEFA over glucose as a substrate. We did not find a direct relation between altered LV diastolic function and myocardial substrate or HEP metabolism in this early stage of non-ischemic DCM. Large-scaled longitudinal studies using similar combined measurements should establish the impact of early metabolic and functional changes on the development of clinically relevant cardiac disease in T2DM over time, including congestive heart failure and cardiac ischemia, and further define the efficacy of targeted interventions to halt the progression of compensated DCM into overt cardiac disease.
Altered Myocardial Substrate Metabolism and Decreased Diastolic Function

Reference List

Pioglitazone improves cardiac function and alters myocardial substrate metabolism without affecting cardiac triglyceride accumulation and high-energy phosphate metabolism in patients with well-controlled type 2 diabetes mellitus
Abstract

BACKGROUND: Cardiac disease is the leading cause of mortality in type 2 diabetes (T2DM). Pioglitazone has been associated with improved cardiac outcome but also with an elevated risk of heart failure. We determined the effects of pioglitazone on myocardial function in relation to cardiac high-energy phosphate, glucose, and fatty-acid metabolism and triglyceride content in T2DM patients.

METHODS AND RESULTS: Seventy-eight T2DM males without structural heart disease or inducible ischemia as assessed by dobutamine-stress echocardiography were assigned to pioglitazone (30mg/day) or metformin (2000mg/day) and matching placebo for 24 weeks. The primary endpoint was change in cardiac diastolic function from baseline relative to myocardial metabolic changes, measured by magnetic resonance (MR) imaging, proton and phosphorus MR-spectroscopy and [18F]-2-fluoro-2-deoxy-D-glucose and [13C]palmitate positron-emission tomography. No patient developed heart failure. Both therapies similarly improved glycemic control, whole-body insulin sensitivity and blood pressure. Pioglitazone versus metformin improved the early peak flow rate (P=0.047) and left ventricular compliance. Pioglitazone versus metformin increased myocardial glucose uptake (P<0.001), but pioglitazone-related diastolic improvement was not associated with changes in myocardial substrate metabolism. Metformin did not affect myocardial function but decreased cardiac work relative to pioglitazone (P<0.006), a change that was paralleled by a reduced myocardial glucose uptake and fatty-acid oxidation. Neither treatment affected cardiac high-energy phosphate metabolism or triglyceride content. Only pioglitazone reduced hepatic triglyceride content (P<0.001).

CONCLUSIONS: In T2DM patients, pioglitazone is associated with improvement in some measures of left ventricular diastolic function, myocardial glucose uptake, and whole-body insulin sensitivity. The functional changes, however, were not associated with myocardial substrate- and high-energy phosphate metabolism.
Chapter 4

Introduction

Cardiac disease is the leading cause of mortality in type 2 diabetes mellitus (T2DM). In asymptomatic patients, cardiac abnormalities exist, even in the absence of coronary artery disease (CAD) or hypertension, due to diabetic cardiomyopathy. Increased left ventricular (LV) diastolic stiffness is a common and early finding. Although diabetic cardiomyopathy is a multi-causal condition, evidence obtained from animal studies indicates that diabetes-related metabolic abnormalities are the major contributors to the observed cardiac defects. Thus, increased non-esterified fatty acid (NEFA) fluxes that result in myocardial triglyceride accumulation, the formation of toxic intermediates, mitochondrial dysfunction and oxidative stress have been implicated. Although NEFAs are the preferred cardiac substrate under physiological conditions, the heart should be able to readily switch to glucose oxidation during stress or ischemia.

Because to prolonged exposure to an abnormal metabolic environment, the diabetic heart may lose its flexibility to switch between NEFA and glucose as substrates as required by the circumstances. Consequently, the initially adaptive mechanism will transform into a maladaptive vicious circle that leads to altered high-energy phosphate metabolism and contractile dysfunction. Mechanistic in vivo studies in humans are limited, but similar mechanisms have been proposed to underlie human diabetic cardiomyopathy.

By targeting lipotoxicity and insulin resistance, the blood-glucose lowering agent pioglitazone may favorably influence cardiac risk in T2DM. In the PROactive Study (PROspective pioglitAzone Clinical Trial in macroVascular Events), pioglitazone reduced cardiovascular disease in high-risk patients with T2DM. However, although pioglitazone improved cardiac function in experimental diabetic cardiomyopathy, its use in patients may result in heart failure due to fluid retention. Inasmuch as the majority of patients in the PROactive study had CAD and longstanding diabetes, it is feasible that cardiac inability to accommodate metabolic changes may have contributed to the pioglitazone-related heart failures. Indeed, substrate manipulation in heart failure due to CAD decreased myocardial efficiency and cardiac function, which reveals the close connection of metabolism and function in the compromised heart. At present, however, it is unknown whether interventions aimed at altering cardiac metabolism will lead to changes in function in the non-ischemic diabetes heart. We studied the effect of pioglitazone, versus metformin, on myocardial function, dimensions and perfusion, in association with cardiac glucose and fatty acid metabolism, as well as triglyceride content and high-energy phosphate metabolism, using magnetic resonance (MR) imaging and spectroscopy and positron emission tomography (PET). To avoid confounding by ischemia, we performed the studies in patients with well-controlled T2DM of short duration and with verified absence of cardiac ischemia.
Pioglitazone improves cardiac function and alters myocardial substrate metabolism

Methods

Study design and patients
The PIRAMID study (Pioglitazone Influence on TriGlyceride Accumulation in the Myocardium In Diabetes) was a 24-week prospective randomized double-blind double-dummy with active comparator, 2-center parallel-group intervention. Males with uncomplicated T2DM, aged 45-65 years, were eligible. Inclusion criteria were a glycated hemoglobin level of 6.5-8.5 % at screening, body-mass index (BMI; weight/[length]^2) of 25-32 kg/m^2, blood pressure not exceeding 150/85 mm Hg (with or without the use of anti-hypertensive drugs). Exclusion criteria were any clinically significant disorder, particularly any history or complaints of cardiovascular and liver disease or diabetes-related complications, prior use of thiazolidinediones or insulin. Written informed consent was obtained from all participants. The protocol was approved by the Medical Ethics Committee of both centers, and performed in full compliance with the Declaration of Helsinki.

Study procedures
Participants underwent a two-step screening procedure consisting of a medical history, physical examination, electrocardiogram (ECG), Ewing tests to exclude autonomic neuropathy and fasting blood- and urine-analysis (screening-visit 1) and dobutamine-stress echocardiography to exclude cardiac ischemia or arrhythmias (screening-visit 2). Following successful screening, participants entered a 10-week run-in period during which they were washed-out from their previous blood-glucose lowering agents (metformin monotherapy 39.8%, sulfonlurea monotherapy 25.6% and metformin and sulfonlurea combination therapy 34.6%), transferred to glimepiride monotherapy, and titrated until a stable dose was reached during 8 weeks prior to randomization. Mean glycated hemoglobin levels at screening and at the end of the run-in period were comparable (data not shown). All patients underwent MRI, the first 60 patients underwent both MRI and PET examinations (see below). Due to the demanding protocol phosphorus magnetic resonance ([^31]P)-MR spectroscopy was offered as an optional test.

Patients were randomized to pioglitazone (15 mg once daily, titrated to 30 mg once daily after 2 weeks) or metformin (500 mg twice daily, titrated to 1000 mg twice daily) and matching placebo, to be taken in addition to glimepiride throughout the study. A randomization code-list, with a block size of 4, was generated by the trial pharmacist (Amsterdam). Treatments were allocated chronologically and stratified for study center. All study investigators and study personnel were unaware of treatment assignment for the duration of the study. If recurrent hypoglycemia occurred, the glimepiride dose was lowered stepwise to levels of non-occurrence. Back-titration to pioglitazone 15 mg once daily or metformin 500 mg twice daily was made if persistent, study-drug related side-effects occurred. Patients were assessed in the fasting state at 2-8-week intervals for 24 weeks and underwent outcome measurements at baseline and at study termination as outlined below. They were requested to adhere to pre-study lifestyle and dietary habits throughout the study.

Cardiac magnetic resonance imaging protocol
MR assessments were performed after an overnight fast at one single site (Leiden), using a 1.5-T whole-body MR scanner (Gyroscan ACS/NT15; Philips, Best, the Netherlands). During MR examinations, blood samples were collected to determine substrates and blood pressure and heart rate were monitored. Rate pressure product (RPP) was calculated as the product of systolic blood pressure and heart rate. The entire heart was imaged in the short-axis orientation using ECG-gated breath-hold balanced steady state free procession imaging.[1] Measures of systolic function were
LV ejection fraction (EF) and cardiac index (CI = Cardiac output/body surface area), LV end-diastolic volume (LVEDV), LV end-systolic volume (LVESV) and stroke volume (SV) were cardiac dimensions. An ECG-gated gradient-echo sequence with velocity encoding was performed to measure blood-flow across the mitral valve for the determination of LV diastolic function parameters, including peak filling rates of the early filling phase (E) and the atrial contraction (A) and their ratio (E/A) were calculated. Also, the peak (E-dec_{170}) and mean deceleration gradient of E (E-dec_{mean}) were calculated. LV filling pressures (E/Ea) were estimated. Images were analyzed quantitatively using dedicated software (MASS® and FLOW®, Medis, Leiden, the Netherlands).

Cardiac and hepatic proton MR spectroscopy
Cardiac and hepatic proton MR spectroscopy ([1H]-MRS) were performed as described previously. Briefly, Myocardial [1H]-MRS spectra were obtained from the interventricular septum carefully avoiding contamination from epicardial fat. Spectroscopic data acquisition was double triggered using electrocardiographical triggering and respiratory navigator echoes to minimize motion artifacts. Water-suppressed spectra were acquired to measure myocardial triglyceride content and spectra without water suppression were acquired and used as an internal standard. [1H]-MRS data were fitted using Java-based MR user interface software (MRUI version 2.2, Leuven, Belgium), as described previously. Myocardial triglyceride content relative to water was calculated as (signal amplitude of triglyceride)/(signal amplitude of water) · 100. [31P]-MR spectra of the liver were performed with an 8 mL voxel positioned in the liver, avoiding gross vascular structures and adipose tissue depots. The twelfth thoracic vertebra was used as a landmark to ensure the same voxel position during both visits. Sixty-four averages were collected with water suppression.

Phosphorus magnetic resonance spectroscopy
A 100-mm-diameter surface coil was used to acquire ECG-triggered [31P]-MR spectra of the left ventricular (LV) anterior wall with subjects in the supine position. Volumes of interest were selected by image-guided spectroscopy with 3D-ISIs. Shimming was performed automatically and tuning and matching of the [31P] surface coil was performed manually. Technical details of data acquisition and spectral quantification were similar as described before. Spectroscopic volume size was typically 7 × 7 × 7 cm. Acquisitions were based on 192 averaged free induction decays, and total acquisition time was 10 min. [31P]-MR spectra were quantified automatically in the time domain using prior spectroscopic knowledge and were corrected for partial saturation effects and for the adenosine-triphosphate (ATP) contribution from blood in the cardiac chambers. The phosphocreatine (PCr)/ATP ratios of the spectra were calculated and used as a parameter representing myocardial high energy phosphate metabolism.

Positron emission tomography imaging protocol
PET examinations were performed after an overnight fast at a single center (Amsterdam) using an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, TN, USA). Patients received two venous catheters, one in the antecubital vein and one in the vein of the opposite hand, the latter being wrapped into a heated blanket to obtain arterialized blood. During procedures patients were monitored by telemetry and blood pressure was measured at 5-minute intervals. PET was used to measure myocardial blood flow (MBF) with H, myocardial fatty acid uptake (MFAU), β-oxidation (MFAO) and esterification (MFAE) with [13C]palmitate, and myocardial metabolic rate of glucose uptake (MMRglu) with [18F]-2-fluoro-2-deoxy-D-glucose ([18F]FDG). Perfusion and NEFA metabolism were assessed in the fasting state, whereas MMRglu was measured during a euglycemic-hyperinsulinemic
Pioglitazone improves cardiac function and alters myocardial substrate metabolism

clamp (see below). Following a 10-min transmission scan, H$_2^{15}$O was injected (t=10 min) and a 10-
min dynamic emission scan (40 frames) was acquired. Subsequently, a 30-min dynamic emission
scan (34 frames) was performed following $[^1]$C-palmitate injection (t=35 min). Hereafter, the clamp
was started (t=65 min), as described previously,$^{22}$ to approximate an isometabolic steady state and
measure whole-body insulin sensitivity. At steady state (around t=155 min), following a new trans-
mission scan, $^{18}$FDG was injected and a 60-min dynamic emission scan (40 frames) was acquired.
Blood samples were collected during $[^1]$C-palmitate and $^{18}$FDG scans at predefined time points to
measure glucose, NEFA, lactate, lipids and insulin levels. In addition, $^{13}$CO$_3$ was measured during the

PET image analysis
PET data were quantitatively reconstructed using filtered backprojection applying all appropriate
corrections. In order to generate myocardial time-activity curves, region of interest (ROIs) were
defined on resliced LV short-axis (summed) $[^1]$C-palmitate and $^{18}$FDG images and subsequently
projected onto the dynamic images. ROIs were drawn as previously described $^{22}$ and grouped for
further analysis. Myocardial segments exposed to liver spill-in were omitted from the analysis of
$[^1]$C-palmitate data. Additional ROIs were defined in left and right ventricular chambers for $[^1]$C
palmitate and H$_2^{15}$O image-derived input functions (IDIF). A separate aorta ascendance ROI was
defined for $^{18}$FDG IDIF.

