At the heart of the matter

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Imaging cardiac metabolism in insulin resistance

Vera de Wit-Verheggen

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Veur mam

Omdat jouw liefde en steun onvoorwaardelijk waren

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CHAPTER 1

General introduction and outline

Diabetic cardiomyopathy

The prevalence of obesity and type 2 diabetes mellitus (T2DM) is still increasing worldwide and is a major public health problem. The related comorbidities, such as cardiovascular disease (CVD), have a huge impact on quality of life and drive-up health care costs (1, 2). CVD is the leading cause of death globally and death rates are about 1.7 times higher amongst diabetic patients compared to non-diabetic patients (3). Even in the prediabetic state, there is an association between fasting blood glucose and the risk of CVD (4, 5). This increased prevalence of CVD is mainly caused by atherosclerosis, induced by the many risk factors that are characteristic for obese and diabetic patients, such as dyslipidemia and hypertension (6-12). Atherosclerosis leading to ischemic heart disease is already extensively discussed in previous literature (13). Though importantly, even when corrected for atherosclerosis, cholesterol values, weight, blood pressure and age, patients with T2DM remain at an increased risk for developing cardiac failure, mainly by suffering from diastolic dysfunction (14). Diastolic dysfunction is defined as abnormal relaxation of the myocardium and may be present years before symptoms occur. This phenomenon has also been described as diabetic cardiomyopathy (DCM) (15). Even in subjects with prediabetes, higher glucose levels were associated with lower cardiac function parameters (16). In fact, when patients with T2DM develop CVD, they have a worse prognosis than CVD patients without T2DM (6).

Although the exact etiology of DCM is unknown, changes occur already during the development of prediabetes, which is typically accompanied by insulin resistance at multiple levels. One of the metabolic changes during the development of insulin resistance and diabetes is a reduction in oxidative capacity (17). While insulin resistance blunts glucose uptake and glucose oxidation in the postprandial state, it is typically associated with a blunted glucose oxidation and increased fat oxidation rate in the fasted state (18, 19). This increased fatty acid oxidation increases oxygen demand, compared to an equal amount of glucose substrates, at the expense of myocardial efficiency making the heart more prone for ischemia.

Since mitochondria are responsible for oxidative metabolism, they are key to a normal function of cardiomyocytes. A low mitochondrial respiration has been reported in cardiomyocytes upon heart failure (20-22), ischemic heart disease (23), atrial fibrillation (24), and T2DM (25-27). A possible explanation for the hampered mitochondrial function may be found in mouse studies, which showed that a high abundance of fatty acids may lead to inefficient substrate oxidation

in the heart (reflected by a reduced ratio of energy production (ATP production) to respiration), resulting in the formation of reactive oxygen species and thereby to mitochondrial damage (26, 28-30). Possibly, a similar mechanism occurs in the human heart in the prediabetic state which ultimately results in mitochondrial dysfunction and reduced ATP synthesis (31). However, human data on cardiac mitochondrial function is scarce, because the gold standard to determine mitochondrial function requires fresh biopsy material for high resolution respirometry measurements.

In addition to metabolic changes in the myocardium, also adipose tissue around the heart has been reported to undergo changes in obesity and T2DM, and have been implicated in decreased cardiac function. Increased pericardial fat (PF) volume is associated with adverse CVD outcomes. Here fore, the relationship and the potential effects of PF on cardiac dysfunction (32, 33) has gained a lot of attention. A proportion of the PF is called the epicardial adipose tissue (EAT) ,which is located between the myocardium and visceral pericardium. When EAT expands during the development of obesity, it may have detrimental effects on cardiac function, specifically on diastolic function.

In view of all these metabolic derangements in the onset of obesity and T2DM contributing to the development of DCM, further research to gain insights in the pathogenesis of DCM is needed. In addition, it may be beneficial to intervene and to counterbalance the altered substrate metabolism in prediabetes and T2DM. PPARα regulates many genes involved in mitochondrial function and fat metabolism. On a whole-body level, activation by PPARα ligands increases HDL cholesterol and stimulates free fatty acid (FFA) uptake and enhances FFA oxidation in the liver and skeletal muscle and therefore diminishes the FA pool to be incorporated into TG-rich lipoproteins (34-36). PPARα agonists are therefore currently used as drugs to treat dyslipidemia and, in particular, hypertriglyceridemia. Activation of PPARα in the liver has effects on liver FA turn-over and metabolism. These effects may be beneficial, since ectopic fat accumulation in the liver is associated with insulin resistance on hepatic and whole-body level (37). Activation in the heart may have beneficial effects on mitochondrial function and fat oxidative capacity, but this has not yet been investigated in the human heart. Due to their non-invasiveness, advanced imaging methodologies can be used to investigate metabolic processes in DCM in more detail.

Advanced imaging methodologies to assess cardiac metabolism in human

Non-invasive imaging techniques, such as magnetic resonance spectroscopy (MRS) can be applied to acquire metabolic information *in vivo*. MRS yields chemical information of tissues, thereby making it a useful technique to study substrate stores and dynamics in different organs. Therefore, MRS has been shown to be of great value in determining cardiac energy metabolism. Using Phosphorus MRS (^{31}P-MRS), spectra containing signals of high-energy metabolites, containing phosphorus atoms in the cardiac muscle can be acquired. Cardiac ^{31}P-MRS as a quantitative technique was validated in the past in animal studies (38). The most abundant phosphorous containing metabolites in the human heart are adenosine triphosphate (ATP) and phosphocreatine (PCr). Since PCr is buffering ATP concentration when ATP demand is increased, the PCr/ATP ratio reflects the myocardial energy status. Furthermore, ATP production in the heart is almost entirely driven by mitochondrial oxidative metabolism, therefore a low PCr/ATP ratio may be a marker of compromised mitochondrial function in cardiac tissue. Indeed, some studies have shown that participants with T2DM (39-42) and heart failure (43) have reduced cardiac PCr/ATP ratios compared to healthy controls. However, whether this measurement is a good reflection of *in vivo* mitochondrial function is so far unknown. Hence, more knowledge upon mitochondrial function is warranted in order to gain more insight into the role of mitochondrial dysfunction in cardiac pathologies.

Using Positron Emission Tomography (PET), we can study substrate uptake and oxidation in detail. With the glucose analogue [^{18}F]-fluorodeoxyglucose (^{18}F-FDG) PET studies can give insight in the myocardial uptake of glucose. It was shown that a lower myocardial glucose uptake correlates with decreased diastolic function (44). However, studies measuring the influx of glucose during insulin stimulation to measure tissue-specific insulin sensitivity are scarce for prediabetic populations. Therefore, presently, little is known about the glucose dynamics in prediabetic humans, even though it may be highly relevant in the development of DCM. Advanced MRS and PET imaging techniques were applied in the current thesis to investigate cardiac substrate metabolism and to better understand the metabolic changes that take place during the development of DCM.

Thesis outline

First, current literature on the changes in cardiac metabolism in prediabetes are reviewed in **chapter 2**. In **chapter 3** we studied the importance of adipose tissue accumulation around the heart in a healthy population, since it is known that the pericardial fat (PF) is strongly intertwined with cardiac function in both obese subjects and subjects with T2DM (45-47), although the underlying mechanisms remain unknown (48-51). Herefore, a large cohort study was used as a tool to explore possible correlations between diastolic cardiac function and PF.

In **chapter 4** we aimed to validate PCr/ATP as assessed by ^{31}P-MRS as a measure of cardiac mitochondrial function in humans. To this end, we compared cardiac energy status (^{31}P-MRS) with *ex vivo* measurements of mitochondrial function by high resolution respirometry in metabolically compromised patients scheduled to undergo cardiothoracic surgery, in order to test the hypothesis that cardiac energy status reflects mitochondrial function.

In literature, PCr/ATP ratios were shown to be negatively correlated with fasting plasma FFA concentrations (41, 52) and to be lower in T2DM. Currently, it is unknown whether PCr/ATP is also low in prediabetes. As in prediabetes FFA in the morning are elevated, a lowered PCr/ATP ratio in prediabetes may be expected, as a hallmark of metabolic changes in the heart in the prediabetic state. This hypothesis was tested in **chapter 5** in prediabetic and healthy overweight volunteers. We determined PCr/ATP in prediabetic volunteers in the fasted state and later during the day when FFA concentrations are typically normalized.

Chapter 6 focusses on the importance of PPARα in cardiac metabolism. Results from animal studies suggest that PPARα agonists can have metabolically beneficial effects, counteracting the negative effects of overweight, however, human data is largely lacking. We evaluated the effects of the PPARα agonist ciprofibrate on cardiac metabolism in prediabetic patients in a randomized cross-over trail.

Finally, in **chapter 7** the main results and conclusions of the previous chapters in this thesis are discussed in a broader perspective. Furthermore, future directions in the field of cardiac metabolism are discussed.

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CHAPTER 2

Changes in cardiac metabolism in prediabetes

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Abstract

In type 2 diabetes mellitus (T2DM) there is an increased prevalence of cardiovascular disease (CVD), even when corrected for atherosclerosis and other CVD risk factors. Diastolic dysfunction is one of the early changes in cardiac function that precedes the onset of cardiac failure and it occurs already in the prediabetic state. It is clear that these changes are closely linked to alterations in cardiac metabolism, however the exact etiology is unknown. In this narrative review, we give an overview of the early cardiac changes in fatty acid and glucose metabolism in prediabetes and its consequences on cardiac function. A better understanding of the relationship between metabolism, mitochondrial function and cardiac function will lead to insights into the etiology of the declined cardiac function in prediabetes.

1. Introduction

Prediabetes, defined as impaired fasting glucose (fasting plasma glucose between 6.1 and 6.9 mmol/l) or impaired glucose tolerance (2-h plasma glucose between 7.8 and 11.0 mmol/l) (1), places individuals at high risk of developing type 2 diabetes mellitus (T2DM) and its cardiovascular disease (CVD)-related complications (2, 3). The increased risk of CVD is proportional to the fasting blood glucose in prediabetes (2, 4, 5) and is mainly caused by atherosclerosis, induced by the many risk factors that are characteristic for prediabetic patients, like for instance dyslipidemia and hypertension (6-12). Atherosclerosis leading to ischemic heart disease has been extensively discussed in previous literature (13). However, even when corrected for atherosclerosis, cholesterol values, bodyweight, blood pressure, and age, patients with prediabetes remain at increased risk for the development of heart failure, mainly through the development of diastolic dysfunction (in T2DM known as diabetic cardiomyopathy (DCM)) (14, 15). This phenomena is also part of the spectrum better known as heart failure with a preserved ejection fraction (HFpEF) (16).

Interestingly, diastolic dysfunction has been shown to be present not only in T2DM but also in prediabetes (17). Evidence associates higher glucose levels with lower cardiac function parameters in prediabetes (17), indicating that changes in cardiac function arise early in the development of T2DM. Changes in cardiac metabolism in response to hyperglycemia are considered to be an important pathway through which T2DM causes DCM (18, 19), and this may already be at play in prediabetes. Recognition of these metabolic changes may help to better understand the underlying etiology of diastolic dysfunction in prediabetes, which provides a window of opportunity for the prevention of DCM in the early development of T2DM. This narrative review will therefore only focus on the possible cardiac metabolic mechanisms behind the declined cardiac function in prediabetes (irrespective of their CVD risk profile), as these changes precede the onset of DCM. We will not discuss other possible pathways such as oxidative stress and inflammation, nor DCM (20-22), which have already been extensively discussed in the literature.

2. Cardiac fat

When energy intake exceeds expenditure it eventually results in body fat accumulation as can be seen in obesity (23). The large surplus of nutrients also leads to the development of fat deposits in organs other than adipose tissue, such as skeletal muscle, the liver and the heart. Such ectopic lipid accumulation has been related to insulin resistance in many tissues. Thus, cardiac fat accumulation may play an important role in the development of diastolic changes in the prediabetic heart. Cardiac lipid content can be studied by both an *in vivo* and an *ex vivo* approach.

In vivo studies use magnetic resonance spectroscopy (MRS) for the relative quantification of metabolites involved in lipid metabolism. Proton MRS (^{1}H-MRS) generates a spectrum wherein multiple lipid signals (CH_2 and CH_3), a creatine (Cr) signal, and a water (H_2O) signal can be distinguished. CH_2/H_2O is generally used as a parameter that reflects myocardial triglyceride content, and this mainly represents neutral lipid storage as triglycerides in the myocardium. With this *in vivo* technique, it was shown that the myocardial triglyceride content is increased in overweight and obese individuals (24) and possibly even more in prediabetes and T2DM (25), in comparison to lean individuals. In addition, myocardial triglyceride content was weakly associated with insulin sensitivity, as determined by the homeostasis model assessment index (25).

Ex vivo studies found increased intramyocardial lipid deposition in patients with the metabolic syndrome (average HOMA score 4.2 ± standard deviation 0.5, which often is considered as prediabetes) (26), obesity or T2DM (27), compared to lean patients. Also, Anderson et al. showed that in nondiabetic and diabetic individuals the cardiac fat content correlated positively with the HbA1c (28). These *ex vivo* studies suggest that cardiac fat already in prediabetic individuals is increased compared to healthy lean individuals (26-28), which is in line with the above mentioned *in vivo* results of McGavock et al. (25).