MBF was determined using the standard single-tissue compartment model.24 $[^1]$C-palmitate time-
activity curves were analyzed using a three-tissue plasma input kinetic model, which, together with
plasma NEFA concentrations, enabled calculation of MFAU, MFAO and MFAE.25 The $[^1]$C-palmitate
IDIF was corrected for $^{13}$CO$_2$ metabolites and difference between plasma and whole blood concen-
trations as described elsewhere.6 This model is similar to that described by Bergmann et al,26 but
with a reduced number of parameters, thereby increasing precision of derived estimates (details online).
MmRglu was calculated by multiplying the net influx constant for $^{18}$FDG, $K_j$, by the mean
plasma glucose concentration. For determination of $K_j$, Patlak graphical analysis was used.27

Study end points
The primary endpoint was change from baseline to follow-up (24 weeks) in diastolic function as
operationalized by the four parameters, i.e. the E-dec$_{max}$ and E-dec$_{max}$, EPFR and E/A ratio. Secondary
efficacy measures included difference in cardiac dimensions, systolic function parameters and
myocardial metabolism and perfusion variables as described above, as well as differences in he-
patic and myocardial triglyceride content, body-mass index, blood pressure, glycated hemoglobin
(reference values 4.3-6.1%), plasma lipids and whole-body insulin sensitivity. Exploratory analyses
included changes in the relation of left ventricular (LV) end-diastolic volume and estimates of LV
filling pressure, including N-terminal probrain natriuretic peptide (NT-proBNP), the ratio of early
diastolic velocity (E) and early diastolic tissue velocity (Ea) and high-energy phosphate metabolism
(PCR/ATP ratio). Blood samples for end-point measurements were analyzed at one central labora-
tory (Amsterdam).

Statistical analysis
Since at the time of study-design, no data were available regarding the effect of thiazolidinediones
on MR-measured cardiac function, we based our sample-size calculations on previous MR studies.2
To detect a subtle 15% (SD 20%) difference in the diastolic functional parameter EPFR with 90% power,
approximately 80 randomized patients were needed (primary end-point). The sample-size
for the PET measurements was based on available PET studies. We calculated that 60 randomized patients would be necessary to detect a difference of 20% (SD 25%); estimated drop-out rate 20% in cardiac metabolism, with 80% power. Values are means ± standard error (SE) or median (interquartile range, IQR), when non-normally distributed. Between-group comparisons were done with analysis of covariance (ANCOVA) with adjustments for treatment group and baseline values. Within-group changes from baseline were assessed using independent-paired t-tests or Wilcoxon signed-ranks tests. Correlations were calculated by Pearson’s or Spearman’s correlation analyses, as appropriate. All statistical tests were two-sided, significance was considered at the level of 0.05. Analyses were done with SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA).

This study was initiated, designed, performed, analyzed and submitted for publication by the investigators at both centers, without any interference of the funding source. The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written. This trial is registered with Current Controlled Trials, number ISRCTN53177482.

Results

Figure 1 shows the trial flowchart. At baseline, the study groups were well-matched (Tables 1 and 2). Glimepiride dose-adjustment was needed in 4 patients randomized to pioglitazone and in 3 assigned to metformin. Two patients required metformin back-titration. No clinically evident fluid retention or heart failure occurred during the study.

At 24 weeks, pioglitazone and metformin similarly improved glycemic control from baseline (Table 2). Pioglitazone versus metformin significantly increased HDL, whereas metformin decreased total and LDL cholesterol levels (Table 2). Pioglitazone, but not metformin, induced weight gain relative to baseline (from 91±2 to 94±2 kg vs 92±2 to 92±3 kg; between-group P<0.001). Both pioglitazone and metformin significantly improved whole-body insulin sensitivity by a median 35.1% and 29.6%, respectively, which was paralleled by reduced NEFA levels during hyperinsulinemia that were more suppressed by pioglitazone (Table 2); however, neither treatment affected fasting NEFA levels. Metformin increased and pioglitazone decreased fasting lactate levels (Table 2). In both groups, similar decreases in systolic blood pressure and rate-pressure product were observed, whereas diastolic blood pressure and heart rate remained unchanged (Table 3).

At follow-up, pioglitazone increased indices of diastolic function, including E-dec, E-dec, and the early peak filling rate (Table 3). Pioglitazone-treated patients showed an increase in LVEDV, whereas NT-proBNP levels and E/Ea, remained unchanged (Tables 2 and 3). In contrast to metformin, pioglitazone shifted the relations of LVEDV and estimates of LV filling pressure towards improved compliance (Fig. 2 A/B). Metformin had no significant effect on the diastolic cardiac parameters measured. Comparisons between groups of diastolic function parameters revealed a significant difference in early peak filling rate, while only a trend was observed for E-dec (Table 3). A significant between group difference in SV, CI and cardiac work was observed, whereas EF remained unaltered in both groups (Table 3). PET examinations were successful in 54 (90%) subjects. At follow up, pioglitazone significantly increased and metformin markedly decreased MMRglu from baseline (between-group P<0.001, Fig. 2 C and 2D). At 24 weeks, pioglitazone and metformin therapy did not significantly change MFAU from baseline, whereas only metformin significantly reduced MFAO (Fig. 2D). MFAE was negligible in both groups; however, increases from baseline were observed after pioglitazone and, to a lesser extent after metformin therapy (Figs. 2C and 2D). These minor changes measured by PET were
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not detected by the $[1\text{H}]$-MRS measurements because myocardial triglyceride content remained unchanged in both groups (pioglitazone: 0.77±0.05 % versus 0.82±0.07 %; metformin: 0.87±0.08 % versus 0.89±0.07 %, between-group $P=0.774$). In contrast, pioglitazone but not metformin decreased hepatic triglyceride content (pioglitazone: 5.9 (2.6-17.4) % vs 4.1 (1.9-12.3) %; metformin: 7.7 (3.7-23.9) % vs 10.7 (5.1-22.0) %, between-group $P<0.001$). PCR/ATP was successfully obtained on both study occasions from 22 patients ($n=13$ in the pioglitazone group), which was similar at baseline in both groups and not influenced by either treatment (pioglitazone: 2.02±0.06 vs 1.99±0.11; metformin: 2.19±0.10 vs 2.04±0.07, between-group $P=0.976$). PCR/ATP of the remaining participants could not be collected at baseline or at follow-up or appeared of insufficient spectral quality (Cramér-Rao standard deviation (rCRSD) >20%). Metformin but not pioglitazone slightly increased MBF from baseline (metformin: 0.86±0.05 gram/ml/min vs 0.93±0.04 gram/ml/min; pioglitazone: 0.94±0.04 gram/ml/min vs 0.92±0.03 gram/ml/min, between-group $P=0.254$).

No associations were observed between pioglitazone-related changes in diastolic function and alterations in myocardial NEFA metabolism, MMRglu or PCR/ATP (data not shown).

Figure 1. Trial profile.

Pioglitazone improves cardiac function and alters myocardial substrate metabolism

4
### Table 1. Patients characteristics at baseline*

<table>
<thead>
<tr>
<th></th>
<th>Pioglitazone (n=39)</th>
<th>Metformin (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td>56.8±1.0</td>
<td>56.4±0.9</td>
</tr>
<tr>
<td><strong>Time since diagnosis of diabetes, y</strong></td>
<td>4 (3-6)</td>
<td>3 (1-5)</td>
</tr>
<tr>
<td><strong>Current smoker (%)</strong></td>
<td>10 (26%)</td>
<td>7 (18%)</td>
</tr>
<tr>
<td><strong>Body-mass index</strong></td>
<td>28.2±0.5</td>
<td>29.3±0.6</td>
</tr>
<tr>
<td><strong>Waist circumference, cm</strong></td>
<td>103.8±1.5</td>
<td>104.9±1.8</td>
</tr>
</tbody>
</table>

**Concomitant medication**

<table>
<thead>
<tr>
<th></th>
<th>Pioglitazone (n=39)</th>
<th>Metformin (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Statin (%)</strong></td>
<td>19 (48.7%)</td>
<td>19 (48.7%)</td>
</tr>
<tr>
<td><strong>Any antihypertensive medication (%)</strong></td>
<td>19 (48.7%)</td>
<td>15 (38.5%)</td>
</tr>
<tr>
<td><strong>Beta-blocker (%)</strong></td>
<td>5 (12.8%)</td>
<td>2 (5.1%)</td>
</tr>
<tr>
<td><strong>Diuretic (%)</strong></td>
<td>6 (15.4%)</td>
<td>6 (15.4%)</td>
</tr>
<tr>
<td><strong>ACE inhibitor (%)</strong></td>
<td>9 (23.1%)</td>
<td>9 (23.1%)</td>
</tr>
<tr>
<td><strong>ARB (%)</strong></td>
<td>6 (15.4%)</td>
<td>3 (7.7%)</td>
</tr>
<tr>
<td><strong>Calcium antagonist (%)</strong></td>
<td>1 (2.6%)</td>
<td>3 (7.7%)</td>
</tr>
</tbody>
</table>

* There were no statistically significant differences between treatment groups. Values are presented as means ±SE, median (IQR) or number and % of total. ACE denotes angiotensin-converting enzyme, ARB angiotensin-receptor blocker.

### Figure 2. Relations of LV end-diastolic volume (LVEDV) and estimates of LV filling pressure and myocardial metabolism

Relations of LV end-diastolic volume (LVEDV) and estimates of LV filling pressure, including E/Ea (A) and NT-proBNP (B) before (black) and after (white) 24 weeks of treatment with pioglitazone (circles) or metformin (squares). Myocardial fatty acid uptake (MFAU), oxidation (MFAO) and esterification (MFAE) and the metabolic rate of glucose uptake (MMRglu) in patients with type 2 diabetes before (black) and after (white) 24 weeks treatment with pioglitazone (C) or metformin (D). P values for between groups differences: MFAU (P=0.056), MFAO (P=0.091), MFAE (P=0.467) and MMRglu (P=0.001). Myocardial fatty acid metabolism was assessed during fasting and myocardial glucose metabolism during hyperinsulinemia.
Table 2: Biochemical and metabolic characteristics and whole-body insulin sensitivity at baseline and 24 weeks

<table>
<thead>
<tr>
<th></th>
<th>Pioglitazone Baseline</th>
<th>24 weeks</th>
<th>P value</th>
<th>Metformin Baseline</th>
<th>24 weeks</th>
<th>P value</th>
<th>P value (between groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>7.1±0.2</td>
<td>6.5±0.1</td>
<td>&lt; 0.001</td>
<td>7.0±0.1</td>
<td>6.3±0.1</td>
<td>&lt; 0.001</td>
<td>0.146</td>
</tr>
<tr>
<td>Plasma glucose, mmol/L</td>
<td>8.4 (7.2-10.3)</td>
<td>7.7 (6.7-9.4)</td>
<td>0.002</td>
<td>8.2 (6.8-9.1)</td>
<td>6.8 (5.8-7.4)</td>
<td>0.001</td>
<td>0.141</td>
</tr>
<tr>
<td>Non esterified fatty acids, mmol/L</td>
<td>0.45 [0.4-0.59]</td>
<td>0.46 [0.34-0.57]</td>
<td>0.369</td>
<td>0.53 [0.39-0.77]</td>
<td>0.49 [0.39-0.56]</td>
<td>0.136</td>
<td>0.933</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>5.8 [3.8-8.3]</td>
<td>7.0 (4.4-7.0)</td>
<td>0.106</td>
<td>8.0 [3.1-9.9]</td>
<td>5.9 [3.2-9.8]</td>
<td>0.377</td>
<td>0.151</td>
</tr>
<tr>
<td>Lactate, mmol/L</td>
<td>1.2 [1.0-1.5]</td>
<td>1.0 [0.8-1.2]</td>
<td>0.001</td>
<td>1.1 [1.0-1.5]</td>
<td>1.5 [1.2-1.8]</td>
<td>0.012</td>
<td>0.001</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.5±0.1</td>
<td>4.6±0.2</td>
<td>0.374</td>
<td>4.9±0.2</td>
<td>4.5±0.2</td>
<td>0.001</td>
<td>0.042</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.5±0.1</td>
<td>2.5±0.1</td>
<td>0.380</td>
<td>2.9±0.1</td>
<td>2.6±0.2</td>
<td>0.001</td>
<td>0.107</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.07 [0.94-1.28]</td>
<td>1.23 [0.99-1.46]</td>
<td>0.003</td>
<td>1.13 [0.90-1.42]</td>
<td>1.02 [0.86-1.26]</td>
<td>0.133</td>
<td>0.009</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.4 [1.0-2.2]</td>
<td>1.4 [0.9-2.3]</td>
<td>0.926</td>
<td>1.5 [0.9-2.1]</td>
<td>1.7 [0.9-2.3]</td>
<td>0.519</td>
<td>0.596</td>
</tr>
<tr>
<td>NT-proBNP, ng/L</td>
<td>24 [20-38]</td>
<td>26 [19-40]</td>
<td>0.731</td>
<td>32 [18-43]</td>
<td>33 [20-43]</td>
<td>0.134</td>
<td>0.505</td>
</tr>
</tbody>
</table>

**During hyperinsulinaemia**

|                      |                       |          |         |                    |          |         |                          |
| Non esterified fatty acids, mmol/L | 0.07 [0.05-0.13] | 0.04 [0.02-0.05] | < 0.001 | 0.09 [0.04-0.16] | 0.06 [0.03-0.14] | 0.006 | 0.036                    |
| Insulin, pmol/L      | 572 [503-620]         | 521 [447-590] | 0.014 | 614 [540-710]   | 520 [472-601] | < 0.001 | 0.292                    |
| Lactate, mmol/L      | 1.1 [1.0-1.3]         | 1.1 [1.0-1.2] | 0.070 | 1.0 [0.9-1.3]   | 1.4 [1.2-1.7] | < 0.001 | 0.001                    |
| M/I value, (mg/kg min)/pmol/L | 0.46 [0.28-0.75] | 0.54 [0.43-0.97] | 0.001 | 0.45 [0.19-0.80] | 0.58 [0.35-1.00] | 0.033 | 0.501                    |

Data are mean (SE) or median (IQR). M/I value = whole body insulin sensitivity adjusted during the steady state. NT-proBNP = N-terminal probrain natriuretic peptide.
Table 3. Hemodynamic parameters and cardiac dimensions and function at baseline and 24 weeks

<table>
<thead>
<tr>
<th></th>
<th>Pioglitazone</th>
<th>Metformin</th>
<th>P value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>24 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemodynamics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>130±2</td>
<td>125±2</td>
<td>0.036</td>
<td>126±2</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>77±1</td>
<td>74±1</td>
<td>0.064</td>
<td>74±1</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>65±1</td>
<td>63±1</td>
<td>0.235</td>
<td>65±1</td>
</tr>
<tr>
<td>Rate pressure product, (beats/min) · mm Hg</td>
<td>850±1</td>
<td>785±195</td>
<td>0.040</td>
<td>820±215</td>
</tr>
<tr>
<td>Cardiac function and dimensions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV mass, g</td>
<td>108±2</td>
<td>105±3</td>
<td>0.171</td>
<td>107±3</td>
</tr>
<tr>
<td>LV end-systolic volume, mL</td>
<td>66±3</td>
<td>66±3</td>
<td>0.821</td>
<td>60±2</td>
</tr>
<tr>
<td>LV end-diastolic volume, mL</td>
<td>160±4</td>
<td>166±5</td>
<td>0.045</td>
<td>152±4</td>
</tr>
<tr>
<td>Stroke volume, mL</td>
<td>94±3</td>
<td>99±3</td>
<td>0.016</td>
<td>92±3</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>59±1</td>
<td>60±1</td>
<td>0.228</td>
<td>61±1</td>
</tr>
<tr>
<td>Cardiac index, (L/min) · m²</td>
<td>2.9±0.1</td>
<td>2.9±0.1</td>
<td>0.845</td>
<td>2.9±0.1</td>
</tr>
<tr>
<td>Cardiac work, mm Hg/L/min</td>
<td>57±2</td>
<td>57±2</td>
<td>0.898</td>
<td>55±2</td>
</tr>
<tr>
<td>E peak filling rate, mL/s</td>
<td>42±15</td>
<td>44±14</td>
<td>0.067</td>
<td>40±14</td>
</tr>
<tr>
<td>E deceleration peak, mL/s² · 10⁻²</td>
<td>3.5±0.2</td>
<td>3.5±0.2</td>
<td>0.034</td>
<td>3.5±0.2</td>
</tr>
<tr>
<td>E deceleration mean, mL/s² · 10⁻²</td>
<td>2.3±0.1</td>
<td>2.3±0.1</td>
<td>0.080</td>
<td>2.3±0.1</td>
</tr>
<tr>
<td>E/A peak flow</td>
<td>1.07±0.05</td>
<td>1.09±0.05</td>
<td>0.583</td>
<td>1.01±0.04</td>
</tr>
<tr>
<td>E/Ea</td>
<td>9.2±7.4</td>
<td>9.1±6.6</td>
<td>0.695</td>
<td>9.3±3.8</td>
</tr>
</tbody>
</table>

Data are means ± SE. LV = left ventricular. E = early diastolic filling phase. A = diastolic atrial contraction. E/Ea = estimate of the left ventricular filling pressure.
Discussion

Pioglitazone but not metformin improved LV diastolic function and compliance in men with well-controlled, uncomplicated T2DM and verified absence of cardiac ischemia. Although both treatments improved whole-body insulin sensitivity, pioglitazone and metformin induced differential alterations in myocardial substrate utilization. These changes in substrate utilization did not affect high-energy phosphate metabolism or myocardial triglyceride content, but only pioglitazone significantly lowered hepatic triglyceride content. The effects of pioglitazone on diastolic function were not related to myocardial metabolism.

Thiazolidinedione-related improvement in cardiac diastolic function were reported by some, but not by others. These contrasting findings may be due to differences in study populations, severity and duration of diabetes, co-morbid conditions including pre-existent cardiac dysfunction and CAD, medication use and the use of echocardiography versus MRI in small populations. The relatively normal cardiac function at baseline may explain the seemingly modest pioglitazone induced diastolic functional improvements in our patients. Given the absence of treatment-related effects on the MR-estimate of LV filling pressure (E/Ea) in both treatment-groups, we hypothesize that pioglitazone-improved LV compliance accounts for the observed favorable changes in the transmural filling pattern. The most important finding in LV functional change, however, is the pioglitazone-related increase in LVEDV at similar estimates of LV filling pressure, which is compatible with an improved LV compliance.

These data are in line with earlier findings in diabetic rats, showing that pioglitazone improved diastolic function by reducing myocardial collagen content and by favorably affecting matrix remodeling. A more recently described mechanism possibly underly the observed LV compliance improvement, may be the pioglitazone-induced inhibition of macrophage chemotaxis, cardiac macrophage expression of pro-inflammatory genes and secretion of the inflammatory glycoprophosphoprotein osteopontin, which is associated with myocardial fibrosis and stiffness.

Only pioglitazone increased SV, as reported by others, possibly due to a decrease in peripheral resistance. Metformin tended to decrease CI, compatible with the reported metformin-related effects on cardiac sympathovagal balance. Both treatments induced similar decreases in systolic blood pressure and RPP, whereas diastolic blood pressure and heart rate remained unchanged. Although previous studies showed comparable decreases in SBP following thiazolidinedione therapy, the observation that SBP was also reduced by metformin suggests that part of the changes in SBP may be attributed to an initial stress response rather than to the effect of therapy.

The present study is timely in the light of the ongoing debate regarding the safety profile of thiazolidinediones. During this short-term trial we observed no cardiac events nor heart failure. In the PROactive population, the majority of whom had a history of CAD, pioglitazone use was associated with an increased risk of heart failure. Our data indicate that when pioglitazone is used in patients with uncomplicated T2DM without cardiac ischemia, it may reverse the process of cardiac concentric remodeling, which is among the hallmarks of diabetic cardiomyopathy, by shifting the LV end-diastolic pressure-volume relation towards improved compliance. However, it is conceivable that in patients with compromised hearts, in particular those with (ischemic) dilated cardiomyopathy, pioglitazone may actually promote the risk of overt heart failure.

Cardiac glucose uptake was assessed under hyperinsulinemic euglycemic conditions to standardize metabolic conditions and to improve the signal-to-noise ratio. As NEFA substrate metabolism was measured in the fasting state, direct reciprocal associations of cardiac glucose and NEFA metabolism are limited. Both treatments induced significant, albeit differential changes in cardiac substrate
metabolism. Pioglitazone increased MMRglu which may be due to the simultaneous reduction of competing substrates, in particular NEFA, but also by direct enhancement of myocardial insulin signaling and expansion of the available pool and translocation of GLUT-4 receptors in the heart. Metformin significantly lowered MMRglu and MFAO. These changes were paralleled by an increase in plasma lactate, whereas NEFA levels decreased during hyperinsulinemia and remained unchanged during fasting. Others showed a trend towards MMRglu decline but no changes in lactate levels in 9 patients after 26-week metformin therapy. The normal human heart may be regarded as a metabolically flexible omnivore that utilizes the most energy-efficient substrate available. Although, NEFA are the preferential substrate due to the highest ATP yield, during stress, increased workload and ischemia, the heart can switch to energetically more favorable substrates, including glucose and lactate. As myocardial lactate uptake has been shown to be directly proportional to circulating lactate levels, it might be speculated that metformin could have increased myocardial lactate utilization as was previously shown for skeletal muscle. However, the observed decreases in cardiac glucose and NEFA metabolism in the metformin-group might also be linked to the treatment-related reduction in cardiac work, as less ATP needs to be generated to maintain adequate high-energy phosphate levels.

Unexpectedly and contrary to findings from animal studies, we found no association between treatment-related cardiac functional and metabolic changes. Few and partly conflicting data exist regarding NEFA uptake/utilization in the human (pre)diabetic heart and its relation to cardiac function. Based on animal studies, it was proposed that the diabetic heart primarily relies on the abundantly supplied NEFA, in the presence of myocardial insulin resistance. Chronically elevated NEFA utilization may lead to impaired β-oxidation, accumulation of toxic intermediates, production of reactive oxygen species and mitochondrial dysfunction and finally, to cardiac functional abnormalities. Because glucose relative to NEFA-oxidation requires less oxygen per mole of ATP produced, therapies enhancing myocardial glucose utilization, including insulin and thiazolidinediones, have been advocated in T2DM patients with cardiac ischemia. However, it is unknown whether enforced myocardial glucose use is beneficial at all occasions.

Similarly, rosiglitazone increased MMRglu in T2DM patients with CAD, without affecting echocardiographically measured function. Additionally, indirect stimulation of myocardial glucose metabolism by acute deprivation of NEFA by acipimox in heart failure patients resulted in depressed cardiac work and efficiency. These findings support the notion that compromised hearts may lose their flexibility to respond to imposed changes in substrate availability and readily switch to another substrate. In contrast, it is likely that the myocardium of patients with uncomplicated T2DM of short duration still possesses sufficient oxidative capacity to benefit from NEFA as the preferential myocardial substrate. Forced glucose utilization in these patients will not necessarily lead to improved cardiac function. Because changes in myocardial metabolism and function in the pioglitazone-treated patients were not related, it is unlikely that the improvement in diastolic function originates from altered metabolism.

Since we did not measure myocardial oxygen consumption, no calculation of treatment-related changes in cardiac efficiency can be made. Nevertheless, since resting perfusion is tightly coupled to oxidative metabolism, the unchanged cardiac work and resting perfusion after pioglitazone may suggest that cardiac efficiency was unaffected by this therapy. In contrast, metformin significantly reduced cardiac work and increased resting perfusion, both of which effects seem compatible with an actual reduction in cardiac efficiency. These changes, however, did not translate into a decrease in PCR/ATP ratio, implying an adaptive cardiac response sufficient to preserve high-energy phosphate metabolism. Additional studies addressing the effects of these pharmacological interven
Pioglitazone improves cardiac function and alters myocardial substrate metabolism

transitions regarding myocardial energetics and efficiency in T2DM are warranted. Pioglitazone decreased hepatic but not myocardial triglyceride content, indicating differential regulation of various body lipid compartments. We recently found an independent association between diastolic function and myocardial triglyceride content in T2DM patients," confirming and extending previous data by McGavock et al. However, as noted earlier, since myocardial triglyceride content was not altered by pioglitazone, this indicates that the improvement in LV filling dynamics and compliance are likely caused by other mechanisms. The actual mechanisms by which pioglitazone improved LV diastolic function and compliance could, however, not be identified in this study using the present technology. Further studies are therefore warranted to define those mechanisms.

The major asset of this study is the combined use of PET and MRI/[^31]P]-MRS and [^1]H]-MRS to evaluate cardiac effects of pioglitazone and metformin. The relatively short intervention-time and exclusion of women and patients with ischemia, however, are limitations precluding generalization of the results.

Conclusion

Only pioglitazone improved LV diastolic function and compliance, whereas both pioglitazone and metformin altered myocardial substrate metabolism, likely due to treatment-specific changes in plasma substrate levels. Pioglitazone-related improvement in diastolic function was not associated with concomitant alterations in myocardial substrate metabolism. Treatment with pioglitazone in patients with uncomplicated, well-controlled T2DM and absence of cardiac ischemia might be beneficial, as demonstrated by the improved diastolic function and LV compliance, in the presence of unaltered myocardial high-energy phosphate metabolism.

Funding sources

This investigator-initiated study was supported by Eli Lilly, The Netherlands. Metformin tablets and matching placebo’s were kindly provided by Merck, The Netherlands.


Pioglitazone improves cardiac function and alters myocardial substrate metabolism

Effects of hepatic triglyceride content on myocardial metabolism in type 2 diabetes

Chapter 5

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Hildo J Lamb

Abstract

OBJECTIVES: The purpose of this study was to investigate the relationship between hepatic triglyceride content and both myocardial function and metabolism in T2DM.

BACKGROUND: Heart disease is the leading cause of mortality in type 2 diabetes (T2DM). Central obesity and hepatic steatosis, both hallmark abnormalities in T2DM, have been related to increased risk of heart disease.

METHODS: Sixty-one T2DM patients underwent myocardial perfusion and substrate metabolism measurements by positron-emission tomography, using [15O]water, [11C]palmitate and [18F]-2-fluoro-2-deoxy-D-glucose. In addition, whole-body insulin sensitivity (M/I) was determined. Myocardial left ventricular (LV) function and high-energy phosphate metabolism were measured using magnetic resonance (MR) imaging and [31P]-MR spectroscopy respectively. Hepatic triglyceride content was measured by [1H]-MR spectroscopy. Patients were divided according to hepatic triglyceride content (T2DM-low ≤5.56% versus T2DM-high >5.56%).

RESULTS: In addition to decreased M/I (P=0.002), T2DM-high patients had reduced myocardial perfusion (P=0.001), glucose uptake (P=0.005) and PCr/ATP ratio (P=0.003), compared with T2DM-low patients whereas cardiac fatty-acid metabolism and LV function were not different. Hepatic triglyceride content correlated inversely with M/I (Pearsons’ r=-0.620, P=0.001), myocardial glucose uptake (r=-0.413,P=0.001) and PCr/ATP (r=-0.442,P=0.027). Insulin sensitivity correlated positively with myocardial glucose uptake (r=0.528,P=0.001) and borderline with myocardial PCr/ATP (r=0.367,P=0.072), whilst a positive association was found between cardiac glucose uptake and PCr/ATP (r=0.481,P=0.015).

CONCLUSIONS: High liver triglyceride content in T2DM was associated with decreased myocardial perfusion, glucose uptake and high-energy phosphate metabolism, in conjunction with impaired whole-body insulin sensitivity. The long-term clinical implications of hepatic steatosis with respect to cardiac metabolism and function in the course of T2DM require further study.
Chapter 5

Introduction

In the past few decades, the prevalence of obesity and type 2 diabetes (T2DM) has grown to epidemic proportions. T2DM patients are at increased risk of cardiovascular disease (CVD), in particular coronary artery disease (CAD) and heart failure, and consequently, heart disease is the most common cause of death in T2DM. Cardiac abnormalities in T2DM patients can, however, develop in the absence of hypertension or coronary artery disease. These myocardial derangements are attributed to diabetic cardiomyopathy (DCM), a disease entity with a high propensity to progress into overt congestive heart failure.