Interestingly, several links between cardiac fat accumulation and cardiac function have been described. Van der Meer et al. showed that in lean individuals with a normal glucose metabolism (NGM) an increased myocardial triglyceride content (following a very low-calorie diet) measured by ^{1}H-MRS was correlated with a decrease in diastolic function (29). In addition, in individuals with the metabolic syndrome (and a high HOMA score) a correlation was found

between the amount of cardiac fat accumulation and the progression of cardiac dysfunction (measured by myocardial performance index and ejection fraction) (26). The increased myocardial triglyceride content in overweight and obese individuals was accompanied by elevated LV mass and suppressed septal wall thickening as measured by cardiac imaging, compared to lean individuals (24). This suggests that possibly in prediabetes an increased cardiac fat storage may influence cardiac function negatively.

3. Adipose tissue surrounding the heart

The fat deposits around the heart (epicardial adipose tissue and pericardial adipose tissue) also typically increase with overweight/obesity and are reported to be more pronounced in diabetic patients. These depots have not been measured specifically in prediabetic populations and although specific data of these separate depots in prediabetes is lacking, it might be expected that the epicardial adipose tissue (EAT) is elevated in prediabetes, since cardiac fat is increased in prediabetes and the thickness of the EAT is strongly correlated with the cardiac fat in healthy males (30). However, quantitative studies are needed to confirm this concept in prediabetic individuals.

From obese individuals it is known that when EAT expands, the balance between the storage and release of fatty acids shifts towards a more active secretion (31). Furthermore, the expanded EAT transforms its secretory profile towards more pro-inflammatory cytokines and chemokines, negatively affecting neighbouring cells (32-34). This results in a chronic inflammatory response which is shown to be present in enlarged EAT tissue (35, 36). Moreover, this local secretion of inflammatory mediators can also inhibit the activity of insulin. Indeed, EAT is positively associated with insulin resistance and the metabolic syndrome (37, 38).

Literature shows that the expansion of EAT has a negative influence on cardiac function (39-41). Although studies on EAT are lacking in prediabetes, this unfavourable effect of increased EAT on function parameters seems to a general phenomenon and was reported in lean, obese, and T2DM individuals. First of all, pericardial fat thickness, measured from the long axis view, is shown to be a predictor of the mobility of the lateral left ventricle wall, known as e' lateral (39). Secondly, the thickness of EAT in morbidly obese individuals is associated with enlarged atria and impaired diastolic filling of the right and left ventricle (42). This is in line with the findings in a healthy population with on average a normal BMI but with a high prevalence of

the metabolic syndrome and T2DM, wherein PF volume was correlated with left atrial diameter and with E/e' (41). In morbidly obese female individuals the adipose tissue volume around the left ventricle did not only correlate with diastolic function parameters (peak early filling velocity (E) and peak late filling velocity (A)), but also with several left ventricular hemodynamic measurements including cardiac output and stroke volume (40). Furthermore, EAT is associated with left ventricular mass (LVM), which is a strong predictor of adverse cardiovascular outcomes (31, 41).

The association between EAT and cardiac function may be explained by several mechanisms. Firstly, EAT is a storage depot for FFA, and may thus provide the heart with nutrients (32), therewith contributing to the changed cardiac lipid metabolism. Secondly, the chronic inflammatory response which is shown to be present in enlarged EAT tissue (35, 36) and the inflammatory cytokines produced by EAT may act locally as paracrine atherogenic factors (32). Finally, mechanistic hindrance may limit the distensibility of the myocardium (43).

As studies on the relationship of EAT with changes in cardiac metabolism and function are lacking in prediabetes, more research is warranted. Especially since it is known that EAT is more flexible and reduces even before the cardiac fat decreases (44). Possibly, EAT contributes to diastolic dysfunction in prediabetes, however to what extend remains to be elucidated.

4. Enhanced cardiac lipid metabolism

Insulin usually inhibits lipolysis and reduces thereby the release of plasma non-esterified fatty acids (NEFAs). However, in individuals with reduced insulin sensitivity, as is the case in prediabetes, the postprandial effect of insulin is impaired, leaving the circulating free fatty acids (FFA) elevated (45). In addition to the increased FFA levels in the circulation, PET studies show that both the FFA uptake and the FFA oxidation in the prediabetic myocardium are increased. Using ^{18}F-Fluoro-6-Thia-Heptadecanoic Acid (FTHA) as a fatty acid tracer and [^{11}C]acetate to determine cardiac perfusion and oxidative metabolic index, Labbé et al. showed that in prediabetic individuals (defined as impaired glucose tolerance) an increased NEFA uptake in the heart and an increased myocardial oxidative metabolism for the first 6 hours postprandially compared to the individuals with a normal glucose metabolism (NGM) (46). This was in contrast to the uptake of fatty acids in liver and skeletal muscle, since these remained similar in prediabetes compared to NGM in the postprandial state (46). These findings

concerning increased FFA availability in the plasma and myocardial FFA metabolism are confirmed by Brassard et al. in normoglycemic first-degree relatives of T2DM individuals (who are therefore at highly increased risk to develop T2DM) in comparison to matched individuals having no increased risk for T2DM. Using the stable isotopic tracers ([1,1,2,3,3-$^{2}H_5$]-glycerol and [U-^{13}C]-palmitate or [1,2-^{13}C]-acetate), they showed that these individuals at high risk for T2DM during enhanced intravascular TG lipolysis at high insulin levels have both an increased plasma appearance of NEFAs and an increased myocardial oxidation of the NEFAs (45).

These findings in the insulin-stimulated condition from Brassard and Labbé point out that already in prediabetes, changes in cardiac fatty acid handling occur, with an increased uptake and oxidation of fatty acids in the heart in comparison to NGM. Moreover, Labbé et al. revealed that these changes in lipid metabolism may be maladaptive regarding cardiac function. The increased uptake and oxidation of NEFA in the prediabetic individuals was associated with a reduced left ventricular ejection fraction (LVEF), reduced left ventricular stroke volume, and tended to display impaired diastolic function (46). This is in line with the findings from Mather et al. in T2DM individuals, who showed that the augmented myocardial fatty acid oxidation under fasted and insulin-treated conditions (measured by16-[^{18}F]fluoro-4-thiapalmitate (FTP) and ^{11}C-acetate) was accompanied by reduced cardiac work efficiency (47). This may not be surprising since increased fatty acid oxidation at the expense of carbohydrate oxidation increases oxygen demand, resulting in reduced myocardial efficiency (47). In addition, in prediabetic individuals with a known increased risk for atherosclerosis this makes the heart more prone for ischemia. The enhanced fatty acid metabolism in prediabetes has therefore implications for contractile performance and ischemia tolerance (47).

It may be beneficial to counterbalance this altered substrate metabolism, in order to prevent DCM in T2DM. The metabolic changes that occur early on in the prediabetic heart seem to be reversible as shown by several studies in prediabetic individuals. Six months after bariatric surgery, individuals with prediabetes showed an improvement in whole-body insulin sensitivity which correlated positively with the decrease in myocardial fasting free fatty acid uptake, but also myocardial function. Although cardiac fat was not reduced, myocardial structure was improved (44). Similar results in prediabetes were observed by Labbé et al. where modest weight loss following a 1-year lifestyle intervention led to changes in substrate metabolism and improved cardiac function (48). However, a short-term diet of 7 days in prediabetes did not

achieve these improvements in cardiac function (49), and thus suggesting that structural changes regarding cardiac metabolism and function take longer to develop.

5. Decreased cardiac glucose metabolism

Together with alterations in cardiac fatty acid metabolism, reciprocal changes in cardiac glucose metabolism may be expected in prediabetes (50). Here, PET studies using the glucose analogue (^{18}F)-fluorodeoxyglucose [^{18}F-FDG] can give insight in the myocardial uptake of glucose. Kim et al. studied a mixed population of NGM, prediabetes, and T2DM and revealed that the visceral fat area and fasting FFA are independent determinants of myocardial glucose uptake in the fasted condition (51). However, both Kim et al. and Hu et al. showed that prediabetes was not associated with decreased myocardial glucose uptake in a fasted condition, whereas T2DM was (51, 52), which is in line with animal studies (53). However, findings might be different in a fed or insulin-stimulated state.

In contrast to the fasted individuals in the study of Kim et al., Nielsen et al. studied the myocardial glucose uptake 1 hour after oral glucose intake in NGM, prediabetes, and newly diagnosed T2DM individuals, all characterized by chronic heart failure and reduced LVEF. Even though the myocardial blood flow and myocardial flow reserve were similar, individuals with prediabetes and newly diagnosed T2DM had - despite of elevated levels of glucose and insulin - a decreased myocardial glucose uptake compared to NGM (54). However, since the authors did not separate analysis for individuals with prediabetes and T2DM, it is unknown whether there were differences between the these groups.

To assess the insulin-stimulated myocardial glucose uptake in a more controlled setting than right after glucose ingestion, one should measure myocardial glucose uptake during a hyperinsulinemic euglycemic clamp (55). Eriksson et al. showed a similar cardiac glucose metabolic rate during such clamp in control, prediabetes, and T2DM individuals matched for age, sex, and BMI (56). Others showed lower myocardial glucose uptake in T2DM compared to (BMI-matched overweight) NGM individuals during a hyperinsulinemic euglycemic clamp (55, 57). These conflicting *in vivo* findings of myocardial glucose uptake in T2DM are also found in *ex vivo* studies. Full thickness myocardial biopsies form the left ventricle of T2DM individuals showed an increase in cardiac insulin receptor substrate 1 (IRS1) – PI 3-kinases (PI3K) activity compared to their overweight controls with NGM (58). This means that the

insulin signalling cascade, even in this state of insulin-resistance, is intact. However, differences between groups can be blunted due to the fact that all individuals in the *ex vivo* studies were characterized by left ventricular dysfunction.

Overall, results on myocardial glucose uptake in prediabetes are conflicting, both in *in vivo* and *ex vivo* studies. Some found no differences in healthy prediabetic individuals compared to NGM or T2DM individuals in fasted state (51) and during a clamp (56); whereas others did find reduced myocardial glucose uptake 1 hour after oral glucose loading (54) in prediabetic patients with chronic heart failure. Also, previous literature is ambiguous whether myocardial glucose uptake is associated with whole-body insulin sensitivity (57) or not (56) measured during a hyperinsulinemic euglycemic clamp. Data is dispersed and the question remains whether prediabetes is characterized with a reduced myocardial insulin sensitivity.

The effect of altered myocardial glucose metabolism on cardiac function has so far, only been studied in patients with heart failure. Animal studies show conflicting results. In diabetic Zucker rats, the decreased glucose utilization (assessed by ^{18}F-FDG as PET tracer) was associated with impaired diastolic and systolic cardiac function (assessed upon ultrasound) (53). Surprisingly, a study in insulin resistant Sprague-Dawley rats showed an increased glucose utilisation of the myocardium accompanied by a higher left ventricular ejection fraction, a smaller left ventricular end systolic volume, and a thicker end systolic wall thickness (59). Hence, the mechanism remains unclear and it is unexplored what the relation between glucose metabolism, insulin, and cardiac function is in prediabetes.

MRS-studies focusing on the tracers hyperpolarized [1-^{13}C]-pyruvate or [2-^{13}C]-pyruvate give mechanistical information and revealed defects in the carbohydrate metabolism on the level of PDH. Although, only a few studies have been performed with this new technique, the first results are promising. Cunningham et al. showed that assessment of the cardiac pyruvate metabolism *in vivo* in humans is feasible (60) and Rider et al. showed a significantly reduced metabolic flux through cardiac pyruvate dehydrogenase in T2DM compared to their age-matched healthy controls (61). Thus, in T2DM, in addition to insulin resistance, a reduced metabolic flux through pyruvate dehydrogenase can explain the decreased glucose uptake. In addition, a significant increase in metabolic flux through pyruvate dehydrogenase was observed in the T2DM individuals after the oral glucose loading (61). The depressed flux through pyruvate dehydrogenase in T2DM individuals is in line with results from various animal models

(62-64). Chatham et al. found a depressed flux in both Zucker diabetic fatty rats (62) and in isolated perfused rat hearts with streptozotocin-induced diabetes (63). Interestingly, PDH flux was associated with diastolic function (63, 64). Hopefully, future studies using this elegant method, may give more insight in the underlying mechanisms potentially modulating glucose and fat oxidation in the prediabetic state in humans.

In cases where changes in glucose metabolism were found in the prediabetic state, these were reversible, similarly as the possible alterations in lipid metabolism. Even within one month after bariatric surgery and subsequent weight loss, severely obese T2DM showed an increase in myocardial glucose uptake (65). Hannukainen et al. studied 46 individuals with T2DM, impaired glucose tolerance, and NGM, before bariatric surgery and six months after the surgically induced weight loss. Not only an improvement in whole-body insulin sensitivity was detected which correlated positively with the increase in myocardial glucose uptake and the decrease in myocardial fasting free fatty acid uptake, also myocardial function, and myocardial structure were improved (44). But like the lifestyle intervention, which had positive effects on lipid metabolism, from this study we neither know whether these changes are due to the whole-body effects of the weight loss. A 16 week intervention with the PPARγ-agonist rosiglitazone has proven to increase myocardial glucose uptake during a hyperinsulinemic euglycemic clamp in both ischemic and non-ischemic regions in individuals with T2DM and coronary artery disease (66), showing that myocardial glucose uptake can not only be affected by bariatric surgery or lifestyle adjustments, but also by drugs.

6. Mitochondrial function

Mitochondria are responsible for oxidative metabolism and are key to a normal function of the cardiomyocytes. It is therefore not surprising that mitochondrial dysfunction is suspected to play a pivotal role the development of DCM (67, 68). Unfortunately, human data in prediabetes in this area is lacking. Though studies in male Long-Evans rats which were high-fat fed and had a streptozotocin treatment as a model for prediabetes, have shown a mild diastolic dysfunction and cardiac hypertrophy associated with early changes in mitophagy (69). This supports the suggestion that mitochondrial dysfunction underlies development of DCM in prediabetes.

However, information from both *in vivo* and *ex vivo* studies performed in obesity and T2DM is available and may explain possible changes in mitochondrial function in prediabetes. From *ex vivo* studies using high-resolution respirometry as a reflection of mitochondrial function, it is known that a lower mitochondrial respiration is associated with T2DM (28, 70). This indicates that the myocardium of T2DM individuals, in spite of their preference for fatty acid oxidation as shown in PET studies, has a decreased maximal capacity for fatty acid-supported respiration, in comparison to non-diabetic individuals.. Moreover, Anderson et al. reported a negative correlation of maximal capacity for fatty acid respiration with the HbA1c (28). The relationship with Hb1A1c may suggest that mitochondrial function may already be affected in prediabetes.

In line with the notion of decreased mitochondrial function in metabolically challenged individuals such as prediabetes, some differences in mitochondrial function are already found between lean and obese individuals. Montaigne et al. found in vitro abnormal respiratory chain complex activities in obese individuals without T2DM, but this did not result in a reduced mitochondrial respiration (70). This is in line with Niemann et al., who showed that obese individuals had disturbed mitochondrial biogenesis and function (respiratory chain complex I) in the right atrial cardiomyocytes compared to lean individuals (71). Also, *ex vivo* contractile performance was decreased in obese individuals already before the onset of clinical cardiomyopathy, although to a lesser extent than in T2DM (70). However, these results suggest that not only chronic hyperglycemia as seen in T2DM, but already the early-stage alterations in glucose homeostasis as seen in obesity, have impact on mitochondrial function and thereby on the intrinsic myocardial contractile function. It is therefore to be expected that these changes in obese individuals are also present in prediabetes, as a prelude to DCM, however this remains to be explored in new cross-sectional studies.

In vivo, concentrations of high-energy phosphates have been suggested to be closely related to cardiac mitochondrial oxidation. This can be measured by ^{31}P-MRS. With this technique, PCr and ATP can be recognized at a specific resonance frequency. As a relative quantification, the ratio of PCr over ATP is used as a measure for myocardial energy status. Mitochondria produce ATP during oxidative phosphorylation, and ATP can in turn be used to convert creatine (Cr) into PCr. In the sarcolemma, the phosphate group of PCr is exchanged with adenosine diphosphate (ADP) to form ATP in case of increased energy demand. In this way, PCr acts to buffer ATP. This PCr shuttle system is also shown in Figure 1. In the normal myocardium, ATP synthesis can be maintained at the rate of ATP demand and PCr levels are sustained. However,

in cardiac disease with a decreased mitochondrial function, ATP demand may outweigh the mitochondrial capacity for ATP production and hence PCr concentrations will fall (72). Hence, the PCr/ATP ratio has been suggested to be a marker of mitochondrial function, however, one should be aware that creatine supply, pH, and oxygen supply may independently influence PCr concentrations in the cardiomyocyte (73).

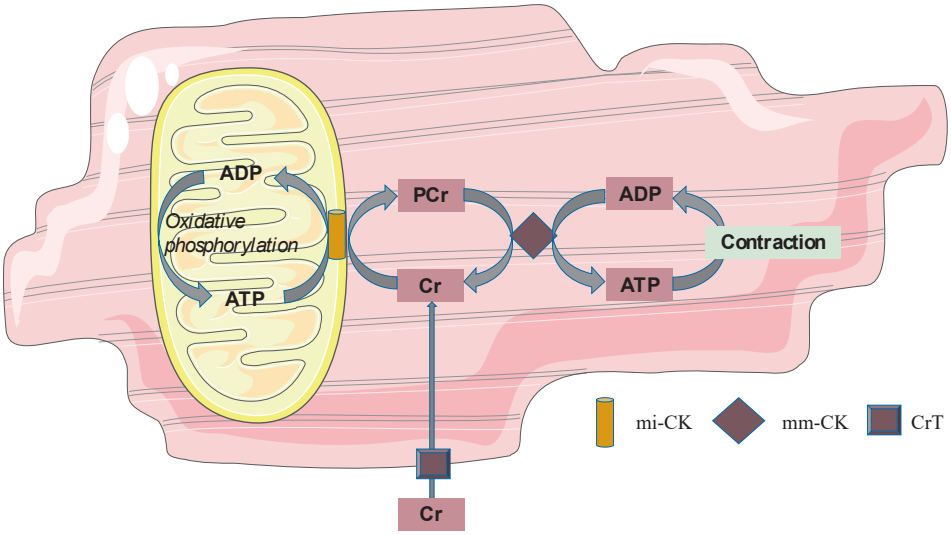

Figure 1. Phosphocreatine shuttle system. Mitochondria produce ATP during oxidative phosphorylation, and this ATP converts Cr at the mitochondrial membrane into PCr through mi-CK. PCr in turn shuttles from the mitochondrial membrane to the sarcolemma where through the mm-CK the phosphorous group of PCr is exchanged with ADP to form ATP in cases of increased energy demand. In this way PCr acts to buffer ATP. ADP adenosine diphosphate; ATP adenosine triphosphate; Cr free creatinine; PCr phosphocreatinine; mi-CK mitochondrial Creatine Kinase; mm-CK myofibrillar Creatine Kinase; CrT creatinine transporter.

Since *ex vivo* studies hint towards a decreased mitochondrial function in prediabetes (28, 70), it can be expected that in prediabetes, the myocardium may have to rely more on its reserves (PCr) for the production of ATP to meet the ATP demand, resulting in a reduced myocardial energy status, as measured by PCr/ATP ratio *in vivo*. Despite that the literature upon cardiac mitochondrial dysfunction in obesity and T2DM is expanding, studies in prediabetes are lacking. ^{31}P-MRS *in vivo* studies performed by Diamant et al. and Scheuermann-Freestone et al. show in T2DM with a relatively high HbA1c (6.1 ± 1.1 and 8.3 ± 0.4, respectively) a lower PCr/ATP ratio compared to NGM, and are thus in line with the decreased mitochondrial function measured *ex vivo* (74, 75). However in T2DM individuals with well-regulated plasma

glucose the PCr/ATP ratio found by Rijzewijk et al. was not different from matched obese controls (76). In addition, Scheuermann-Freestone et al. showed that the PCr/ATP ratio correlated negatively with plasma FFA concentrations in T2DM and NGM, and that PCr/ATP correlated positively with the plasma glucose concentrations in the individuals with T2DM, thus showing that metabolic dysregulation is a hallmark of a disturbed cardiac energy status (75) and thereby implying that this already could be the case in prediabetes.

Both in nondiabetic and in T2DM individuals a lower PCr/ATP ratio is shown to be inversely associated with diastolic cardiac function parameters, like for instance E acceleration peak, E deceleration peak, and E peak filling rate (74). This human *in vivo* MRS-data supports the hypothesis that the early alterations in mitochondrial energy metabolism in the prediabetic state do increase the susceptibility to diastolic heart failure, as seen in DCM.

However, since *in vivo* studies in prediabetes are currently lacking, the expected decreased PCr/ATP ratio in prediabetes is a speculation based on data in obesity and T2DM. Therefore, the complex pathology of changes in mitochondrial function and metabolism in prediabetes remains incompletely understood. As already described in the section on cardiac fat, a potential mechanism may involve detrimental effects of the excessive bioavailability of nutrients. Possibly these nutrients, or more specifically fatty acids, may influence mitochondrial function. In mouse studies, it has been shown that a high abundance of fatty acids may lead to inefficient substrate oxidation in the heart (reflected by a reduced ratio of energy production (ATP production) to respiration), resulting in the formation of reactive oxygen species (ROS) and thereby to mitochondrial damage (67, 68, 77, 78). Possibly, a similar mechanism occurs in the human heart in the prediabetic state.

Secondly, gene regulatory pathways may affect mitochondrial function by influencing the interplay between the supply and oxidation of the various substrates. Not only in individuals with T2DM, also in first degree relatives of individuals with T2DM, it has been shown that the expression of peroxisome proliferator-activated receptor (PPAR) coactivator PGC-1α is decreased in skeletal muscle (79). PGC-1 is usually increased upon cellular ATP demand (80), leading to transcription of NRF-1, PPARα, and PPARγ, and may thereby have indirect effects on mitochondrial metabolism. Since NRF-1 regulates the expression of many mitochondrial genes, including the OXPHOS genes, a decreased expression of PGC-1α will result in a lower mitochondrial content of the OXPHOS complexes. These data found in skeletal muscle may be

translated to heart, though data in prediabetes are lacking. Yet, in T2DM, Montaigne et al. found a downregulation of NRF-1 and mitochondrial function is the heart (70).

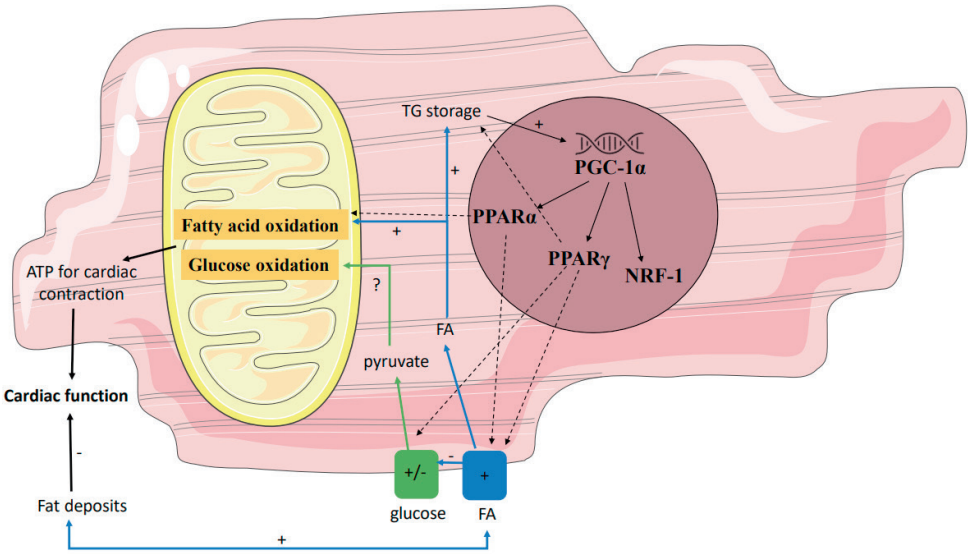

Figure 2. Gene regulatory pathways affecting mitochondrial function. Several genes influence the interplay between the supply and oxidation of the various substrates. Down- and/or upregulation of these genes in prediabetes may affect the mitochondrial function. Partly because of this, in prediabetes fatty acid oxidation may be stimulated, resulting in a net reduction of ATP and thus reduced myocardial efficiency. PPAR peroxisome proliferator-activated receptor; ATP adenosine triphosphate.