Several mechanisms have been proposed to underlie DCM, in particular the metabolic hallmarks of the T2DM phenotype such as insulin resistance, dyslipidemia and hyperglycemia. These diabetes-related metabolic derangements are collectively thought to contribute to altered myocardial substrate handling and, subsequently, to the observed cardiac (diastolic) dysfunction. The underlying mechanisms include oxidative stress, mitochondrial dysfunction and compromised high-energy phosphate metabolism.

Hepatic steatosis is a common finding in patients with (uncomplicated) T2DM. It is associated with a cluster of metabolic abnormalities, including insulin resistance, hyperglycemia, dyslipidemia and a pro-inflammatory state, all factors known to adversely affect the cardiovascular system. Indeed, several studies in humans showed an association of fatty liver with an increased carotid intima media thickness (cIMT), impaired flow-mediated vasodilation, advanced atherosclerosis, and CVD. Interestingly, high liver fat was associated with alterations in myocardial high-energy phosphate metabolism in healthy obese individuals, and myocardial glucose metabolism in T2DM patients with CAD. These studies have provided hypothesis generating preliminary data regarding the potential mechanisms that could link liver steatosis to cardiac disease as a risk factor for CVD. However, whether high liver fat adversely affects metabolic or functional aspects of the heart in asymptomatic patients with uncomplicated T2DM, still remains to be determined.

Using state-of-the-art imaging techniques, the aim of the present study was to assess the relationship between liver triglyceride content and myocardial metabolism in T2DM patients with verified absence of clinical ischemic heart disease.

Methods

Participants

Sixty-one T2DM patients were selected from a previous study based on availability of PET measurements. This two-center study was approved by the Medical Ethics Committee of both centers, and performed in compliance with the Declaration of Helsinki. All patients signed informed consent prior to inclusion. Patients were recruited by advertisements in local newspapers. Male T2DM patients, aged 45-65 years, without diabetes-related complications were eligible. Inclusion criteria were a glycated hemoglobin $A_1c$ (HbA$_1c$) level of 6.5-8.5 % at screening, BMI of 25-32 kg/m$^2$, blood pressure not exceeding 150/85 mm Hg (with or without the use of anti-hypertensives). Patients were excluded if they had a history or current hepatic or cardiovascular disease and if they used insulin, fibrates, thiazolidinediones or other hormonal replacement therapy. Screening of patients consisted of medical history, physical examination, echocardiogram and fasting blood and urine analyses. In addition, patients underwent dobutamine-stress echocardiography to confirm absence of inducible ischemia. After successful screening, participants commenced with a 10-week
run-in period during which their regular bloodglucose lowering agents were changed to gliclazide monotherapy, and titrated until a stable dose was reached during 8 weeks prior to assessments, to exclude possible confounding effects on myocardial metabolism of differential agents. The present baseline data were derived from a previously reported intervention study, in which participants were randomized to pioglitazone or metformin after baseline measurements, to study the effects of these agents on cardiac function and metabolism.

Imaging
The study protocol was performed during two visits within the same week. During one visit, cardiac perfusion and substrate metabolism were measured using PET, whereas during the other visit myocardial function was measured by MRI and hepatic triglyceride content and myocardial PCR/ATP ratio by [1H]- and [13C]-spectroscopy respectively. At both occasions, patients visited the clinical research unit at 08:00 in the morning following an overnight fast of approximately 12-15 hours. Glucose-lowering agents were not taken in the morning before assessments.

PET
All PET examinations were performed at one single center (Amsterdam) using an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, TN, USA). Patients received two cathers; one in an antecubital vein and one in a contralateral hand vein being wrapped into a heated blanket to obtain arterialized blood. Myocardial perfusion (MBF) studies were performed in 2D acquisition using [18O]water (1100 MBq) as tracer. Myocardial glucose metabolism (MMRglu) and fatty-acid uptake studies were performed in 3D acquisition mode using [18F]-2-fluoro-2-deoxy-D-glucose ([18F]FDG, 170 MBq) and [13C]palmitate (185 MBq) as tracers, respectively. MBF and fatty-acid uptake were assessed in the fasting state, whereas MMRglu was measured under hyperinsulinemic euclidean conditions. The scan protocol was as follows. Following a 10-min transmission scan for attenuation correction, [18O]water was injected (t=10 min) and a 10-min dynamic emission scan consisting of 40 frames with progressively increasing frame length was acquired. Subsequently, a 30-min dynamic emission scan consisting of 34 frames with increasing frame length was performed following [13C]-palmitate injection (t=35 min). Hereafter, the clamp was started (t=65 min), as described previously, to approximate an isometabolic steady state (plasma glucose level 5 mmol/l) and measure whole-body insulin sensitivity (M value). At steady state (t=155 min), following a new transmission scan, [18F] FDG was injected and a 60-min dynamic emission with increasing frame length of 40 frames was acquired. Blood samples were collected during all three scans at predefined time points to measure glucose, NEFA, lactate, lipids and insulin levels. In addition, [13CO2] was measured during the [13C]palmitate scan. Total radiation exposure of the entire sequence of scans was 4.87 mSv.

PET data analysis
PET data were reconstructed using filtered backprojection applying all appropriate corrections. In order to generate myocardial time-activity curves, region of interest (ROIs) were defined on resliced LV short-axis (summed) [13C]-palmitate and [18F]FDG images and subsequently projected onto the corresponding dynamic images. ROIs were drawn and grouped for further analysis as previously described. Myocardial segments exposed to liver spill-in were omitted from the analysis of [13C]-palmitate data. Additional ROIs were defined in right and left ventricular chambers for [13C] palmitate and [18O]water image-derived input functions (IDIF). A separate aorta ascendence ROI was defined for [18F]FDG IDIF. Myocardial perfusion was determined using the standard single-tissue compartment model. As resting myocardial perfusion is related to the rate pressure prod
uct (RPP = heart rate × systolic blood pressure), corrected resting MBF (1000×MBF/RPP) was also calculated.\textsuperscript{24} Moreover, myocardial vascular resistance was calculated by dividing the mean arterial pressure by myocardial rest perfusion.\textsuperscript{25} \textsuperscript{11}C]palmitate time-activity curves were analyzed using a three-tissue plasma input kinetic model, which, together with plasma NEFA concentrations, enabled calculation of Myocardial fatty acid uptake, oxidation and esterification (MFAU, MFAO and MFAE, respectively).\textsuperscript{26–28} The \textsuperscript{11}C]palmitate IDIF was corrected for \textsuperscript{11}C]CO, metabolites and differences between plasma and whole blood concentrations as described elsewhere.\textsuperscript{15, 26} MMRglu was calculated by multiplying the net influx constant for \textsuperscript{18}F]FDG, $K_r$, with the mean plasma glucose concentration. For determination of $K_r$, Patlak graphical analysis was used.\textsuperscript{29}

**MRI**
The heart was imaged in short-axis orientation using electrocardiographically (ECG)-gated breath-holds with a sensitivity encoding balanced turbo-field echo sequence. LV ejection fraction (LVEF), cardiac output (CO), stroke volume (SV), LV end-diastolic (LVEDV) and LV end-systolic volume (LVESV), LV mass and their indexes calculated by dividing each parameter through the body surface area (CI, LVEDVI, LVESVI and LVMI), were determined by analyses of end-diastolic and end-systolic images.\textsuperscript{21} An ECG-gated gradient-echo sequence with velocity encoding was performed to measure blood flow across the mitral valve in order to determine LV diastolic function. Measures included the peak filling rate of the early filling phase (E) and of the atrial contraction (A), the ratio of the peak filling rates (E/A) and the peak deceleration gradient of the early filling phase (E deceleration peak). Additionally, an estimation of LV filling pressure (E/ Ea) was calculated.\textsuperscript{22} During MR imaging, blood pressure and heart rate were measured.

To quantify the amount of visceral and subcutaneous abdominal fat, three consecutive transverse images were obtained during 1 breath hold with the middle image at a level just above the fifth lumbar vertebra.\textsuperscript{23} All images were analyzed quantitatively using dedicated software (FLOW or MASS, Medis, Leiden, the Netherlands).

**Hepatic \textsuperscript{1H}-MRS**
Hepatic \textsuperscript{1H}-MR spectroscopy was performed as described previously.\textsuperscript{21–24} Briefly, to obtain hepatic \textsuperscript{1H}-MR spectra, an 8 ml voxel was positioned in the liver avoiding gross vascular structures and adipose tissue depots. Both spectra with and without water suppression were obtained, to calculate hepatic triglyceride (TG) content as a percentage relative to water (100×TG/water).\textsuperscript{24} To quantify hepatic triglyceride content, the methylene and water signals were corrected for $T_2$ decay by use of the exponential relaxation equation $S = S_0 \exp (-T_2/T_1)$, where $S_0$ represents the corrected signal, $T_2$ the echo time, $T_1$ the longitudinal relaxation time and $S_0$ the signal after application of the 90° pulse. $T_1$ relaxation times for water and triglyceride of 50 and 60 ms, respectively, were used as described by Szczepaniak et al.\textsuperscript{24} A fatty liver was defined as hepatic triglyceride content exceeding 5.56%.\textsuperscript{24, 25}

**Myocardial \textsuperscript{31}P]-MRS**
ECG-triggered \textsuperscript{31}P]-MR spectra of the LV anterior wall were acquired using a 100-mm-diameter surface coil. Volumes of interest were selected by image-guided spectroscopy with 3D-ISIS. Shimming was performed automatically and tuning and matching of the \textsuperscript{31}P] surface coil was performed manually. Spectroscopic volume size typically was 7 × 7 × 7 cm. Acquisitions were based on 192 averaged free induction decays, and total acquisition time was 10 min. \textsuperscript{31}P]-MR spectra were corrected for partial saturation effects and for the adenosine-triphosphate (ATP) contribution from
blood in the cardiac chambers. The myocardial phosphocreatine (PCr)/ATP ratios of the spectra were calculated and used as a parameter representing myocardial HEP metabolism.\(^{26,27}\)

Biochemical Analyses

Samples were analyzed at one certified central laboratory (Amsterdam, The Netherlands). HbA\(_1c\) was determined by high-performance liquid chromatography (HPLC; Menarini Diagnostics, Florence, Italy; reference values: 4.3-6.1%). Plasma glucose was quantified by the use of a hexokinase-based technique (Roche Diagnostics, Mannheim, Germany). Plasma triglycerides, total cholesterol and high-density lipoprotein cholesterol were determined using enzymatic colorimetric methods (Modular, Hitachi, Japan). Levels of low-density lipoprotein cholesterol were calculated using Friedewald's formula (reference values: 2.0-4.6 mmol/l). Plasma insulin levels were quantified by an immunoradiometric assay (Bayer Diagnostics, Mijdrecht, The Netherlands). Plasma free-fatty acids were measured by an enzyme-linked immunosorbent assay (Wako Chemicals, Neuss, Germany). Ultra-sensitive C-reactive protein (us-CRP) was determined by ELISA (DSL, Webster, Texas, USA). The sensitivity was 1.6 \(\mu\)g/l and the interassay coefficients of variations ranged from 3 to 5%. In duplo determinations of plasma malondialdehyde, a marker of oxidative stress, were performed by HPLC after alkaline hydrolysis and reaction with thiobarbituric acid.\(^{28}\) The intra-assay COV was 5.7%.

Statistical analysis

Values are expressed as mean±(standard error; SE) or median (with interquartile range; IQR). Normality was assumed if the histogram showed a normal distribution, the Kolmogorov-Smirnoff test was > 0.05 and skewness and kurtosis were < 1.0. Non-normally distributed data were log-transformed. Comparisons between groups were made using the independent t-tests. Linear regression was used to adjust for BMI differences between groups. The chi-square test was used for nominal parameters. Univariate and multiple analyses with a forward selection procedure were performed. The aim of these analyses was to determine which variables influence liver triglyceride content, MMRglu, PCr/ATP. Those variables with an \(P<0.1\) were subsequently entered in a forward multivariable regression analysis and those variables with a \(P < 0.05\) were considered independently related to the dependent variable. As liver triglyceride content was a skewed variable, this variable was log-transformed. Moreover, several independent variables were log-transformed. Analyses were performed with SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA). A two-tailed probability value <0.05 was considered significant.

Results

Assessment of myocardial function, hepatic \(^1\)H-MR spectroscopy and measurements of MMRglu were successfully completed in all 61 patients. Because of technical reasons \(^1\)Owater and \(^1\)H-C palmitate data were not available for 1 and 8 subjects, respectively. Due to the demanding nature of the protocol myocardial PCr/ATP was offered as an optional test. Therefore measurements were available only in a subgroup of 25 patients. These subjects did not differ in clinical characteristics form the other 36 patients.
Subject characteristics, hemodynamics and myocardial function

Table 1 shows characteristics of the entire study population, divided into groups with high and low hepatic triglyceride content. Both groups did not differ with respect to age, glycemic control, duration of diabetes and use of medication. BMI and plasma triglycerides, however, were higher in the T2DM-high group. Metabolic characteristics are given in Table 2, showing higher fasting plasma insulin and lactate and a borderline significant increase in CRP in T2DM-high patients. Under hyperinsulinemic euglycemic clamp conditions plasma fatty-acid and insulin levels were higher and M/I was lower in T2DM-high patients. No differences were observed in myocardial hemodynamics, and LV systolic and diastolic function and dimensions between T2DM-high and T2DM-low patients (Table 3).