In addition to the downregulation of NRF-1 following a decreased expression of PGC-1α, also PPARα and PPARγ can be influenced by NRF-1. These PPAR receptors are known to coordinate the expression of the most key regulators of the fatty acid metabolism and are thereby responsible for determining substrate preference in the heart. Polymorphisms within the human PPARα and PPARγ gene have been reported to influence risk markers for CVD, including BMI, cholesterol, and the incidence of T2DM (81). Seen as PPAR polymorphisms are associated with the incidence of T2DM, possible difference in PPAR expression in healthy compared to prediabetic and T2DM individuals may be expected. However, Marfella et al. studied biopsies of the left ventricular septum, and did not find significant differences in myocardial PPARα expression between individuals with and without metabolic syndrome, although PPARγ was lower in the healthy individuals (26). Moreover, the expression of PPARγ was correlated with LVEF and the accumulation of cardiac fat (26). This is in line with the

results of Anderson et al. who found a decreased mitochondrial respiration upon fatty acid stimulation, but did not find differences in expression of PGC-1α nor PPARα in the heart atria in T2DM (28). However, this was a relatively small and heterogenous group of patients. Similarly, a large observational study in T2DM individuals (the FIELD study), showed that the use of a PPARα agonist (fenofibrate) did not reduce the risk of coronary events, however it did reduce the total cardiovascular events, mainly due to fewer non-fatal myocardial infarctions and revascularizations (82), implicating that PPARα stimulation may have a beneficial effect in T2DM. The mechanism behind this advantageous effect in human is unknown, but treatment with a PPARα agonist (GW7647) showed also in mice a protective effect on myocardial contractile function after induction of cardiac ischemia (83) and treatment with a PPARα agonist (fenofibrate or ciprofibrate) in different animal models of insulin resistance (high fat diet induced in C57BL/6 mice or genetic induced in obese Zucker rats) showed a slight improvement of glucose metabolism (84). The latter may possibly explain the beneficial effect of fenofibrate in the FIELD study, if findings from animal studies can be translated to humans. On the contrary, other mouse studies showed that an increased availability of fatty acids led to activation of the PPARα gene regulatory pathway, which resulted in an increase uptake of fatty acids and cardiac dysfunction in diabetic mice (85-87). Thus, the effect on PPARα agonists on the cardiac function are contradictory in different animal studies, and intervention studies in prediabetes and T2DM are not performed. Hence, the order of events remains unclear and thus are studies in bigger populations needed to pin-point the relevance of PPAR gene regulatory pathways in the development of cardiac metabolic aberrations and cardiac dysfunction in prediabetes.

7. Conclusion

Data from clinical studies on cardiac metabolism in prediabetes is scarce. Myocardial triglyceride content is associated with insulin sensitivity (25) and is increased in prediabetes (25-28). Although the different fat deposits around the heart have not been measured in prediabetic populations, it might be expected that the epicardial adipose tissue is elevated (30, 37, 38). During insulin stimulated conditions both the FFA uptake and the FFA oxidation in the prediabetic myocardium are increased during insulin stimulated conditions (45, 46). Although, a vastly decreased glucose uptake and glucose oxidation have been shown in T2DM, the few studies in prediabetes show conflicting results and the question remains whether prediabetes is characterized with a reduced myocardial insulin sensitivity (51, 52, 56, 57). Mitochondrial

function is also not well studied in prediabetes, but it is likely that not only hyperglycemia as seen in T2DM, but already the early-stage alterations in glucose homeostasis as seen in obesity, have impact on mitochondrial function as HbA1c is negatively correlated to maximal fatty acid respiratory capacity (28) and plasma glucose concentrations are correlated with PCr/ATP (75). It is therefore to be expected that a decreased mitochondrial function is also present in prediabetes, as a prelude to mitochondrial dysfunction in DCM (67, 68).

The metabolic changes have consequences in prediabetic individuals, as metabolic influences on cardiac function are often seen in different patient populations. Cardiac function was negatively influenced in both healthy and metabolically compromised individuals by an increased fat storage (24, 26, 29), increased epicardial adipose tissue (39-41), increased FFA uptake and oxidation (46, 47), and a lower PCr/ATP ratio (74). Although studies in prediabetic individuals are lacking, these results do support the notion that metabolic changes in prediabetes might contribute to the development of diastolic dysfunction, as seen in DCM.

The metabolic changes and the associated functional impairment in the prediabetic heart do seem to be reversible (44, 48, 65, 66). Hence, it seems to be important to counterbalance these changes in substrate metabolism in the early (pre)diabetic state and improve mitochondrial function, as these changes precede DCM in T2DM. This emphasizes the need for studies intervening in the prediabetic state, to allow a better cardio-protection in the development of T2DM and the metabolic syndrome.

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CHAPTER 3

Pericardial fat and its influence on cardiac diastolic function

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Abstract

Background Pericardial fat (PF) has been suggested to directly act on cardiomyocytes, leading to diastolic dysfunction. The aim of this study was to investigate whether a higher PF volume is associated with a lower diastolic function in healthy subjects.

Methods 254 adults (40-70 years, BMI 18-35 kg/m^2, normal left ventricular ejection fraction), with (a)typical chest pain (otherwise healthy) from the cardiology outpatient clinic were retrospectively included in this study. All patients underwent a coronary computed tomographic angiography for the measurement of pericardial fat volume, as well as a transthoracic echocardiography for the assessment of diastolic function parameters. To assess the independent association of PF and diastolic function parameters, multivariable linear regression analysis was performed. To maximize differences in PF volume, the group was divided in low (lowest quartile of both sexes) and high (highest quartile of both sexes) PF volume. Multivariable binary logistic analysis was used to study the associations within the groups between PF and diastolic function, adjusted for age, BMI, and sex.

Results Significant associations for all four diastolic parameters with the PF volume were found after adjusting for BMI, age, and sex. In addition, subjects with high pericardial fat had a reduced left atrial volume index ($p=0.02$), lower E/e ($p<0.01$) and E/A ($p=0.01$), reduced e' lateral ($p<0.01$), reduced e' septal $p=0.03$), compared to subjects with low pericardial fat.

Conclusion These findings confirm that pericardial fat volume, even in healthy subjects with normal cardiac function, is associated with diastolic function. Our results suggest that the mechanical effects of PF may limit the distensibility of the heart and thereby directly contribute to diastolic dysfunction.

Background

Diastolic heart failure is a major cause of morbidity and mortality (1) and is preceded by diastolic dysfunction, which is often present in patients with obesity and type 2 diabetes mellitus (T2DM). Diastolic dysfunction is defined as abnormal relaxation of the myocardium and may be present years before symptoms occur. It can be diagnosed by quantifying diastolic tissue motion and intracavitary filling pressures. The guidelines for diagnosing diastolic function combine measurement of diastolic tissue motion, diastolic blood flow quantification, and structural abnormalities such as the presence of left atrial dilation (2). Meeting 2 or more criteria results in the diagnosis of diastolic dysfunction.

Despite the clear definition, the understanding of the pathological mechanism of diastolic dysfunction remains poor. Various potential mechanisms have been suggested, but none of them can adequately explain the pathological process. Since increased pericardial fat (PF) volume is associated with adverse cardiovascular disease (CVD) outcomes, interest has peaked into this relationship and the potential effects of PF on cardiac dysfunction (3, 4).

PF is divided into two fat components: the Epicardial Adipose Tissue (EAT) and the Cardiac Adipose tissue (CAT). It is presumed that the EAT, due to its anatomical proximity to the myocardium, has the most effects on the myocardium. In normal physiology, EAT may have positive metabolic effects as it has an important function in lipid storing, and it also secretes endocrine factors (5). It demonstrates a great flexibility in the storage and release of fatty acids, which has been suggested to protect the heart from lipotoxicity, whilst–simultaneously providing energy to the myocardium during high energy demand (6, 7). As a metabolically active endocrine organ, EAT also produces adipokines which may protect the heart from cardiovascular disease (8). However, when EAT expands, the balance between the storage and release of fatty acids shifts towards a more active secretion, as seen in obese subjects in comparison to lean subjects (9). The expanded EAT transforms its secretions into pro-inflammatory cytokines and chemokines (6, 8, 10). Cho et al. showed that the thickness of EAT at the right ventricle wall was associated with inflammation represented by hs-CRP level, LV mass, and subclinical myocardial dysfunction in males (11). This is also confirmed in EAT biopsies taken from patients undergoing coronary artery bypass grafting (CABG) (12, 13). Some of these mediators are known to have profibrotic properties, linking the inflammation of enlarged EAT with fibrosis (14). From studies performed in (morbidly) obese subjects with a

high prevalence of T2DM, we know that PF, EAT, and CAT are linked to several diastolic function parameters (15-17). However, studies associating PF directly with diastolic function in healthy subjects are scarce, and the underlying mechanisms remain unknown (18-21).

Moreover, Ng et al. found an association between EAT volume index and interstitial myocardial fibrosis in an overweight to obese population (19). This association suggests that enlarged EAT may be related to asymptomatic cardiac remodeling, and hence, the enlarged EAT may be involved in the development of cardiac diastolic dysfunction as is seen in overweighed subjects. Most studies on EAT have focused on the effects of EAT on systolic function, whereas in fact, in obese and diabetic populations, diastolic function are the first cardiac function parameters to change in obesity and metabolic syndrome (22). In addition, Yang et al. showed an increased EAT burden in pre-diabetic and diabetic subjects, compared to normoglycemic subjects (23). Also, Christensen et al. found that high levels of EAT were associated with the composite of incident CVD and mortality in subjects with T2DM (24). EAT may possibly play a more central role in the development of asymptomatic diastolic cardiac dysfunction than previously assumed, underlining the importance of a better understanding of the relationship between EAT and early changes in cardiac diastolic function. Hence, further studies focusing on exploring the relationship between EAT and diastolic dysfunction in a relatively healthy population, independently of their metabolic profile, are warranted.

In summary, it is unknown whether PF and / or EAT influences diastolic cardiac function in healthy subjects before any symptoms of diastolic failure occur. Most studies looking into the associations between PF or EAT with diastolic function have been performed in subjects with heart failure, CVD, overweight, or (pre-)diabetes (18-20, 25). This may possibly confound the relationship, as many structural and metabolic changes may interfere. Therefore, in this study, we aim to determine whether a higher PF volume is associated with subclinical but lower diastolic function in a healthy population. Secondly, we aim to examine if this lower diastolic function is solely derived from the EAT compartment, or if it is associated to the PF compartment as a whole.

Methods

Ethics approval and consent to participate

This study was approved by the Institutional Review Board (IRB) and Ethics Committee. Involved data were collected on a routine basis from within the Maastricht biomarker CT study (ClinicalTrials.gov NCT01671930, MEC 08-4-057) and analysed anonymously in accordance with Institutional Review Board guidelines. The study complies with the ethical principles of the Helsinki Declaration.

Study cohort

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This study cohort is comprised of patients from the cardiology outpatient clinic presenting with (a)typical chest pain, who were according to the standard care protocol referred for coronary computed tomographic angiography (CCTA) for the evaluation of stable CVD, in accordance with the current guidelines (26, 27). Inclusion criteria for the Maastricht biomarker CT study were a recent history of cardiac typical or atypical chest pain, dyspnea, or collapse; at least 1 mL of serum for determination of biomarkers; and a diagnostic CCTA-scan, defined as 7 or more interpretable coronary segments. The exclusion criteria were hsCRP concentration >10 mg/L (indicating underlying inflammatory disease), severe renal dysfunction, or dialysis (due to application of contrast fluids).

254 patients enrolled in the echocardiography subgroup of the Maastricht Biomarker CT study were retrospectively included in this study (28, 29). A flowchart of inclusion is provided in Figure 1. In the present subgroup analysis (n=254), patients aged 40-70 years with a BMI between 18 and 35 kg/m^2 without history or diagnosis of acute coronary syndrome at the time of CCTA were included. Exclusion criteria for this subgroup study were: left ventricular ejection fraction (LVEF) <45%, diastolic dysfunction, atrial fibrillation, and diabetes mellitus.

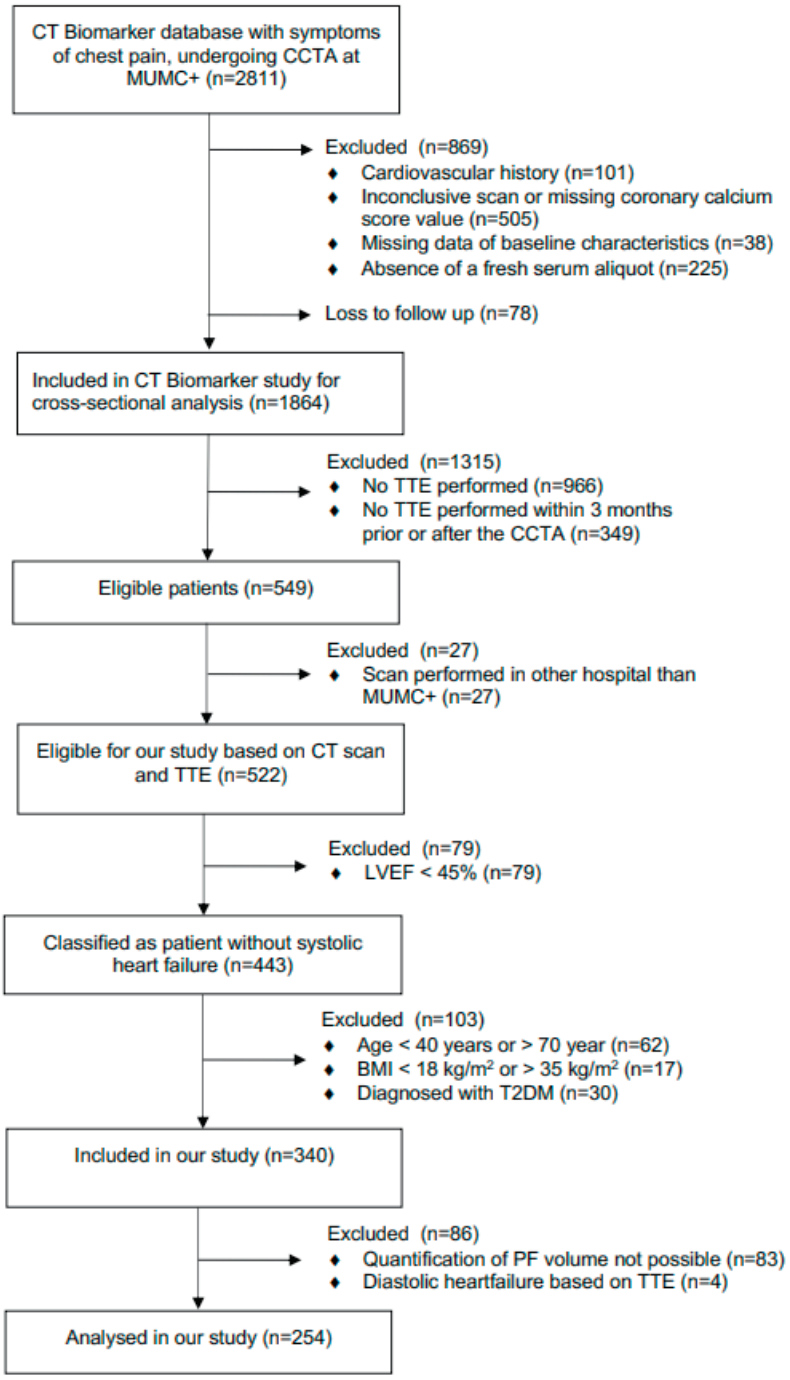

Fig. 1. Flowchart of inclusion (n=254). 254 patients from the Maastricht biomarker CT study were eligible for the analysis of the association of PF and diastolic function in healthy subjects.