<table>
<thead>
<tr>
<th>Table 1. Subject characteristics</th>
<th>T2DM-low N=29</th>
<th>T2DM-high N=32</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>57.3±0.9</td>
<td>56.4±1.0</td>
<td>0.507</td>
</tr>
<tr>
<td>Time since diagnosis of diabetes (yrs)</td>
<td>4 (2-6)</td>
<td>4 (3-5)</td>
<td>0.858</td>
</tr>
<tr>
<td>Current smoker – %</td>
<td>21 (6/29)</td>
<td>22 (7/32)</td>
<td>0.230</td>
</tr>
<tr>
<td>Body mass index (kg·m⁻²)</td>
<td>27.1±0.6</td>
<td>30.1±1.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>101±2</td>
<td>107±2</td>
<td>0.015</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>7.0±0.2</td>
<td>7.3±0.2</td>
<td>0.306</td>
</tr>
<tr>
<td>Total cholesterol (mmol·L⁻¹)</td>
<td>4.3±0.1</td>
<td>4.5±0.1</td>
<td>0.212</td>
</tr>
<tr>
<td>LDL cholesterol (mmol·L⁻¹)</td>
<td>2.6±0.1</td>
<td>2.7±0.1</td>
<td>0.46</td>
</tr>
<tr>
<td>HDL cholesterol (mmol·L⁻¹)</td>
<td>1.05 (0.85-1.23)</td>
<td>0.96 (0.81-1.10)</td>
<td>0.131</td>
</tr>
<tr>
<td>Triglycerides (mmol·L⁻¹)</td>
<td>1.2 (0.8-1.6)</td>
<td>1.8 (1.2-2.4)</td>
<td>0.054</td>
</tr>
<tr>
<td>ALT (U·L⁻¹)</td>
<td>26 (21-33)</td>
<td>36 (29-49)</td>
<td>0.004</td>
</tr>
<tr>
<td>y-GT (U·L⁻¹)</td>
<td>21 (18-37)</td>
<td>41 (33-48)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Medications – %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>38 (11/29)</td>
<td>47 (15/32)</td>
<td>0.481</td>
</tr>
<tr>
<td>Any antihypertensive medication</td>
<td>38 (11/29)</td>
<td>44 (14/32)</td>
<td>0.644</td>
</tr>
<tr>
<td>β-blockers</td>
<td>10 (3/29)</td>
<td>9 (3/32)</td>
<td>0.899</td>
</tr>
<tr>
<td>Diuretics</td>
<td>7 (2/29)</td>
<td>16 (5/32)</td>
<td>0.285</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>17 (5/29)</td>
<td>19 (6/32)</td>
<td>0.876</td>
</tr>
<tr>
<td>Angiotensin II blocker</td>
<td>14 (4/29)</td>
<td>13 (4/32)</td>
<td>0.881</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>3 (1/29)</td>
<td>6 (2/32)</td>
<td>0.613</td>
</tr>
<tr>
<td>Omega-3</td>
<td>1 (0/29)</td>
<td>0 (0/32)</td>
<td>0.337</td>
</tr>
</tbody>
</table>

Data are mean±SE or median (IQR). T2DM-high or low: Type 2 diabetes patients with high or low liver triglyceride content. ACE, angiotensin-converting enzyme.
Effects of hepatic triglyceride content on myocardial metabolism in type 2 diabetes

Table 2. Biochemical and metabolic characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>T2DM-low N=29</th>
<th>T2DM-high N=32</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasting</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma glucose (mmol·L⁻¹)</td>
<td>8.3 (6.8-10.7)</td>
<td>8.3 (6.9-9.6)</td>
<td>0.600</td>
</tr>
<tr>
<td>Plasma non-esterified fatty acids (mmol·L⁻¹)</td>
<td>480 (365-685)</td>
<td>510 (425-595)</td>
<td>0.986</td>
</tr>
<tr>
<td>Plasma lactate (mmol·L⁻¹)</td>
<td>1.0 (0.9-1.3)</td>
<td>1.2 (1.0-1.5)</td>
<td>0.030</td>
</tr>
<tr>
<td>Plasma insulin (pmol·L⁻¹)</td>
<td>5±17</td>
<td>90±10</td>
<td>0.006</td>
</tr>
<tr>
<td>usCRP (mg·L⁻¹)</td>
<td>3.6±0.6</td>
<td>7.0±1.4</td>
<td>0.064</td>
</tr>
<tr>
<td>Malondialdehyde (µmol·L⁻¹)</td>
<td>9.9±0.5</td>
<td>9.8±0.4</td>
<td>0.976</td>
</tr>
<tr>
<td><strong>During hyperinsulinemia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma non-esterified fatty acids (mmol·L⁻¹)</td>
<td>71±11</td>
<td>130±13</td>
<td>0.001</td>
</tr>
<tr>
<td>Plasma lactate (mmol·L⁻¹)</td>
<td>1.1 (0.9-1.3)</td>
<td>1.1 (1.0-1.3)</td>
<td>0.754</td>
</tr>
<tr>
<td>Plasma insulin (pmol·L⁻¹)</td>
<td>508 (458-611)</td>
<td>615 (571-744)</td>
<td>0.001</td>
</tr>
<tr>
<td>M/I value (mg·kg⁻¹·min⁻¹)/(µmol·L⁻¹)</td>
<td>0.02 (0.44-1.07)</td>
<td>0.37 (0.17-0.46)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Data are mean±SE or median (IQR). T2DM-high or low. Type 2 diabetes patients with high or low liver triglyceride content. nsCRP = ultrasensitive C-reactive protein. M/I value = whole body insulin sensitivity adjusted during the steady state.

Table 3. Hemodynamic parameters, cardiac dimensions and function in the study population

<table>
<thead>
<tr>
<th></th>
<th>T2DM-low N=29</th>
<th>T2DM-high N=32</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hemodynamics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>122±12</td>
<td>125±12</td>
<td>0.443</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>76±1</td>
<td>76±1</td>
<td>0.646</td>
</tr>
<tr>
<td>Heart rate (beats·min⁻¹)</td>
<td>63±2</td>
<td>62±1</td>
<td>0.794</td>
</tr>
<tr>
<td>Rate pressure product (beats·min⁻¹·mmHg)</td>
<td>774±1288</td>
<td>781±1243</td>
<td>0.857</td>
</tr>
<tr>
<td><strong>Systolic function and dimensions</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV mass (g)</td>
<td>104±11</td>
<td>111±13</td>
<td>0.114</td>
</tr>
<tr>
<td>LV mass index (g·m⁻²)</td>
<td>5±1</td>
<td>5±2</td>
<td>0.519</td>
</tr>
<tr>
<td>LV end-systolic volume (mL)</td>
<td>62±2</td>
<td>62±2</td>
<td>0.974</td>
</tr>
<tr>
<td>LV end-systolic volume index (mL·m⁻²)</td>
<td>30±1</td>
<td>30±1</td>
<td>0.449</td>
</tr>
<tr>
<td>LV stroke volume (mL)</td>
<td>93±3</td>
<td>94±3</td>
<td>0.630</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>60±1</td>
<td>60±1</td>
<td>0.717</td>
</tr>
<tr>
<td>Cardiac index (min⁻¹·m⁻²)</td>
<td>3.0±0.1</td>
<td>2.9±0.1</td>
<td>0.512</td>
</tr>
<tr>
<td>Cardiac work (mm Hg·mL⁻¹·min⁻¹)</td>
<td>52.6±0.2</td>
<td>53.7±0.2</td>
<td>0.677</td>
</tr>
<tr>
<td><strong>Diastolic function and dimensions</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV end-diastolic volume (mL)</td>
<td>155±4</td>
<td>157±4</td>
<td>0.719</td>
</tr>
<tr>
<td>LV end-diastolic volume index (mL·m⁻²)</td>
<td>76±2</td>
<td>73±2</td>
<td>0.359</td>
</tr>
<tr>
<td>E peak filling rate (mL·s⁻¹)</td>
<td>409±15</td>
<td>413±16</td>
<td>0.829</td>
</tr>
<tr>
<td>E deceleration peak (mL·s⁻²·10⁻¹)</td>
<td>5.57±0.13</td>
<td>5.99±0.39</td>
<td>0.439</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.0±0.05</td>
<td>1.0±0.04</td>
<td>0.618</td>
</tr>
<tr>
<td>E/Ed</td>
<td>9.8 (7.7-13.0)</td>
<td>8.5 (6.6-11.4)</td>
<td>0.196</td>
</tr>
</tbody>
</table>

Data are mean±SE or median (IQR). T2DM-high or low. Type 2 diabetes patients with high or low liver triglyceride content. LV = left ventricular. E = early diastolic filling phase. A = diastolic atrial contraction. E/Ed = estimate of the left ventricular filling pressure.
Myocardial perfusion and metabolism

MBF was lower in T2DM-high than in T2DM-low patients (Figure 1A), also after correction for RPPP (1.07±0.04 versus 1.26±0.05 mL·g⁻¹·mmHg⁻¹·10.000⁻¹, P=0.006). In contrast, myocardial vascular resistance was higher (118±5 versus 98±4 Hg·ml⁻¹·min⁻¹·ml⁻¹, P=0.004). Both MMRglu (Figure 1B) and PCr/ATP ratio (Figure 1C) were lower in T2DM-high than in T2DM-low patients. The alterations in myocardial fatty-acid metabolism in T2DM-high patients did not reach statistical significance (MFAU: 83±5 versus 92±7 nmol·min⁻¹·ml⁻¹, P=0.266; MFAO: 82±5 versus 89±6 nmol·min⁻¹·ml⁻¹, P=0.361; MFAE: 1±1 versus 3±1 nmol·min⁻¹·ml⁻¹, P=0.368).

**Figure 1.** Myocardial perfusion, glucose uptake and high-energy phosphate metabolism

Myocardial resting perfusion (A, n=61), Metabolic rate of glucose metabolism (MMRglu) (B, n=61), and myocardial phosphocreatine (PCr)/ATP ratio (C, n=25) in T2DM patients with low [□] and high [■] liver triglyceride content.

**Hepatic triglyceride content and associations**

According to the definitions, T2DM-high versus T2DM-low patients had higher median hepatic triglyceride content (14.4 (9.6-18.9) % versus 2.0 (1.2-3.8) %). Hepatic visceral fat (2.6 (2.5-2.8) versus 2.4 (2.5-2.6) mL, P=0.001) and subcutaneous fat (743±46 vs 604±51 mL, P=0.045) were higher in T2DM-high versus T2DM-low patients. Univariable and multivariable regression analyses of liver triglyceride content and myocardial metabolism are shown in Table 4, revealing M/I and visceral fat volume to be independently related to liver triglyceride content, plasma fatty-acid and lactate levels to be independently related to MMRglu and only MMRglu to be independently related to PCr/ATP. Amongst others, significant associations were seen between both myocardial glucose and fatty-acid metabolism and plasma levels of malondialdehyde. Correlations between liver triglyceride content, MMRglu and myocardial PCr/ATP ratio are shown in Figure 2.
### Table 4. Univariable and multivariable linear regression analysis of liver triglyceride content, myocardial metabolic rate of glucose and PCr/ATP ratio

<table>
<thead>
<tr>
<th></th>
<th>Liver Triglyceride Content</th>
<th>MMRglu-10</th>
<th>PCr/ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariable</td>
<td>Multivariable</td>
<td>Univariable</td>
</tr>
<tr>
<td></td>
<td>retransformed coefficient</td>
<td>P value</td>
<td>retransformed coefficient</td>
</tr>
<tr>
<td>Plasma triglycerides (mmol L(^{-1})) (\ast)</td>
<td>4.943</td>
<td>0.004</td>
<td>-</td>
</tr>
<tr>
<td>Plasma lactate (mmol L(^{-1})) (\ast)</td>
<td>19.953</td>
<td>0.004</td>
<td>-</td>
</tr>
<tr>
<td>Plasma fatty acids (mmol L(^{-1})) (\ast)</td>
<td>1.690</td>
<td>0.604</td>
<td>-</td>
</tr>
<tr>
<td>Plasma insulin (pmol L(^{-1})) (\ast)</td>
<td>0.155</td>
<td>&lt; 0.001</td>
<td>-</td>
</tr>
<tr>
<td>HbA(_1c) (%)</td>
<td>1.318</td>
<td>0.058</td>
<td>-</td>
</tr>
<tr>
<td>Malondialdehyde (μmol L(^{-1}))</td>
<td>1.057</td>
<td>0.301</td>
<td>-</td>
</tr>
<tr>
<td>Myocardial PCr/ATP</td>
<td>0.245</td>
<td>0.027</td>
<td>-</td>
</tr>
<tr>
<td>M/I value (10 mg/kg (\cdot) min (^{-1}))/ (pmol L(^{-1}))</td>
<td>0.136</td>
<td>&lt; 0.001</td>
<td>0.201</td>
</tr>
<tr>
<td>Liver Triglyceride Content (%) (\ast)</td>
<td>NA</td>
<td>NA</td>
<td>-</td>
</tr>
<tr>
<td>MMRglu (10 mmol (\cdot) mL (^{-1}) (\cdot) min (^{-1}))</td>
<td>0.706</td>
<td>0.001</td>
<td>-</td>
</tr>
<tr>
<td>MFAU (10 mmol (\cdot) mL (^{-1}) (\cdot) min (^{-1}))</td>
<td>0.668</td>
<td>0.405</td>
<td>-</td>
</tr>
<tr>
<td>MFAO (10 mmol (\cdot) mL (^{-1}) (\cdot) min (^{-1}))</td>
<td>0.713</td>
<td>0.497</td>
<td>-</td>
</tr>
<tr>
<td>Visceral fat volume (mL)</td>
<td>15.68</td>
<td>&lt; 0.001</td>
<td>4.036</td>
</tr>
<tr>
<td>Subcutaneous fat volume (mL)</td>
<td>1.002</td>
<td>0.009</td>
<td>-</td>
</tr>
<tr>
<td>Plasma uric acid (mg/L) (\ast)</td>
<td>1.999</td>
<td>0.037</td>
<td>-</td>
</tr>
<tr>
<td>Body mass index (kg/m(^{2}))</td>
<td>1.164</td>
<td>&lt; 0.001</td>
<td>-</td>
</tr>
</tbody>
</table>

*Legend on next page*
NOTE: For those independent variables with an asterisk (*), the regression coefficient is estimated for a difference of one unit in the log of the independent variable. The transformed regression coefficient can be interpreted as follows: A difference of 1 unit log HbA1c is related to a 1.318 x higher liver triglyceride content. HbA1c = glycated hemoglobin. M/I value = whole body insulin sensitivity adjusted during the steady state. \(^2\)RR = MMRglu was significantly related with MFAU (P=0.010) and MFAO (P=0.009) in those patients in whom PCr/ATP was available. MMRglu = myocardial metabolic rate of glucose uptake. MFAU/O = myocardial fatty-acid oxidation. \(\text{ucCRP} = \text{ultrasensitive C-reactive protein.}\)

Figure 2. Correlations between liver triglyceride content, myocardial metabolism and PCr/ATP ratio

Correlation between liver triglyceride content and myocardial metabolic rate of glucose (MMRglu) (A), between liver triglyceride content and myocardial phosphocreatine (PCr)/ATP ratio (B), and between MMRglu and myocardial PCr/ATP (C) in those T2DM patients in whom myocardial PCr/ATP was available.

Discussion

In the present study, reduced myocardial perfusion, glucose uptake and PCr/ATP ratio were found in T2DM patients with high liver triglyceride content with verified absence of inducible ischemia. LV function and dimensions, however, were similar as in T2DM patients with low liver triglyceride content. Moreover, liver triglyceride content was inversely associated with myocardial substrate and high-energy phosphate metabolism. Furthermore, to the best of our knowledge, this study is the first to show a direct positive relationship between myocardial glucose metabolism and levels of myocardial PCr/ATP in human T2DM in vivo.

In the present study, T2DM patients with increased liver triglyceride content were characterized by lower HDL cholesterol, higher plasma triglyceride and CRP levels, and lower insulin sensitivity as
compared with T2DM patient with low liver triglyceride content. None of the patients had CVD or diabetes-related complications, allowing the assessment of early myocardial abnormalities in the absence of potentially confounding effects of CAD and hypertension.

Myocardial glucose uptake was decreased in T2DM patients with high versus low liver triglyceride content, confirming previous data from Lautamaki et al,27 who reported decreased PET-measured myocardial glucose consumption in non-stenotic myocardial segments of T2DM patient with CAD and high liver triglyceride content. In the present study, plasma fatty-acid levels were inversely related to myocardial glucose metabolism, therefore increased substrate levels may explain reduced myocardial glucose metabolism by reverse substrate competition. A more pronounced impairment of insulin signalling and reduced membrane bound GLUT-4 in T2DM patients with high liver triglyceride content, however, may also have contributed to decreased myocardial glucose metabolism.28, 29 Decreased resting myocardial perfusion and increased myocardial vascular resistance, observed in the present study, may indicate an early alteration in myocardial tissue and/or vascular properties in T2DM patients with high liver triglyceride content. Although age, gender and RPPP are known to influence resting myocardial perfusion,11 these were similar in the two groups, and therefore cannot explain the differences in myocardial perfusion between both groups. Induced hyperinsulinaemia has been shown to significantly increase myocardial perfusion at rest in T2DM patients with CAD, also in non-affected cardiac regions, emphasizing the role of insulin action on myocardial perfusion.30 The observed difference in myocardial perfusion between both groups in the present study may be related to the same mechanism as T2DM patients with high liver triglyceride content were significantly more insulin resistant. Previously, resting myocardial perfusion under hyperinsulinaemia in non-stenotic cardiac segments was not different in T2DM patients with high- versus low liver triglyceride content and CAD, whereas myocardial glucose uptake was lower in patients with high liver triglyceride content.12 As myocardial perfusion and glucose metabolism were measured under fasting and hyperinsulinaemic conditions, respectively, the present study design does not allow direct comparisons. Nevertheless, it is likely that the relatively small decrease in myocardial perfusion is responsible for only a minor fraction of the decrease in myocardial glucose metabolism in the T2DM patients with high liver triglyceride content. The major fraction of this decrease presumably is an intrinsic effect or related to substrate competition as mentioned above.

High liver triglyceride content in T2DM patients was also associated with a decreased myocardial PCr/ATP ratio. Myocardial PCr/ATP is known to be decreased in a variety of diseases, among others obesity and T2DM.31-33 The present results in T2DM patients with verified absence of inducible ischemia are in line with a previous study by Perseghin et al,34 who reported a reduced myocardial PCr/ATP ratio in young non-diabetic men with high-versus low liver triglyceride content. Fatty-acids, glucose and lactate are the primary energy substrates of the heart, with a substrate preference depending on myocardial workload and substrate supply in conjunction with feeding status.35 Glucose, however, is the most energy efficient substrate.6 Experimental data suggest that increased fatty-acid relative to glucose metabolism leads to the formation of toxic lipid signaling molecules and oxidative stress, ultimately resulting in mitochondrial dysfunction and reduced ATP synthesis.4

In the present study inverse relationships between myocardial glucose and fatty-acid metabolism and plasma levels of malondialdehyde were found. Moreover, a positive relationship between myocardial glucose metabolism and myocardial PCr/ATP ratio was found in T2DM patients. The present study design precludes an assessment as to whether those associations describe a cause and effect relationship. It may, however, be speculated that when glucose relative to fatty-acid metabolism contributes more to total ATP synthesis, this may results in a more favorable myocardial energy level.
Although myocardial metabolism differed between T2DM patients with high and low liver triglyceride content, no such differences were seen in myocardial systolic and diastolic function or dimensions. This finding corresponds with the earlier work of Perseghin et al, who found differences in PCr/ATP ratios, but not in myocardial function and dimensions between non-diabetic men with high versus low liver triglyceride content.

Limitations

The following limitations need to be considered in the present study. Firstly, PET measurements were performed under different conditions, i.e. fasting perfusion and fatty-acid measurements, but euglycemic hyperinsulinemic clamp conditions for measurement of glucose metabolism. An euglycemic hyperinsulinemic clamp is mandatory, as under fasting conditions virtually no glucose metabolism will be present in insulin resistant myocardium. Differences in myocardial perfusion and glucose metabolism in the two groups are therefore not readily interpretable. Secondly, there were substantially less myocardial PCr/ATP data available than for the other measurements. Thirdly, only men were included, which limits generalizibility of the results to both genders.

Conclusion

In the absence of diabetes related complications and inducible ischemia, T2DM patients with high liver triglyceride content showed decreased myocardial perfusion, glucose uptake, high-energy phosphate metabolism and whole-body insulin resistance compared with similar patients with low liver triglyceride content. The long-term clinical implications of this association between liver steatosis and altered cardiac metabolism require further study in T2DM.
Reference List

Chapter 5

PART 2 – Diabetes and The Liver
Liver fat content in type 2 diabetes: relationship with hepatic perfusion and substrate metabolism
Abstract

OBJECTIVE: Hepatic steatosis is common in type 2 diabetes mellitus (T2DM). It is causally linked to the features of the metabolic syndrome, liver cirrhosis and cardiovascular disease. Experimental data have indicated that increased liver fat may impair hepatic perfusion and metabolism. The aim of the present study was to assess hepatic parenchymal perfusion, together with glucose and fatty-acid metabolism in relation to hepatic triglyceride content.

METHODS: Fifty-nine men with well controlled T2DM and 18 age matched healthy normoglycemic men were studied using positron emission tomography (PET) to assess hepatic tissue perfusion, insulin stimulated glucose and fasting fatty-acid metabolism respectively in relation to hepatic triglyceride content, quantified by proton magnetic resonance (MR) spectroscopy. Patients were divided into two groups with hepatic triglyceride content below (T2DM-low) or above (T2DM-high) the median of 8.6%.

RESULTS: T2DM-high patients had the highest BMI, HbA1c and lowest whole-body insulin sensitivity (ANOVA, all P<0.001). Compared to controls and T2DM-low patients, T2DM-high patients had the lowest hepatic parenchymal perfusion (P=0.004) and insulin-stimulated hepatic glucose uptake (P=0.013). The observed decrease in hepatic fatty acid influx rate constant, however, only reached borderline significance (P=0.088). In T2DM patients, hepatic parenchymal perfusion (r=0.360, P=0.007) and hepatic fatty acid influx rate constant (r= -0.407, P=0.007) correlated inversely with hepatic triglyceride content. In a pooled analysis, hepatic fat correlated with hepatic glucose uptake (r= -0.329, P=0.004).

CONCLUSIONS: In conclusion, T2DM patients with increased hepatic triglyceride content showed decreased hepatic parenchymal perfusion and hepatic insulin mediated glucose uptake, suggesting a potential modulating effect of hepatic fat on hepatic physiology.
Introduction

Obesity and type 2 diabetes (T2DM) have grown to epidemic proportions in virtually all parts of the world due to a sedentary lifestyle and positive energy balance. (1) Hepatic steatosis is a common finding in T2DM, which is causally linked to features of the metabolic syndrome, liver cirrhosis and cardiovascular disease. (2;3) The pro-atherogenic serum lipid profile associated with hepatic steatosis is a consequence of an increased synthesis of very low density lipoproteins (VLDL). (4) Moreover, hepatic steatosis is associated with impaired insulin signaling in insulin responsive tissues by promoting the formation of humoral factors. (5) and it plays a role in atherogenesis via induction of systemic inflammation. (6)

The liver is the central organ for lipid and glucose metabolism, both of which are additionally regulated by insulin. (7-9) Liver steatosis is associated with impaired inhibition of hepatic glucose output, but also with impaired insulin clearance. (10;11) Using splanchnic catheterization in patients with T2DM and healthy controls, glucose and fatty-acid fluxes into the liver have been characterized. (12-15) However, those techniques can not discriminate between the effects of the liver versus those of the other splanchnic tissues. More recently, positron emission tomography (PET) was introduced to non-invasively assess hepatic substrate fluxes. (16-18) To date, however, only a few studies have addressed effects of gluco-metabolic disorders on hepatic disposal of glucose and fatty-acids in humans using PET. (19-21)

Hepatic steatosis has also been associated with alterations of hepatic hemodynamics. Using non-invasive Doppler sonography, decreased portal vein hemodynamics were demonstrated in patients with fatty liver disease. (22;23) Human donor livers, studied during organ retrieval using laser Doppler flowmetry, showed diminished microcirculation compared with control livers. (24) Moreover, animal data revealed that graded steatosis decreased parenchymal microcirculation. (25) In addition to these highly invasive methods, non-invasive in vivo studies of hepatic perfusion have also been performed using PET. (26-28) However, little is known about the relationship between liver triglyceride content with hepatic perfusion or substrate metabolism in human T2DM.

The purpose of the present study was to measure hepatic perfusion and metabolism and to investigate the relationship with hepatic fat content in T2DM patients without diabetes related complication and age matched healthy male subjects.

Methods

Participants

Fifty-nine T2DM patients and 18 healthy controls participated in this two-center study, which was approved by the Medical Ethics Review Committees of both centers and performed in compliance with the Declaration of Helsinki. All subjects signed informed consent prior to inclusion. Patients and controls were recruited by advertisements in local papers. Male T2DM patients, aged 45-65 years, without diabetes related complications were eligible. Inclusion criteria were glycated hemoglobin (HbA1c) level of 6.5-8.5 % at screening, BMI of 25-32 kg/m2; blood pressure not exceeding 150/85 mm Hg (with or without the use of anti-hypertensives). In addition, only moderate alcohol intake was allowed. Patients were excluded if they had a history of or current hepatic or cardiovascular disease. Other exclusion criteria were the use of insulin, fibrates, thiazolidinediones or other hormonal replacement therapy. Healthy males, aged 45-65 years, with normal glucose metabolism, as assessed by a 75 g oral glucose tolerance test, were eligible as controls. Inclusion criteria
Liver fat content in type 2 diabetes: relationship with hepatic perfusion and substrate metabolism

were BMI of 25-32 kg/m² and blood pressure below 150/85 mm Hg. Patients and healthy controls underwent a screening consisting of medical history, physical examination, electrocardiogram and fasting blood- and urine analyses. In addition, patients underwent dobutamine-stress echocardiography to confirm absence of inducible ischemia. All eligible patients entered a 10 week run-in period in which their blood glucose lowering agents were stopped. Subsequently all patients were transferred to a comparable dosage of glimepiride monotherapy. Data on myocardial perfusion and substrate uptake have previously been published elsewhere. (29)

Study design
The study protocol was performed during two visits, within the same week. At one of the visits, hepatic triglyceride content was measured using 1H-MRS. In addition, subcutaneous and visceral fat volumes were measured using MRI. At the other visit, hepatic perfusion and metabolism were measured using PET. At both occasions, patients visited the clinical research unit in the morning at 08:00 following an overnight fast of approximately 12-15 hours and no glucose-lowering agents were taken on the day of the assessments.

Magnetic resonance imaging and spectroscopy
All MR studies were performed at a single center (Leiden) on the same 1.5 Tesla whole body MR scanner (Gyroscan ACS/NT15, Philips, Best, the Netherlands) with subjects at rest and in supine position. Hepatic 1H-MR spectra were obtained as described previously. (30) In short, 1H-MRS of the liver was performed with an 8 ml voxel positioned in the right lobe of the liver avoiding gross vascular structures and adipose tissue deposits. Sixty-four averages were collected with water suppression. Spectra were obtained with an echo time (TE) of 26 ms and a repetition time (TR) of 3000 ms. 1024 data points were collected using a 1000 Hz spectral. Without changing any parameter, spectra without water suppression, with a TR of 10 s and with 4 averages were obtained as an internal reference. 1H-MRS data were fitted using Java-based MR user interface software (JMRUI version 2.2, Leuven, Belgium), as described previously. (31) Hepatic triglyceride content relative to water was calculated as 100 · (signal amplitude of triglyceride)/(signal amplitude of water). T2DM patients were divided according to the median liver fat content in a low (≤ 8.6 %; T2DM-low) and high (> 8.6 %; T2DM-high) liver triglyceride group. Abdominal visceral and subcutaneous fat depots were quantified using MRI. (32) A turbo spin echo imaging protocol was used and imaging parameters included the following: TE = 11 ms, TR = 168 ms, flip angle = 90°, slice thickness 10 mm. Three consecutive transverse images were obtained during 1 breath hold, with the middle image at a level just above the fifth lumbar vertebra. The volumes of the visceral and subcutaneous fat depots of all slices were calculated by converting the number of pixels to square centimeters multiplied by the slice thickness. The total volume of the fat depots was calculated by summing fat volumes of all three slices.