Biochemical analysis

Serum samples were collected just before CCTA, processed within 2 hours and directly stored at -80°C until analysis. Total cholesterol (CV 2.0%), triglycerides (CV 2.5%), high-density (CV 3.0%) and low-density lipoprotein concentrations were measured as previously described (Cobas 6000, Roche Diagnostics) (28). Serum creatinine (CV 2.5%) and cystatin C concentrations were measured in a fresh aliquot (Cobas 6000; Roche Diagnostics). Creatinine concentrations were assessed using the enzymatic method (Cobas 6000, Roche Diagnostics). Cystatin C was measured using a new particle-enhanced turbidimetric assay (Gentian AS), which was standardized against the certified ERM-DA471/IFCC cystatin C reference material (30). Glomerular filtration rate was estimated by the Chronic Kidney Disease Epidemiology Collaboration equations using serum creatinine and cystatin C concentrations (31).

Cardiac computed tomographic angiography

All 254 patients had undergone a standardized non-enhanced scan to determine the calcium score using the Agatston method (32) at our center prior to CCTA assessment.

Semi-automatic segmentation determined the PF volume by dedicated software (SyngoVia, Siemens Healthineers, Forcheim, Germany) using a threshold from -150 to -50 Hounsfield Units to distinguish visceral adipose tissue, as set by the software (33). Because of the large sample size, only in a random sample of 10% of the subjects the pericardium was marked manually to separate the PF into EAT and CAT (depicted in Figure 2), and thereafter, the software calculated the separate 3D volumes of EAT and CAT.

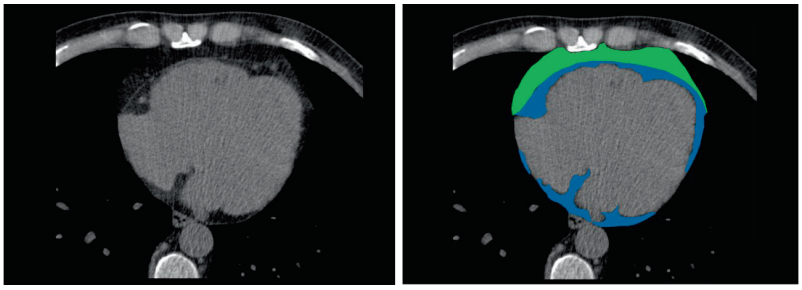

Fig. 2. Definition of pericardial fat (PF) and the related adipose tissues. The adipose tissue surrounding the heart is defined as the pericardial fat (PF) and is a combination of epicardial and cardiac fat components. Within the PF, the pericardium demarcates the epicardial adipose tissue (EAT) from the cardiac adipose tissue (CAT). EAT (depicted in blue) is located between the myocardium and visceral pericardium, CAT (depicted in green) is located adherent and external to the parietal pericardium.

Echocardiography

Echocardiography was performed within a period of 3 months from the CCTA by an experienced echocardiographist. Transthoracic images of the left ventricle (LV) were acquired to assess morphology, function and mass (Philips IE 33, Philips Healthcare). LV function and -mass were calculated by off-line analysis using Xcelera software package (Philips), according to current ESC/AHA guidelines (34).

Only four diastolic parameters are decisive in the evaluation of diastolic function according to the American Society of Echocardiography (ASE)/European Association of Cardiovascular Imaging (EACVI) guidelines, namely, left atrial volume index (LAVI) , e' septal, e' lateral (mobility of the septal and lateral left ventricle wall respectively), and peak velocity of tricuspid regurgitation (TR) (2). Therefore, most of the analyses will focus upon these diastolic function parameters. But, in addition, also mitral peak A and E velocity, E/A ratio, and E/e' ratio, were determined.

Statistical analysis

Baseline characteristics of the sample were summarized using mean and standard deviation or median and interquartile range (IQR) for normally distributed and skewed continuous variables, respectively. Categorical data were presented as absolute number and percentage. To assess the independent association of PF and diastolic function parameters in these 254 patients, linear regression analysis was performed with either LAVI or e' septal or e' lateral or TR as the dependent variable. These models were adjusted for BMI, age, sex, and their interaction terms with PF, since it is known that these parameters are strongly associated with PF (9, 35, 36). Results of the linear regression analysis are presented as regression coefficient with 95% confidence interval (95% CI).

This study is based on a sample of healthy participants without diastolic dysfunction, therefore, only mild differences in diastolic function were expected. To maximize the differences in PF volume, the group was divided into low PF (lowest quartile of both sexes separately) and high PF (highest quartile of both sexes separately). The lowest and highest quartile groups were matched for cardiovascular risk factors, i.e., sex, systolic and diastolic blood pressure, total and LDL cholesterol, and kidney function. Differences in other baseline characteristics across these extreme quartiles of PF volume were investigated using the independent-samples t-test for continuous variables with a normal distribution, or the Mann-Whitney U-test for non-normal

distributed continuous variables. Pearson's chi-square test was used for categorical variables. Data are presented as proportions, means ± standard deviations, and data with a non-normal distribution are presented as the median (interquartile range, IQR).

To assess the independent association of PF and diastolic function parameters in these extreme quartiles (n=130), also multivariable linear regression analysis was performed with either LAVI, or e' septal, or e' lateral, or E/e', or TR as the dependent variable. These models were adjusted for BMI, age, and sex. Results are presented as regression coefficient with 95% confidence interval (95% CI).

To investigate the association of EAT with the total PF and EAT with diastolic function, Pearson's correlation coefficient was computed. Because only in 10% of the subjects an EAT volume was known, this subgroup was considered too small to perform regression analysis. All statistical analyses were performed with IBM SPSS Statistics Version 25.0 (SPSS, Inc.). Two-sided p-values of ≤ 0.05 were considered statistically significant.

Results

The baseline characteristics for the total sample and the lowest and highest quartile groups of PF volume are presented in Table 1.

Distribution and determinants of the PF volume

Median (IQR) PF volume in the total cohort were 1.411 (IQR 1.035, 2.057) dl. Since males have a higher PF volume than females (median 1.729 dl, IQR 1.202,2.492; median 1.215 dl, IQR 0.909,1.552; respectively), the upper and lower PF volume quartiles of males and females were combined for the analysis (Figure 3A).

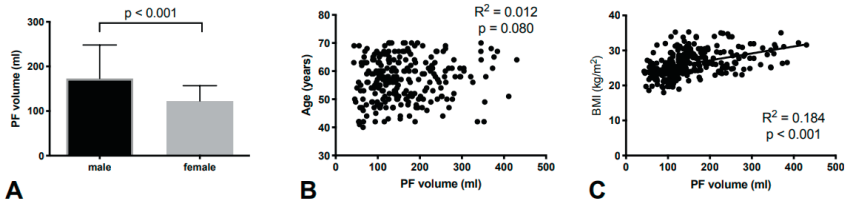

Fig. 3. The variation of PF volume to sex, age and BMI in a healthy population. PF volume is higher in males as in females (A), PF volume is not related to age (B) and PF volume is associated with BMI (C).

There was a significant difference between the lowest and highest quartile groups for age (55.7 ± 8.0 versus 59.1 ± 7.4, p = 0.015), BMI (23.7 ± 2.7 versus 28.1 ± 2.9, p value <0.001), glucose (5.5 ± 0.8 versus 5.9 ± 1.2, p = 0.025), HDL cholesterol (1.5 ± 0.4 versus 1.2 ± 0.4) and triglycerides (1.5 ± 1.1 versus 2.4 ± 2.0, p = 0.001), see table 1. The CAD findings were not different between the two groups of high and low PF. However, Framingham Risk Score was higher in the high PF group, possibly due to the association of PF with age and BMI.

Table 1. Baseline characteristics of the study sample, and divided into highest and lowest quartiles of PF.

	Total sample (n=254)	**PF low (n=65)**	**PF high (n=65)**	**P-value**
Demographics				
Age (years)	57.0 ± 7.5	55.7 ± 8.0	59.1 ± 7.4	0.015
Sex (% female)	48	46	48	0.860
Cardiovascular risk factors				
Framingham Risk Score	18.0 ± 13.2	14.4 ± 10.1	21.4 ± 16.1	0.004
Glucose (mmol/L)	5.6 ± 0.9	5.5 ± 0.8	5.9 ± 1.2	0.025
Body mass index (kg/m2)	26.4 ± 3.7	23.7 ± 2.7	28.1 ± 2.9	<0.001
Systolic bloodpressure (mmHg)	142 ± 20	141 ± 23	146 ± 20	0.139
Diastolic bloodpressure (mmHg)	81 ± 11	80 ± 12	82 ± 11	0.254
Total cholesterol (mmol/L)	5.6 ± 1.1	5.5 ± 1.2	5.8 ± 1.2	0.148
HDL cholesterol (mmol/L)	1.3 ± 0.4	1.5 ± 0.4	1.2 ± 0.4	0.001
LDL cholesterol (mmol/L)	3.6 ± 1.0	3.4 ± 1.0	3.6 ± 1.1	0.405
Triglycerides (mmol/L)	1.5 (1.0, 2.2)	1.2 (0.8, 1.5)	1.7 (1.3, 2.5)	<0.001
Creatinine (µmol/L)	76 ± 17	76 ± 15	75 ± 18	0.769
eGFR (MDRD) (ml/min/1.73m2)	88 ± 18	89 ± 16	90 ± 21	0.619
CRP (mg/L)	2.3 ± 2.7	2.1 ± 2.5	2.8 ± 3.8	0.470
Coronary Artery Disease				
No Plaque (%)	39.4 ± 4.9	46.2 ± 5.0	35.4 ± 4.8	0.215
Mild (%)	37.0 ± 4.8	33.8 ± 4.8	36.9 ± 4.9	0.716
Moderate (%)	10.20 ± 3.0	7.7 ± 2.7	10.8 ± 3.1	0.548
Severe (%)	11.8 ± 3.2	9.2 ± 2.9	15.4 ± 3.6	0.289
Multi-vessel (%)	1.6 ± 1.3	3.1 ± 1.7	1.5 ± 1.2	0.563

Data are presented as means ± standard deviation, percentage, or as median (interquartile range, IQR).

Distribution and determinants of diastolic function

The association between diastolic function and PF volume was investigated, as some of the diastolic parameters are expected to deteriorate during the development of diastolic dysfunction before clinical criteria for diastolic dysfunction are met (Figure 4).

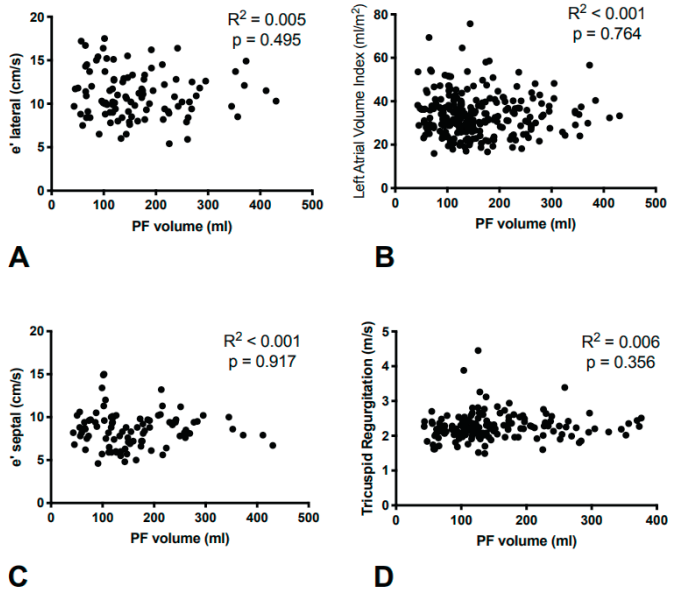

Fig. 4. PF is not associated with diastolic function parameters in a healthy population. Data of the entire cohort (n=254) are displayed. No correlations are found.