Positron emission tomography
All PET studies were performed at a single center (Amsterdam) using an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, TN, USA). Patients received three venous catheters; one in both antecubital veins and one in a hand vein being wrapped into a heated blanket to obtain arterialized blood during the [18F]FDG scan. Hepatic tissue perfusion was performed in 2D mode and quantified using [18O]H₂O (1100 MBq). Hepatic glucose and fatty-acid uptake were performed in 3D mode and quantified using [18F]FDG (170 MBq) and [14C]palmitate (185 MBq), respectively. Perfusion and fatty-acid uptake were assessed in the postabsorptive state, whereas glucose uptake was performed under
hyperinsulinemic euglycemic conditions. The following scan protocol was used for all studies. Following a 10 minutes transmission scan for attenuation correction, [11]O]bO2 was injected and a 10 minutes dynamic emission scan, consisting of 40 frames with progressively increasing frame length, was acquired. Subsequently, a 30 minutes dynamic emission scan, consisting of 34 frames with progressively increasing frame length, was performed following [11]C]palmitate injection. Next, a euglycemic hyperinsulinemic clamp procedure was started using an insulin infusion rate of 40 mU·m−2·min−1 as previously described.(33) Euglycemia was maintained by adapting the glucose infusion rate to maintain a plasma glucose level of 5 mmol/l. Whole body insulin sensitivity (M/I value) was calculated as the mean plasma glucose level between 90-120 minutes from start of the clamp procedure and then divided by the mean plasma insulin levels in the same time interval. The insulin clearance rate was estimated by dividing the exogenous insulin infusion rate by the steady-state plasma insulin concentrations during the clamp. Under these conditions, the described ratio corresponds to the metabolic clearance rate of systemically administered insulin, minus a small (though variable) part represented by residual insulin secretion. The posthepatic insulin delivery rate of insulin is then calculated as the product of the insulin clearance rate and fasting plasma insulin levels. At steady state (approximately 90 minutes after start of clamp) and following a second transmission scan, [11]C]FDG was injected and a 60 minutes dynamic emission of 40 frames with progressively increasing frame length was acquired. Blood samples were collected during all three scans at predefined time points to measure glucose, NEFA, lactate, lipids and insulin levels. In addition, [11]CO2 was measured during the [11]C]palmitate scan.(29,34)

PET data analysis

Emission data were corrected for physical decay of the respective tracers and for dead time, scatter, randoms and photon attenuation. In order to generate myocardial time-activity curves, large regions (2 by 5 cm) of interest (ROIs) were defined in the right lobe of the liver on 4-5 consecutive planes of OSEM reconstructed (summed) images and then copied to the three dynamic images to obtain one tissue time-activity curve per tracer for each subject. Additionally, circular ROIs (15 mm ø) were drawn on 10 consecutive planes of the respective dynamic images in the aorta ascendens and grouped to obtain one image derived input function (IDIF) for each tracer. To quantify hepatic parenchymal perfusion, it was assumed that [11]O]bO2 in liver can be described by a single tissue compartment model as proposed and validated by Kudomi et al. (27;28)

\[
\frac{dC_l(t)}{dt} = F_a C_l(t) + F_p C_p(t) - \frac{F_a + F_p}{V_T} C_l(t)
\]

(1)

Here, C_l(t), C_a(t) and C_p(t) represent liver, arterial and portal venous blood time-activity curves, respectively, F_a and F_p arterial and portal venous perfusion, respectively, and V_T the partition coefficient of water in liver. The model assumes that C_l(t) can be described as a delayed and dispersed version of C_l(t) following passage through a notional gut compartment:

\[
C_l(t) = k_g C_l(t - \Delta t) \otimes e^{-k_g t}
\]

(2)

Finally, delay Δt, dispersion constant k_g, V_T, F_a, F_p, and fractional hepatic blood volume V_p were determined by non-linear regression using the following operational equation in which the right hand side of eq (2) was substituted for C_l(t):

\[
C_l(t) = (1 - V_p)(F_a C_a(t) + F_p C_p(t)) \otimes e^{-k_g t} + V_p \left( \frac{F_a C_a(t) + F_p C_p(t)}{F_a + F_p} \right) e^{-k_g t}
\]

(3)
Plasma and tissue time activity curves for $^{18}$F-FDG and $^{11}$C-palmitate were quantified using Patlak graphical analysis, as previously described(18-20) and validated in a pig model(16) in this analysis, a graph is produced by plotting $C_l(t)/C_r(t)$ against $[C_l(t)]_p/C_r(t)$, where $C_l(t)$ and $C_r(t)$ are liver and arterial plasma time-activity curves, respectively. The model presupposes irreversible tracer kinetics and, following exclusion of the first few minutes when there is no equilibrium, a linear relationship is obtained. The hepatic influx rate constant ($K$) is then derived from the slope of a linear fit of the latter part of this plot (10-60 minutes). Hepatic glucose uptake (HGU) was calculated by multiplying $K$ with the plasma glucose concentration. Under hyperinsulinemic conditions, as used in the present study, hepatic glucose output and dephosphorylation of FDG-6-phosphate are considered to be essentially absent(21) and reflux will be minimal. Nevertheless, in order to account for reversible tracer uptake, data were additionally analyzed by introducing a rate constant parameter ($K_i$), accounting for tracer outflow as previously described.(21) The $K$ of $^{11}$C-palmitate was not multiplied by fasting fatty-acid levels, as these may not accurately reflect portal vein concentrations, hence only $K$ is provided. Patlak analysis of $^{11}$C-palmitate was confined to the interval from 3 to 10 minutes after tracer injection, as a previous study in the liver has shown that labeled triglyceride metabolites of $^{11}$C-palmitate become detectable after 10 minutes.(35) Although for this time interval no correction for labeled triglycerides was necessary, a correction of $^{11}$C-palmitate IDIFs for $^{11}$C-CO$_2$ was still performed, as described elsewhere.(29;34) In addition, the validity of using the Patlak method for analyzing $^{11}$C-palmitate data was assessed using spectral analysis. (36) Spectral analysis allows for 1) assessment of the number of tissue compartments identifiable in the data, and 2) whether these compartments represent irreversible or reversible tracer kinetics, without prior assumptions about the underlying tracer kinetics. Use of this approach showed the validity of the Patlak method, as in all scans only one irreversible compartment was detected for the time interval selected (data not shown).

**Biochemical analyses**

Samples were analyzed at one certified central laboratory (Amsterdam). Plasma glucose was quantified using a hexokinase based technique (Roche Diagnostics, Mannheim, Germany). HbA$_c$ was determined by high-performance liquid chromatography (HPLC; Menarini Diagnostics, Florence, Italy; reference values: 4.3-6.1%). Plasma triglycerides, total cholesterol and high-density lipoprotein cholesterol were determined using enzymatic colorimetric methods (Modular, Hitachi, Japan). Levels of low-density lipoprotein cholesterol were calculated using Friedewald’s formula (reference values: 2.0-4.6 mmol/l). Plasma free-fatty acids were measured by an enzyme-linked immunoabsorbent assay (Wako Chemicals, Neuss, Germany). Plasma insulin levels were quantified by an immunoradiometric assay (Bayer Diagnostics, Midrrecht, The Netherlands). Ultra-sensitive C reactive protein (us-CRP) was determined by ELISA (DSL, Webster, Texas, USA). The sensitivity was 1.6 μg/l and the interassay coefficient of variation (CV) ranged from 3 to 5%. In duplo determinations of plasma malondialdehyde, a marker of oxidative stress, were performed by HPLC after alkaline hydrolysis and reaction with thiobarbituric acid.(37) The intra-assay CV was 5.7%.

**Statistical analyses**

Values are expressed as mean±standard error or as median (interquartile range) in case of skewed distribution. Non-normally distributed data were log-transformed. Comparisons between controls, T2DM-low and T2DM-high patients were performed using Analysis of Variance (ANOVA), including the Bonferroni posthoc multiple comparisons test. Pearson’s and Spearman’s (where appropriate) univariate correlation coefficients were calculated and linear regression was used to control for covariates. Statistical analysis was performed using SPPS for Windows version 15.0 (SPSS Inc., Chicago, IL, USA). A two-tailed probability value <0.05 was considered significant.
Results

The $^1$H-MRS protocol was successfully completed in all participants. Due to technical reasons, 4 ($^{2}$H$_2$O, 2 $^{15}$F-FDG and 12 $^{13}$C-palmitate scans in T2DM patients were not available for analysis and 1 $^{15}$O$_2$O and 2 $^{13}$C-palmitate scans in healthy controls.

Subject characteristics

Baseline characteristics of patients, categorized according to liver fat content, and controls are listed in Table 1. All groups were similar with respect to age, and both T2DM groups had comparable disease duration and medication use. As expected, anthropometric and hemodynamic parameters (which were all in the normal range) differed significantly among groups. Plasma lipid profiles and liver enzymes were different between groups (Table 1). Metabolic characteristics under postabsorptive and hyperinsulinemic conditions are displayed in Table 2 and showed differences between groups. Plasma fatty-acids (postabsorptive state) and plasma lactate (hyperinsulinemia), however, were similar between groups.

Table 1. Subjects characteristics

<table>
<thead>
<tr>
<th>Demography</th>
<th>Controls</th>
<th>T2DM-low</th>
<th>T2DM-high</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>54.7 ± 1.3</td>
<td>57.1 ± 0.9</td>
<td>56.8 ± 1.0</td>
<td>0.304</td>
</tr>
<tr>
<td>Time since diagnosis of diabetes, yrs</td>
<td>NA</td>
<td>4 (2-8)</td>
<td>4 (3-5)</td>
<td>0.426</td>
</tr>
<tr>
<td>Anthropometry and hemodynamics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m$^2$</td>
<td>27.3 ± 0.6</td>
<td>26.7 ± 0.5</td>
<td>30.0 ± 0.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Body surface area, m$^2$</td>
<td>2.1 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>2.1 ± 0.1</td>
<td>0.085</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>102 ± 2</td>
<td>99 ± 2</td>
<td>107 ± 2</td>
<td>0.005</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>118 ± 2</td>
<td>124 ± 2</td>
<td>130 ± 2</td>
<td>0.002</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>72 ± 2</td>
<td>73 ± 1</td>
<td>78 ± 1</td>
<td>0.004</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>56 ± 2</td>
<td>64 ± 2</td>
<td>66 ± 1</td>
<td>0.001</td>
</tr>
<tr>
<td>Metabolic characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA$_1c$, %</td>
<td>5.4 ± 0.1</td>
<td>7.0 ± 0.2</td>
<td>7.3 ± 0.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>5.0 ± 0.2</td>
<td>4.3 ± 0.1</td>
<td>4.5 ± 0.2</td>
<td>0.006</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/l</td>
<td>3.2 ± 0.1</td>
<td>2.6 ± 0.1</td>
<td>2.7 ± 0.7</td>
<td>0.007</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
<td>1.24 (1.10-1.63)</td>
<td>1.05 (0.85-1.29)</td>
<td>0.96 (0.82-1.09)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>0.8 (0.6-1.2)</td>
<td>1.1 (0.8-1.6)</td>
<td>1.8 (1.2-2.3)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ALT, U/l</td>
<td>25 (18-33)</td>
<td>26 (21-33)</td>
<td>37 (30-51)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AST, U/l</td>
<td>24 (20-30)</td>
<td>28 (21-36)</td>
<td>28 (24-38)</td>
<td>0.139</td>
</tr>
<tr>
<td>γ-GT, U/l</td>
<td>23 (17-29)</td>
<td>23 (18-37)</td>
<td>42 (35-48)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>usCRP, mg/l</td>
<td>3.0 (1.7-6.3)</td>
<td>2.9 (1.6-4.5)</td>
<td>4.7 (3.5-6.8)</td>
<td>0.007</td>
</tr>
<tr>
<td>Malondialdehyde, μmol/l</td>
<td>6.0 ± 0.1</td>
<td>9.7 ± 0.5</td>
<td>10.0 ± 0.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Medications – % (n/N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>NA</td>
<td>35 (8/23)</td>
<td>47 (17/36)</td>
<td>0.423</td>
</tr>
<tr>
<td>Any antihypertensive medication</td>
<td>NA</td>
<td>35 (8/23)</td>
<td>47 (17/36)</td>
<td>0.423</td>
</tr>
</tbody>
</table>

Note: Data are mean ± SE, median (IQR), N.A., not applicable. T2DM-low = T2DM patients with liver triglyceride content ≤ 8.6 %, T2DM-high = T2DM patients with liver triglyceride content > 8.6 %, # = significant difference between controls and T2DM-low, § = significant difference between controls and T2DM-high, $ = significant difference between T2DM groups. HbA$_1c$ = glycated hemoglobin. LDL = low-density lipoprotein. HDL = high-density lipoprotein. ALT = alanine aminotransferase. AST = aspartate aminotransferase. γ-GT = γ-glutamyl trasnferase. usCRP = ultra sensitive C-reactive protein.
Table 2. Metabolic characteristics in controls and type 2 diabetic patients with low and high hepatic triglyceride content

<table>
<thead>
<tr>
<th>Metabolic characteristic (fasting state)</th>
<th>Controls</th>
<th>T2DM-low</th>
<th>T2DM-high</th>
<th>P value ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose, mmol/l</td>
<td>5.2 (4.9-5.4)</td>
<td>8.3 [6.7-10.1] *</td>
<td>8.0 (7.1-8.7) *</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Plasma non-esterified fatty acids, umol/l</td>
<td>470 (360-540)</td>
<td>450 (410-570)</td>
<td>500 (370-590)</td>
<td>0.624</td>
</tr>
<tr>
<td>Plasma lactate, mmol/l</td>
<td>0.8 (0.7-0.9)</td>
<td>1.1 (0.9-1.3) *</td>
<td>1.2 (1.0-1.5) *</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Plasma insulin, pmol/l</td>
<td>28 (19-33)</td>
<td>39 (28-62) *</td>
<td>78 (62-99) *</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

| Metabolic characteristics (hyperinsulinemic state) | | | |
|-----------------------------------------------------|----------|----------|-----------|--------------|
| Plasma non-esterified fatty acids, umol/l           | 40 (20-48) | 50 (30-85) * | 115 (70-173) * | < 0.001 |
| Plasma lactate, mmol/l                              | 1.1 (0.9-1.3) | 1.0 (0.9-1.2) | 1.1 (1.0-1.4) | 0.560 |
| Plasma insulin, pmol/l                              | 511 ± 67 | 513 ± 24 | 643 ± 26 * | < 0.001 |
| M/I value, mg/(kg·min)/(pmol/l)                     | 1.13 (0.73-1.66) | 0.68 (0.46-1.0) * | 0.37 (0.17-0.45) * | < 0.001 |
| Insulin clearance rate, ml/min                      | 1101 (1017-1270) | 1029 (951-1262) | 945 (816-1053) | < 0.001 |
| Post-hepatic insulin delivery rate, pmol/min        | 29 (21-40) | 48 (30-77) * | 78 (56-97) * | < 0.001 |

NOTE: Data are mean ± SE, median [IQR]. T2DM-low = T2DM patients with liver triglyceride content ≤ 8.6 %, T2DM-high = T2DM patients with liver triglyceride content > 8.6 %, # = significant difference between controls and T2DM-low. * = significant difference between T2DM-low and T2DM-high; M value = whole body insulin sensitivity. M/I value = M value adjusted for insulin during the steady state.