Table 2. Cardiac function measured by transthoracic echocardiography.

	Total population (n=254)	**PF low (n=65)**	**PF high (n=65)**	**P-value**
Left ventricular ejection fraction (%)	61 ± 5	62 ± 5	61 ± 5	0.213
Left ventricular mass index (g/m^2)	84.7 ± 16.9	80.6 ± 15.6	88.0 ± 16.0	0.008
Left atrial volume index (ml/m)	33.7 ± 0.7	36.8 ± 10.3	32.7 ± 8.4	0.015
e' lateral (cm/s)	11.0 ± 2.7	12.2 ± 2.9	10.3 ± 2.0	0.005
e' septal (cm/s)	8.5 ± 2.0	9.5 ± 2.1	8.4 ± 1.8	0.034
E/A	1.1 ± 0.4	1.1 ± 0.4	1.0 ± 0.4	0.013
Peak E velocity (cm/s)	72 ± 20	73 ± 24	70 ± 18	0.425
Peak A velocity (cm/s)	72 ± 18	66 ± 16	74 ± 17	0.004
E/e'	7.9 ± 2.1	6.8 ± 1.7	8.3 ± 2.3	0.009
Tricuspid regurgitation (m/s)	2.3 ± 0.4	2.2 ± 0.4	2.3 ± 0.3	0.416

Data are presented as means ± standard deviation.
Reference values: LVEF >=45%, LAVI <34 ml/m^2, e' lateral >10 cm/s, e' septal >7 cm/s, E/A 0.8 – 2.5, E/e' 8 – 14, TR 2.0 – 2.8 m/s.

Although still in the normal range, significant differences in the diastolic function parameters were found between the lowest and highest PF quartiles. As shown in table 2, a reduced LAVI and E/e' was found in the lowest PF quartile (p=0.02, p<0.01, respectively); and a reduced e' lateral, e' septal, and E/A in the highest PF quartile (p<0.01, p=0.03, p=0.01, respectively); and an increased peak A velocity in the highest PF quartile (p<0.01). Peak E velocity and TR did not differ significantly between the two extreme PF volume quartiles. Together, these differences reflect a diminished, although still normal, diastolic cardiac function in the highest PF quartile compared to the lowest PF quartile.

Association of PF with diastolic function

In the total sample (n=254), significant associations for all four diastolic parameters with the PF volume were found after adjusting for BMI, age, and sex. These data are depicted in Table 3. Analyses of the interactions with BMI, age, and sex, did not improve the model. In addition, in the extreme quartiles of PF volumes (n=130) a significantly negative association between high PF and LAVI, high PF and e' lateral, and high PF and TR, were found after adjusting for BMI, age, and sex. However, the difference in the mobility of the septal wall between the extreme quartiles of PF volume and between E/e' the extreme quartile of PF volume were no longer evident after the model was adjusted for these factors. These regression data are depicted in Table 4.

Table 3. Multivariable linear regression analysis in the total population exploring associations between PF and parameters of diastolic cardiac function.

	Unadjusted regression coefficient (95% CI)	**p-value**	**Adjusted regression coefficient * (95% CI)**	**p-value**
Left atrial volume index (ml/m2)	-0.24 (-1.79; 1.32)	0.764	-2.05 (-3.92; -0.19)	0.001
e' septal (cm/s)	-0.03 (-0.52; 0.47)	0.917	-0.13 (-0.68; 0.43)	0.020
e' lateral (cm/s)	-0.21 (-0.84; 0.41)	0.496	-0.02 (-0.71; 0.67)	<0.001
E/e'	7.45 (6.49; 8.42)	0.335	0.16 (-0.42; 0.74)	0.003
Tricuspid regurgitation (m/s)	0.04 (-0.04; 0.12)	0.356	-0.02 (-0.12; 0.07)	0.001

Abbreviations: CI – confidence interval.
* Adjusted for body mass index, age, and sex

Table 4. Multivariable linear regression analysis in the extreme PF quartiles (0=low, 1=high) exploring associations between PF and parameters of diastolic cardiac function.

	Unadjusted regression coefficient (95% CI)	p-value	Adjusted regression coefficient * (95% CI)	p-value
Left atrial volume index (ml/m2)	-4.13 (-7.47; -0.80)	0.015	-7.85 (-12.13; -3.56)	0.001
e' septal (cm/s)	-1.17 (-2.25; -0.10)	0.034	-0.96 (-2.28; 0.36)	0.088
e' lateral (cm/s)	-1.97 (-3.33; -0.60)	0.005	-1.39 (-3.13; 0.34)	0.020
E/e'	1.52 (0.40; 2.64)	0.009	1.33 (-0.11; 2.77)	0.118
Tricuspid regurgitation (m/s)	0.06 (-0.09; 0.22)	0.416	0.01 (-0.18; 0.20)	0.004

Abbreviations: CI – confidence interval.
* Adjusted for body mass index, age, and sex

Distribution and determinants of the different components of the PF volume

In 10% of the total sample (n=24), the EAT volume was studied by manually dividing the PF into the different CAT and EAT volumes. This random selection of 6 patients per PF quartile was made since the manual subdivision of the PF is extremely laborious, and to ascertain that the sample reflects the entire population. The data showed that with an increasing PF, no similar increase in the relative volume of EAT and CAT can be expected, as the relationship with the relative amount of EAT and CAT is lacking (p>0.7). These data are illustrated in Figure 5.

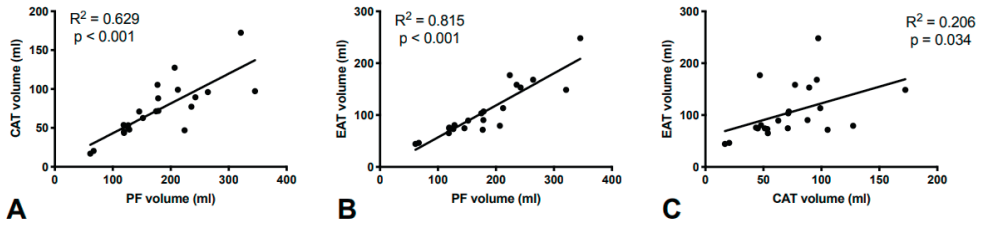

Fig. 5. No relation of PF to its CAT and EAT component. The amount of CAT (A) and EAT (B) are not related to PF. Although EAT and CAT volume show a wide variation, they are linearly associated to each other (C), indicating that both increase with an increase of PF.

To gain further insight into whether EAT is the major culprit in hampering diastolic function as suggested because of its anatomic proximity to the myocardium, separate correlations of EAT were made with the different diastolic parameters. Despite the small number, a direct correlation of the percentage of EAT and e' lateral was found. There was no correlation with EAT and the other diastolic function parameters (Figure S4 in the Supplementary Appendix).

Discussion

Studies associating PF or EAT with diastolic function are scarce and often contradictive. A partial explanation may be that most studies so far were performed in a non-healthy population, which may confound the reported associations of PF or EAT with diastolic function (15-20). Here, we studied the association between PF and diastolic function in a lean to obese middle-aged population, with normal systolic and diastolic cardiac function. We evaluated these relationships independently of their metabolic profile as correction for metabolic risk factors was applied. Furthermore, we explored the association of EAT with PF and EAT with diastolic function.

We report that PF was significantly associated with the diastolic function parameters LAVI, e' lateral, e' septal, E/e', and TR, when corrected for age, BMI, and sex. Adjustment for sex alone was already sufficient to render the association significant, since PF is different distributed between male and female. The reported associations indicate that even in our healthy population with a normal diastolic function, PF – independently of CVD risk factors related to age, BMI, and sex – is associated with diastolic function parameters.

In the analyses focusing on low and high PF volume, high PF was associated with a decrease in LAVI and e' lateral, and an increase in TR (as depicted in Figure 5). The decrease in e' lateral is in line with previous research performed in (morbid) obese subjects with a high prevalence of T2DM (15). The lower e' lateral in the highest PF quartile reflects a slower relaxation of the lateral wall of the left ventricle, necessary for an effective diastolic filling phase. The lower LAVI in the highest PF quartile is not known to be a sign of lower diastolic function. We do not know what underlies these findings, but they may indicate that PF causes mechanical hindrance that compromises not only the mobility of the lateral left ventricle wall (e' lateral), but also compresses the left atrium, and thereby reducing its volume (LAVI). This hypothesis needs further work.

Although EAT was only determined in a small subpopulation (n=24), insights in the compartmental distribution of PF and its consequences on diastolic function can be gathered. We found that at increased PF volumes, the EAT and CAT compartments increased at a same amount relatively to the whole fat depot. This is surprising as Wu et al. reported that subjects after bariatric surgery showed a great loss of CAT and only a small decrease in EAT (37).

Therefore, the regional distribution of adipose tissue remains an important subject for further research, taking into account that this distribution plays an important role in the development of metabolic syndrome and CVD (38).

The association of high PF with e' lateral suggests that in a healthy population the mechanical effects of PF limit the distensibility of the heart first, which subsequently contributes to diastolic dysfunction. This study suggests that secondly, after progression of this relaxation problem of the lateral wall, the LAVI might increase despite the compression of the PF mass, as seen in diastolic dysfunction. But this remains speculative, as we did not measure the mobility of the lateral wall of the left ventricle during the systolic phase. However, during systole the PF mass will be less restrictive than during diastole, which is in line with our hypothesis. Most notably, a mechanically limited heart is accompanied by pressure changes within the cardiomyocytes, which in turn can affect the metabolism of these cells, and thereby, negatively influence diastolic function.

Most of the research on PF so far focused on adipokine release and a potentially causal role in the formation of fibroses. Pressure changes due to increased PF leading to an altered metabolism are an alternative pathway how PF can influence cardiac function. Thus, although the underlying mechanism remains unknown, the idea that mechanical effects of high PF cause a diminished mobility of the myocardium is supported by the current data. As others already suggested, this diminished mobility may provoke fibrosis, which has been associated with diastolic dysfunction, however this remains to be elucidated. In our population changes in diastolic function parameters were associated with an increase in PF, however, the diastolic function was within normal range; hence no causality with fibrosis could be made.

Limitations

As we performed a cross-sectional retrospective study, our study has some limitations by design. Due to the retrospective design, the low and high PF groups were not matched on all relevant characteristics. However, we did adjust our analyses for age, BMI, and sex. Although we corrected for age, BMI, and sex, some of metabolic characteristics such as glucose, HDL-cholesterol, and triglycerides, may confound the associations, although these metabolic characteristics were within normal range. Also, because of the retrospective design, there was timeframe of a maximum of three months between the CCTA and TTE, this may have

influenced our association. In addition, since the manual subdivision of the PF is extremely laborious, we only separated the PF components in 10% of the total cohort, following random selection. Thus, the power was limited for exploring the metabolic effects of EAT, independently of PF, on diastolic function. The cross-sectional outline of this study does not allow any conclusions regarding possible causality. Future work should therefore include a prospective approach to evaluate causal relationships.

Finally, it is important to bear in mind that our study population consisted of relatively healthy subjects, whose cardiac diastolic function was considered to be good. We only studied the associations between PF and small variations in normal diastolic function, which also explains why we did not find correlations between the diastolic parameters and age, BMI, or sex, in our sample (Figures S1, S2, S3 in the Supplementary Appendix). There were no subjects with clinically defined diastolic failure to assess the relationships between PF and diastolic dysfunction. This, of course, remains an important question for future research.

Conclusion

The purpose of the current study was to determine the association of PF and cardiac diastolic function in a healthy population. Linear regression analysis revealed that PF, independently of age, BMI, and sex, is associated with the four diastolic ultrasound parameters which are decisive in the evaluation of diastolic function. A potential underlying mechanism of this may be that increased PF may compress the heart, leading to a limited distensibility in the diastole and fibrosis as seen in cardiac remodeling, and thus, may lead to diastolic dysfunction. This study adds to the growing body of research that explores possible mechanisms in the development of diastolic failure. Concluding, we confirm that PF, even in healthy subjects with normal cardiac function and without diabetes, does hinder diastolic function. The exact causality of this effect and the relationship with fibrosis remains to be determined.