Hepatic and abdominal fat

T2DM-high patients had, compared with T2DM-low patients and controls, the highest hepatic triglyceride content: 21.6 (12.9-29.4) versus 2.6 (1.5-5.2) and 2.5 (1.0-4.2) %, respectively. ANOVA P<0.001. Figure 1 shows a representative MRI image and spectrogram. Subcutaneous and visceral fat volumes were statistically different between groups (736±47 versus 572±39 and 598±52 ml, P=0.020) and (440 (333-578) versus 318 (248-404) and 264 (203-340) ml, P<0.001), respectively.

Hepatic parenchymal perfusion and substrate uptake

Figure 2 shows representative OSEM (summed) reconstructed PET images of the liver for [11C]palmitate, [18F]FDG and fits on the data for the respective tracers. T2DM-high patients had, compared with T2DM-low patients and controls, the lowest hepatic perfusion (0.647±0.038 versus 0.795±0.042 and 0.850±0.047 ml·min⁻¹·cm⁻³, ANOVA P=0.004, Figure 3A). T2DM-high patients had, compared with T2DM-low patients and controls, the lowest insulin mediated HGU (20.4±1.9 versus 24.12±1.1 and 30.7±3.0 μmol·min⁻¹·cm⁻³, respectively, P=0.013. Figure 3B). No tracer loss from the liver could be detected during scan time. The mean hepatic fatty-acid influx rate constant (Figure 3C) was lower in T2DM-high patients compared with T2DM-low patients and controls, but only reached borderline significance (P=0.088).
Figure 1. Reconstructed PET images of the liver and Patlak plots

Representative images of OSEM (summed) reconstructed PET images of the liver with $^{11}$C-palmitate (A) and $^{18}$F-FDG (B) with ROIs used for analysis indicated. Images show uptake in the liver on the left and uptake in the heart on the upper-right. Time course of $^{18}$O$\text{H}_2$O concentration (C) in the liver (circles), with hepatic perfusion model fit (straight line). Patlak plots of $^{11}$C-palmitate (D) and $^{18}$F-FDG (E) data, respectively. The first couple of dots in Figure 2 D&E were excluded from the analysis. The slope of the linear fits equals the net rate of influx $K_i$. Note the correspondence between the respective $K_i$ values and the uptake seen in figures A and B, which is much higher for $^{11}$C-palmitate (fasting state) than for $^{18}$F-FDG (hyperinsulinemic state).

Figure 2. Hepatic perfusion and metabolism

Hepatic perfusion (A), hepatic glucose uptake (HGU) (B), hepatic fatty-acid influx rate constant (C), in controls (□), T2DM patients with low liver triglyceride content (■) and T2DM patients with high liver triglyceride content (○). $K_i$ = hepatic influx rate constant. P values are from Bonferroni post-hoc analysis. For P ANOVA see text.
Correlations between hepatic fat content, parenchymal perfusion and substrate uptake

In a pooled analysis, hepatic triglyceride content correlated inversely with hepatic perfusion (r=-0.402, P=0.001; Figure 3A) and hepatic fatty-acid influx rate constant (r=-0.335, P=0.004; Figure 3B), which both remained significant after correction for diabetic status, HbA1c, BMI, visceral fat content, plasma fatty-acid and lactate levels. Hepatic triglyceride content also correlated inversely with HGU (r=-0.329, P=0.004; Figure 3C), which remained significant after correction for diabetic status, HbA1c, BMI and visceral fat content, but not when additionally correcting for plasma fatty-acid or lactate. Hepatic triglyceride content, but not hepatic perfusion or hepatic fatty-acid influx rate constant, were correlated with M/I value (r=-0.684, P<0.001), malondialdehyde (r=0.427, P<0.001), usCRP (r=0.326, P=0.005), insulin clearance rate (r=0.459, P<0.001), visceral (r=0.612, P<0.001) and subcutaneous fat volume (r=0.392, P<0.001). The hepatic glucose influx rate constant correlated inversely with plasma fatty-acid levels (r=-0.246, P=0.036), HbA1c (r=-0.310, P=0.007) and malondialdehyde (r=-0.434, P<0.001).

In T2DM patients alone, hepatic fat content correlated inversely with hepatic perfusion (r=-0.360, P=0.007) and hepatic fatty-acid influx rate constant (r=-0.407, P=0.007), whereas borderline significant associations were found with HGU (r=-0.245, P=0.057). Hepatic fat content, but not hepatic perfusion or hepatic fatty-acid influx rate constant, correlated with M/I value (r=-0.657, P<0.001) and usCRP (0.375, P=0.005), insulin clearance rate (r=-0.436, P=0.001), visceral (r=0.540, P<0.001)
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and subcutaneous fat ($r=0.375$, $P=0.003$) volumes. The hepatic glucose influx rate constant rate, inversely correlated with malondialdehyde ($r=-0.380$, $P=0.004$) and borderline with plasma fatty-acids ($r=-0.251$, $P=0.059$). None of these correlations were observed in controls alone.

Discussion

Using MRS and PET in the same patients, the present study provides evidence for a potential modulating effect of hepatic fat content on hepatic physiology in T2DM patients. Reduced hepatic parenchymal perfusion, insulin-mediated HGU and a borderline decrease in hepatic fatty-acid influx rate constant were observed in T2DM patients with increased hepatic triglyceride content. Moreover, hepatic triglyceride content was directly and inversely related to hepatic perfusion, hepatic glucose and fatty-acid metabolism.

Hepatic fat content and relationship with hepatic parenchymal perfusion

Although flow through portal vein and hepatic artery is readily accessible using Doppler sonography,(22;23) in vivo studies on human hepatic (parenchymal) perfusion are limited due to the often (highly) invasive methodology required. Indirect methods for measuring hepatic blood flow have been used and include the assessment of clearance or dilution of a dye or marker (gas or microspheres), which have a wider range of clinical applicability than the direct methods.(38) Moreover, non-invasive measurements of hepatic perfusion using PET with the freely diffusible flow tracer [15O]H2O have been shown to provide reliable estimates of hepatic blood flow, when taking into account the dual input from hepatic artery and vena porta.(27;28) In the present study decreased hepatic parenchymal perfusion was observed in T2DM patients with increased liver triglyceride content, but not in those T2DM patients with low liver triglyceride content, as compared with controls, implying a potential modulating effect of liver fat per se.

These results extend data from previous studies suggesting a modulating effect of increased hepatic fat content on hepatic blood flow velocity and perfusion. It has been shown that the level of fatty infiltration in humans alters portal vein hemodynamics in a graded way.(22;23) Especially under stress conditions, such as during ischemia-reperfusion or transplantation, the fatty liver has shown decreased adaptability and hence increased risk of failure.(39) In addition to changes in hepatic macrocirculation, alterations in the hepatic microvasculature have been implicated. In steatotic livers of human donors, laser Doppler flowmetry revealed a significant decrease in hepatic parenchymal perfusion.(24) In New Zealand white rabbits with diet-induced hepatic steatosis, Seifalian et al(25) found that graded steatosis progressively reduced hepatic blood flow velocity and hepatic parenchymal perfusion. Moreover, they observed an inverse correlation between degree of fat in filtration and both total hepatic blood flow and the hepatic parenchymal perfusion was found, with the biggest effect on the latter.

The mechanisms by which increased liver fat affect hepatic perfusion include factors like structural changes in the liver, a microvascular inflammatory response and possibly vascular insulin resistance. Experimental studies in several animal models of diet and genetically induced hepatic steatosis have shown that reductions in sinusoidal perfusion are initially due to enlarged hepatic parenchymal cells overloaded with lipids.(40-43) Consequently, parenchymal cell plates become wider, which results in narrowing and deformation of the lumen of sinusoids, reducing their volume. This eventually leads to sinusoidal dysfunction and impaired hepatic perfusion.(42) Increased leukocyte adherence to endothelial cells, expression of adhesion molecules and upregulation of NF-kappaB
have been shown to promote reactive oxygen species (ROS) generation, with subsequent inflammation and formation of vasoactive metabolites, all of which may be implicated in decreased hepatic parenchymal perfusion. Moreover, insulin resistance, one of the hallmarks of T2DM pathology and strongly associated with hepatic steatosis, may additionally decrease hepatic microcirculatory flow by impaired insulin receptor signalling via the PI3-kinase/Akt/eNOS cascade, which in turn may result in decreased nitric oxygen (NO) synthesis by endothelial cells and hence decreased NO-mediated vasodilation. In addition, stimulated signaling through the insulin-receptor mediated MAPK/ERK pathway may additionally favor vasoconstriction and abnormal angiogenesis, contributing to impaired microvascular hepatic perfusion. Although in the present study no direct relationships were found between hepatic parenchymal perfusion and whole-body insulin sensitivity, oxidative stress or usCRP, the hepatic parenchymal perfusion was inversely correlated with hepatic fat content. Therefore, more studies are warranted to further explore these interrelations.

Hepatic triglyceride content and relationship with substrate metabolism

Interestingly, only a borderline significant difference was found in the fasting hepatic fatty-acid influx rate constant across groups, caused by the lower uptake rate in T2DM-high, but not T2DM-low patients. Depending on the condition, fatty-acid extraction or uptake has been reported to be unaltered (13;14), decreased (15;19) or increased.(14,47) Using PET with the fatty-acid analogue tracer 14(R,S)-3H-fluoro-6-thia-heptadecanoic acid, Loizzo et al found decreased fatty-acid extraction in 10 fasting patients with impaired glucose tolerance (IGT) compared with 8 healthy controls. These findings were primarily explained by reverse substrate competition, as plasma glucose sampled from arterialized blood correlated inversely with fatty-acid uptake. In the present study, during the [13C]palmitate PET scan, only venous sampling was performed and hence this relation could not be tested reliably.

In the present study previous findings were confirmed, indicating that both T2DM and liver fat content are inversely related to insulin-stimulated hepatic glucose-uptake. Hepatic glucose influx and output are directly regulated by insulin through several enzymes. Insulin stimulates the up-regulation of glucokinase and glycogen synthase and conversely inhibits glucose-6-phosphatase and glycogen phosphorylase in hepatocytes. In hepatic insulin resistance, impaired activity of these key enzymes may therefore lead to decreased insulin-stimulated HGU. An indirect mechanism underlying the negative relationship between liver fat and HGU may be increased fatty-acid fluxes related to increased lipolysis from insulin-resistant adipose tissue. The inverse association between plasma fatty-acids and HGU rate is in line with this assumption. Furthermore, other studies have shown that a combined intralipid/heparine infusion increased plasma fatty-acids and reduced splanchnic and peripheral glucose uptake in T2DM patients. Moreover, although the present study is aimed at HGU, it should be mentioned that hepatic glucose uptake only constitutes a small percentage of net change in glucose metabolism during the clamp. Finally, the liver is the main site involved in insulin clearance and degradation. Recently, Kotro-Nen et al(11) found that increased hepatic fat was associated with impaired insulin clearance in 80 nondiabetic subjects. The present inverse relationship between liver fat content and insulin clearance is in line with those results.
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Limitation

In the present study, we used 1H-MRS to measure hepatic triglyceride content. To that purpose, only three MR slides of the liver were made for voxel localization and hence total liver volume could not be calculated. Thus the study’s conclusions are limited to liver tissue studied within the volume of the voxel. Although liver volume was probably increased in the T2DM-high group, the effect of an increased liver volume on our findings cannot be established. From animal studies, however, it seems less likely that an increase in liver volume substantially influenced our findings (25;52). In addition to a decrease in the total number of hepatocytes, many structural changes in the fatty liver may negatively influence hepatic metabolism and parenchymal perfusion.

Conclusion

T2DM patients with high liver triglyceride content have a poorer metabolic profile than age-matched controls and T2DM patients with a liver triglyceride content in the normal range. In addition, T2DM patients with high liver triglyceride content show decreased hepatic parenchymal perfusion and insulin-mediated glucose uptake. Finally, hepatic triglyceride content is inversely related to hepatic parenchymal perfusion, HGU and hepatic fatty-acid influx rate constant suggesting a potential modulating effect of hepatic fat on hepatic physiology.
Liver fat content in type 2 diabetes: relationship with hepatic perfusion and substrate metabolism

Reference List