Supplementary

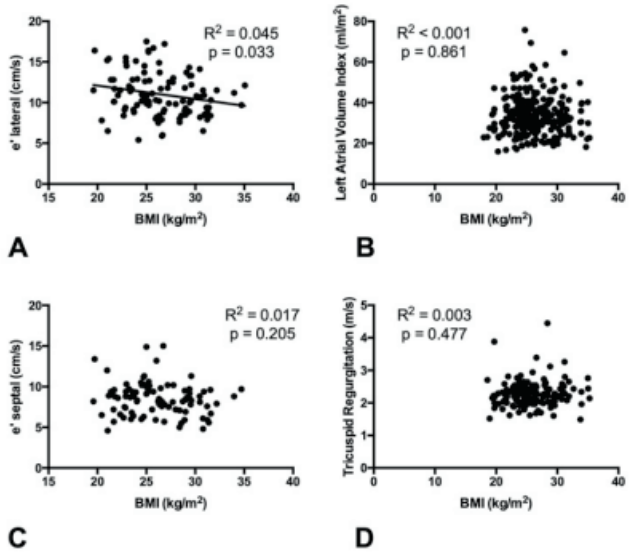

Fig.S1. Associations between BMI and diastolic function parameters in a healthy population. BMI is negatively associated with e' lateral (A), but not with LAVI (B), e'septal (C) or TR (D) in a healthy population.

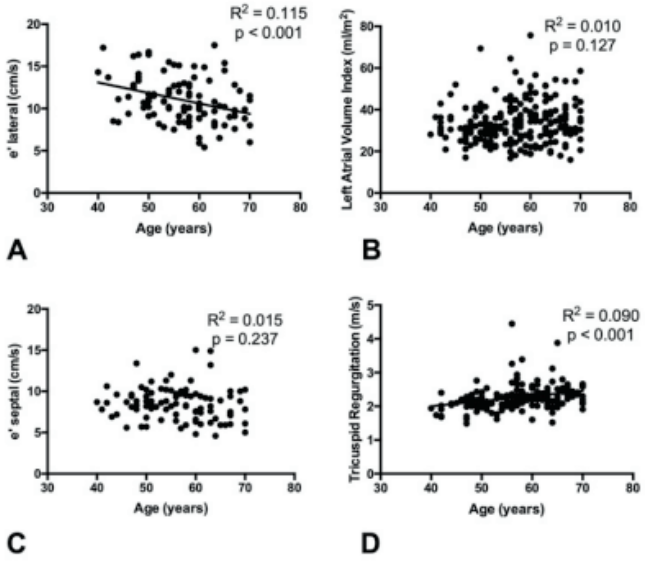

Fig S2. Associations between age and diastolic function parameters in a healthy population. Age is negatively associated with e' lateral (A), and positively associated with TR (D) in a healthy population. Age is not associated with LAVI (B), nor with e' septal (C).

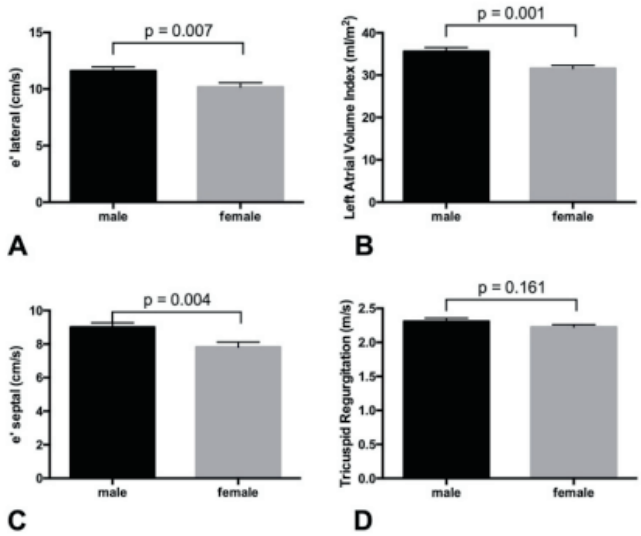

Fig. S3. Associations between sex and diastolic function parameters in a healthy population. Males are associated with higher e' lateral (A), higher LAVI (B), and higher e' septal (C), compared to females. No sex difference is observed in TR (D).

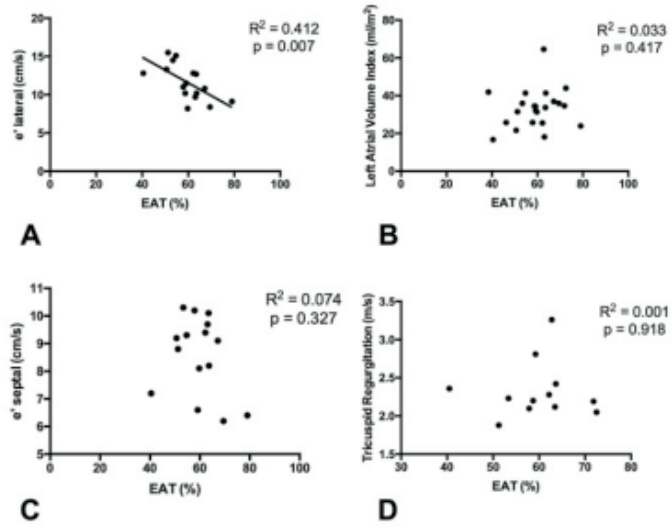

Fig. S4. Associations between EAT volume (as % of PF volume) and diastolic function parameters in subjects with low or high PF volume within a healthy population. Only e' lateral (B) was associated with the relative amount of EAT volume; no associations were found for LAVI (B), e' septal (C), nor TR (D).

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CHAPTER 4

PCr/ATP ratios and mitochondrial function in the heart. A comparative study in humans.

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CHAPTER 5

Cardiac energy metabolism is decreased in prediabetes and does not normalize during the day

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CHAPTER 6

The tissue-specific metabolic effects of the PPARα agonist ciprofibrate in males with prediabetes

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Submitted

CHAPTER 7

General discussion and conclusion

The prevalence of type 2 diabetes mellitus (T2DM) is strongly increasing and is associated with many severe comorbidities and early mortality (1). In fact, many health risks are already apparent in a prediabetic state. Prediabetes is characterized by increased concentration in fasting plasma glucose (but still within the non-diabetic range), and/or glucose intolerance after a meal (2). Whole-body metabolic changes during the development of insulin resistance and a reduction in oxidative capacity (3) are typically seen, which are associated with a blunted fat oxidation rate in the fasted state (4, 5). In combination with the high availability of free fatty acids, this favours the accretion of ectopic fat accumulation in muscle, heart and liver.

In the heart, prediabetes is associated with concentric remodelling (6, 7), cardiac insulin resistance (8), and an increasing oxygen demand, which results in reduced myocardial efficiency (9) and decreased cardiac function (10). Histological analysis showed that patients with the metabolic syndrome have increased intramyocellular lipid deposition in cardiomyocytes, which in turn was correlated with a depressed cardiac function (11). Eventually, this may develop into diabetic cardiomyopathy, a condition that has only been considered an independent disease since 1972 when Rubler et al. studied post-mortem four patients with diabetes who manifested heart failure symptoms (12). Diabetic cardiomyopathy is currently defined as an obvious change in myocardial structure and function in diabetic patients, excluding ischemic diseases, hypertension, or other diseases that can induce myocardial damage (13). These structural changes such as cardiomyocyte hypertrophy, interstitial fibrosis, and lipid deposition in diabetic cardiomyopathy are characterized by an impaired diastolic function without an obvious decrease in systolic function (HFpEF, Heart Failure with preserved Ejection Fraction) (14).

The metabolic disturbances and structural abnormalities that result in diastolic dysfunction have a long asymptomatic latent period (15). Initially, in the development of diabetic cardiomyopathy left ventricular hypertrophy, remodelling, myocardial fibrosis, and diastolic dysfunction are central in the development of heart failure with normal ejection fraction (HFpEF). At later stages of diabetic cardiomyopathy, clinical heart failure will progress towards a reduced ejection fraction (HFrEF). But long before these signs, even in the prediabetic state, higher glucose levels are already associated with lower cardiac function parameters in prediabetes (10). This suggests that changes in cardiac function arise early in the development of T2DM.

Thus, as diabetic cardiomyopathy has become an increasingly recognized entity among clinicians, a better understanding of its pathophysiology is necessary for early diagnosis and the development of treatment strategies for diabetes-associated cardiovascular dysfunction. Since metabolic abnormalities such as cardiac lipid accumulation (11) and cardiac insulin resistance (8) seem to be the primary triggers for the cellular and molecular events that produce structural and functional changes in diabetic cardiomyopathy, the research in this PhD thesis focusses on the use of different imaging methods to study cardiac metabolism in health and insulin resistance. The use of advanced magnetic resonance (MR) methodology and PET-MRI helped us to better understand the metabolic changes that take place during the development of diabetic cardiomyopathy. Specifically, the importance of glucose metabolism is investigated.

How can cardiac metabolism be investigated?

Cardiac energy metabolism is mainly driven by fatty acid and glucose oxidation, meaning that mitochondria are largely fuelled by fatty acyl-coenzyme A and pyruvate (16). The contribution of ketone bodies and amino acids to overall cardiac oxidative metabolism is considered to be minor because of the low availability of these substrates under normal physiological conditions (16). The balance between glucose and fatty acid oxidation is important as the latter requires more oxygen, and myocardial efficiency thus depends on this balance. Therefore, an *in vivo* indication of cardiac mitochondrial function would be to image the substrate uptake and/or oxidation by PET MRI or PET CT. This makes it possible to dynamically monitor cardiac metabolism.

To this end, the injection of ^{18}F fluorinated substrates is a good choice as the half-time of ^{18}F is relatively long (109.8 minutes), making it an isotope that is rather easy to handle. The use of the glucose analogue [^{18}F]-2-fluoro-2-deoxy-D-glucose ([^{18}F]FDG) and the long-chain fatty acid analogue 14(*R,S*)-[^{18}F]fluoro-6-thia-heptadecanoic acid ([^{18}F]FTHA) during PET imaging can give insight in the myocardial uptake of glucose and fatty acids, since this tracer gets phosphorylated and converted into [^{18}F]FDG-6-phosphate ([^{18}F]FDG-6-P) and label-carrying metabolites of [^{18}F]FTHA respectively, which cannot be metabolized further and is thereby trapped in cardiomyocytes. The cardiac uptake of the tracer [14-^{18}F]-fluoro-6-thia-heptadecanoic acid provides information about the fatty acid uptake since the tracer, after the uptake, remains trapped in the tissue, and is proven to be closely linked to fatty acid oxidation rates (17). Furthermore, the FDG tracer can be used in combination with insulin stimulation

(e.g. during a hyperinsulinemic euglycemic clamp), and the uptake of FDG tracer in the tissue equals tissue-specific glucose uptake and thus directly visualizes tissue-specific insulin sensitivity. Such protocol can strongly increase our knowledge about insulin sensitivity of various tissues, not limited to the heart. We have also applied these techniques in **chapter 6** where we investigated how PPARα agonists influence tissue-specific insulin sensitivity in the prediabetic state.

Importantly, fluorinated tracers are important to investigate uptake of substrate, but oxidation cannot be investigated. To this end, other tracers must be used, such as the long-chain fatty acid [^{11}C]-palmitate tracer which, after uptake enters the mitochondrion and undergoes β-oxidation and is hereafter fully metabolized in the citric acid cycle. This means that the tracer [^{11}C]-palmitate is fully metabolized and thus completely metabolically cleared, allowing a detailed investigation of the cardiac palmitate uptake, oxidation, and esterification (18). However, practical use of ^{11}C tracers is limited for most clinics, because of the short half-life of 20 minutes and therefore the need of center-specific cyclotron for its production (17).

A pivotal aspect of cardiac metabolism is cardiac mitochondrial function as oxidative substrate oxidation is the main driver of cardiac metabolism. However, measuring cardiac mitochondrial function is not an easy task. The gold standard to measure mitochondrial function is through *ex vivo* high resolution respirometry, a technique that measures oxygen consumption rate of muscle fibers and which is used in **chapter 4**. This technique requires invasive tissue sampling, limiting its application in human research. As the tissue is isolated and tested in circumstances under a high concentration of substates, it not necessarily mimics the mitochondrial function under normal physiological circumstances. Hence, there is need for an *in vivo* - preferably non-invasive - method for the measurement of cardiac mitochondrial function.

Interestingly, a few reports assessed mitochondrial function *in vivo* using radio-active [^{99}M]-Technetium SPECT imaging (19, 20). [^{99}M]-Technetium washout rate may be affected by several factors, including age, sex, and sympathetic nerve function; though an increase in [^{99}M]-Technetium washout rate is thought to reflect impairment of mitochondrial function as a result of a decrease in the mitochondrial transmembrane potential. However, quantification of [^{99}M]-Technetium washout rate needs to be established by further investigations, since the cut-off value between normal and abnormal mitochondrial function is not yet determined (21).

Although it is often considered as a low risk, these imaging techniques occur at the costs of radiation burden of the isotope-labelled tracer and potentially also the CT (22). In this context, *in vivo* methods without exposure to ionizing radiation to measure cardiac mitochondrial function are warranted. Here, MRS can be an interesting technique, as it enables the *in vivo* quantification of endogenous metabolites and some metabolite concentrations may be related to mitochondrial function. In **chapter 4** we hypothesized that cardiac energy status, measured with the PCr/ATP ratio, may be associated with mitochondrial function. We assumed this since PCr buffers ATP concentration when ATP demand is increased, which means that the PCr/ATP ratio reflects the equilibrium between ATP synthesis and utilization. As energy (ATP) supply in the heart is almost entirely driven by mitochondrial oxidative metabolism, PCr/ATP may be an indicator of mitochondrial function. This hypothesis was supported by the fact that patients with T2DM and heart failure have both, a lower mitochondrial function and a lower PCr/ATP ratio (23-29). However, we did not find any correlations between PCr/ATP ratio determined *in vivo* and any of the mitochondrial respiration states measured *ex vivo*. Apparently, the PCr/ATP ratio is not solely influenced by mitochondrial function but also by additional factors that do not affect mitochondrial function such as variations in substrate and creatine availability, factors we were not able to study in this comparative study. Therefore, even though PCr/ATP remains an interesting parameter of cardiac health and is of prognostic value in heart failure (30), it is a non-specific reflection of mitochondrial function.

In summary, we used invasive and non-invasive techniques to investigate cardiac metabolism and mitochondrial function. The detection of insulin-stimulated FDG uptake is an elegant way to determine tissue-specific insulin sensitivity, while we conclude that PCr/ATP is not a valid surrogate marker for cardiac mitochondrial function and we largely depend on *ex-vivo* determination of mitochondrial function in tissue samples. Further technical development is important to improve these techniques in quality and also in practical applicability. This is necessary to gain more insight into metabolic changes and the role of mitochondrial dysfunction in cardiac pathologies, such as diastolic dysfunction in diabetic cardiomyopathy.

Is cardiac metabolism altered in the prediabetic state?

Diabetic cardiomyopathy is characterized by diastolic dysfunction, a condition which is shown to be present already in prediabetes (10). Evidence even indicates that higher glucose levels in prediabetes are associated with lower cardiac function parameters (10), suggesting that changes in cardiac function arise early in the development of T2DM. Changes in cardiac metabolism in response to hyperglycemia are considered an important pathway through which T2DM causes diabetic cardiomyopathy (31, 32). These metabolic alterations may be part of changes in the prediabetic state (to which we first conducted a literature review in **chapter 2** and later found indications in the cardiac energy status in **chapter 5**) and have been suggested to be associated with diastolic dysfunction in the prediabetic state. In **chapter 2** we aimed to summarize the current literature upon the metabolic alterations, which occur already in prediabetes and the relationship with cardiac function. Though the metabolic changes of prediabetes in cardiac metabolism are not yet unambiguous in literature, more is known about the consequences. In **chapter 5** we showed no differences in left ventricular ejection fraction between volunteers with prediabetes and healthy obese controls, which is in accordance with literature stating that systolic function is still preserved in prediabetes (33). Although systolic cardiac function is still normal in prediabetes, diastolic function may be compromised. As shown in **chapter 3**, the fat deposits around the heart (known as epicardial adipose tissue) are even in a healthy population strongly correlated with diastolic function parameters, as mechanistic hindrance possibly limits the distensibility of the myocardium (34).

Importantly, in **chapter 5** we found a decline in cardiac PCr/ATP in prediabetes in comparison to healthy overweight or obese individuals, showing that cardiac metabolism is already changed in the prediabetic state. Considering earlier evidence, we suspected that this may be related to increased plasma free fatty acids (FFA). However, as FFA did change considerably from the fasted to the fed state in the prediabetes group, and as FFA concentrations were not different between the prediabetes group and the healthy overweight or obese individuals, plasma FFA does not seem to be the underlying reason for lower PCr/ATP in prediabetes. Further research is needed, to better understand the underlying factors leading to a diminished PCr/ATP in prediabetes.

What are the metabolic consequences for the heart and liver of the altered substrate metabolism in prediabetes?

One of the metabolic changes during development of insulin resistance is a reduced oxidative capacity (3), which is typically associated with a blunted fat oxidation rate in the fasted state (4, 5). In combination with the elevated plasma FFA levels in obesity and prediabetes (35), this favours the accretion of fat accumulation ectopically (in skeletal muscle, heart and liver) (1, 36, 37).. In order to modify fat oxidative capacity, stimulating PPARα is a good option since PPARα controls genes involved in lipid and lipoprotein metabolism, and are abundantly expressed in tissues that require high rates of FA oxidation, such as in the heart and liver parenchymal cells (38). The results of PPARα agonism are described in **chapter 6** and show the importance of substrate competition for insulin sensitivity. Normally in prediabetes, substrate competition is due to overabundance of substrate FFA and glucose and may lead to ectopic fat accumulation. With PPARα stimulation substrate competition is driven by the stimulated fat oxidation and not by substrate oversupply, which may be the reason that despite the accumulation of fat in the liver, the metabolic effects were mild and that the measured increase in liver fat storage did not seem to affect whole body insulin sensitivity.

With the double-blind, randomized, placebo-controlled, crossover study described in **chapter 6** we showed that the changes in liver metabolism caused by insulin resistance are not immutable. Other researchers showed that just as the liver, the changes in cardiac metabolism and cardiac function are also reversible in the prediabetic state (39-41). Hence, it seems to be important to correct the changes in substrate metabolism in the early (pre)diabetic state, thereby avoiding long-term consequences, as these changes otherwise may precede towards NAFLD and diabetic cardiomyopathy. This emphasizes the need for studies intervening in the prediabetic state, to allow a better cardio-protection in the development of T2DM and the metabolic syndrome.

Future perspectives

Comparing the cardiac energy status (PCr/ATP ratio) with mitochondrial respiratory capacity indicates that PCr/ATP ratio does not necessarily reflect mitochondrial respiratory capacity (**chapter 4**). It is however important to keep in mind that these results are only associative and that the value of cardiac energy status remains indisputable in many cardiac pathologies, as it has been shown to be of prognostic value in heart failure (30). Future research is needed for a non-invasive methods to measure cardiac metabolism and cardiac mitochondrial function. The use of specific drugs can then be tested to prevent this mitochondrial function decline and thereby possibly preventing the reduction in cardiac function.

Another interesting finding in this thesis that requires more investigation is the reduction in glucose uptake in the liver accompanied by the increase of hepatic lipid content after 5 weeks of treatment with a PPARα agonist (**chapter 6**). It needs to be investigated in more detail to determine whether inhibiting glucose uptake in the liver is detrimental for hepatic function. It could be that specifically in prediabetes an augmentation of the fatty acid uptake and oxidation leads to a greater uptake relative to oxidation, and therefore contributes to NAFLD, and that this is not the case in other patient groups. It would therefore first of all be interesting to study how hepatic lipid content responds in healthy individuals without NAFL when treated with a PPARα agonist. Furthermore, measuring the long-term effects of the increased hepatic lipid content after PPARα treatment in prediabetes may tell us whether hepatic lipid accumulation caused by PPARα stimulation is detrimental or that the metabolic parameters such as insulin sensitivity stay unaffected as is the case after 5 weeks of treatment (**chapter 6**). Above all, more research into the metabolic effect of PPARα agonism in prediabetes may help us to understand the complex metabolic interplay between the heart and liver in prediabetes.

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Addendum

Impact

What is the main aim of the research described in this thesis and what are the most important results and conclusions?

The aim of this thesis is to investigate how cardiac metabolism is changed in prediabetes and how these changes contribute to diastolic dysfunction. Specifically, this thesis focusses on cardiac energy metabolism and cardiac insulin resistance in prediabetes, with special attention for the role of PPARα in regulating cardiac and hepatic metabolism.

Cardiac metabolism is subject of all chapters in this thesis and therefore this gives the reader an indication of where the gaps of our current understanding are, which is valuable for future research. In **chapter 2** the current literature on cardiac fat, lipid and glucose metabolism, and mitochondrial function in the prediabetic heart were reviewed. In prediabetes the increased cardiac fat accumulation is accompanied by an increased FFA uptake and oxidation. Although a vastly decreased glucose uptake, glucose oxidation, and a declined mitochondrial function, have been observed in T2DM, the few studies in prediabetes show conflicting results and the contribution of insulin resistance and mitochondrial inefficiency to the development of cardiac dysfunction remains unclear. It appeared that most studies focus on heart failure and type 2 diabetes mellitus (T2DM), and that the number of studies investigating cardiac metabolism in prediabetes are limited. Even less is known about whether PPARα can influence cardiac metabolism in prediabetes.

The urgency of broadening our knowledge of cardiac metabolism is also shown in **chapter 3**. It shows that even in healthy individuals with a normal cardiac function and without diabetes, an increase in the adipose tissue surrounding the heart (pericardial fat) is associated with a decline in diastolic function, possibly due to a mechanical hindrance. Nonetheless, the changes occur in an overweight, but metabolically rather healthy population and may therefore precede the onset of the metabolic syndrome. The importance of this pericardial fat depot, even in seemingly healthy people, might be underestimated in current literature that mostly focusses on individuals with T2DM.

To gain more insight in cardiac mitochondrial function, we investigated in **chapter 4** whether the cardiac energy status reflects cardiac mitochondrial function, since the ratio of phosphocreatine (PCr) over adenosine triphosphate (ATP) measured by ^{31}P-Magnetic Resonance Spectroscopy (^{31}P-MRS) is a non-invasive surrogate marker of cardiac energy status *in vivo*. However, our results do not support the use of cardiac energy status (PCr/ATP) as a surrogate marker of mitochondrial function in the heart. The dissociation of the two parameters in the present study suggests that mitochondrial function is not the only determinant of cardiac energy status.

While type 2 diabetes is recognized as CVD risk factor and some changes in cardiac metabolism have been reported, the role of metabolic changes in prediabetic heart remain elusive. This is studied in **chapter 5** where we find that cardiac energy status is already reduced in prediabetes. This is an important finding, because although cardiac energy status does not reflect mitochondrial function, it remains an important marker of cardiovascular health.

In **chapter 6** we explore the effects of stimulation of the PPARα pathway in prediabetes. Here, five weeks of treatment with PPARα tended to alter cardiac glucose metabolism and decreased insulin-stimulated glucose uptake in the liver. This is consistent with the expectation that PPARα stimulates fat uptake and oxidation, and through substrate competition inhibits glucose uptake in the liver and possibly in the heart. This is not accompanied by effects on whole body insulin resistance, nor on whole body glucose or fatty acid oxidation rates. Hence, these changes do not seem to be detrimental, despite the stimulatory effect on fat metabolism by the PPARα agonism. New research should be performed to explore the effects of prolonged treatment to see whether the changes in metabolism are permanent in the long term, and more specifically into fat metabolism since we did not study this specifically. It may be the case that through increasing fat oxidative capacity the accumulated fat in the liver is temporary, leading to a higher insulin sensitivity in prediabetes in the end. However, this remains speculative and a new study may lead to novel insights in the PPARα pathway in prediabetic humans.

What is the contribution of the results to science and societal changes?

The prevalence of prediabetes is extremely high worldwide and diastolic dysfunction is frequently present in people with prediabetes. Changes in cardiac metabolism are associated with diastolic dysfunction in type 2 diabetes and quite likely, prediabetic individuals are similarly affected. At present, knowledge about cardiac metabolism in people with prediabetes and the health consequences of disturbed cardiac metabolism are very limited.

The results of the current thesis can guide future research in this area. Firstly, this thesis can help with the choice of suitable methodology. ^{31}P-MRS measures cardiac energy status, which appears to be decreased in prediabetes indicating an increased CVD risk. In addition, a dynamic PET scan measuring glucose uptake during insulin stimulation provided insights upon the PPARα pathway in prediabetes and also highlighted the interplay between fatty acid and glucose metabolism in heart and liver. This study may stimulate future researchers to also use this dynamic PET technique, possibly even with other tracers than a glucose analogue.

In addition, the results of the research described in this thesis contribute to our understanding of human cardiac metabolism in prediabetes and its importance for cardiac function. Future studies can extend the findings presented in this thesis by investigating what effects PPARα agonism may have on the long term and investigate whether diastolic function improves after normalisation of pericardial fat volume. This can give guidance on which pathways are important to influence for the prevention or treatment of cardiac dysfunction in (pre)diabetes. Ultimately, this will contribute to improving prevention of T2DM, reducing health care costs and relieving the pressure on the health care system.

For whom are the results interesting and of relevance?

The results and conclusions presented in this thesis are interesting for other researchers, who can set-up new studies further investigating cardiac metabolic alterations in prediabetes and how these changes contribute to the development of diastolic dysfunction. These studies can make use of the techniques described in this thesis, specifically the dynamic PET-MRI technique during a hyperinsulinemic euglycemic clamp to specifically determine insulin-stimulated fat and glucose uptake and oxidation with different tracers. Ultimately, this knowledge could help in the prevention and treatment of diastolic dysfunction in diabetic

cardiomyopathy. In terms of prevention, this knowledge would be of interest for people with overweight and obesity, as these are at increased risk for prediabetes and diabetes.

Fibrates are known to treat hypertriglyceridemia and thereby reduce health risks in patients at high risk for cardiovascular diseases. Our study does not change the current indication for fibrates and has therefore no consequences for current fibrate users. In this thesis, we studied ciprofibrate as a modulator of PPARα, which has given more insights in the PPARα pathway in prediabetes. The fact that liver glucose uptake decreases and cardiac glucose uptake tends to decrease, demonstrates the effect of stimulating fat oxidation by PPARα. Knowledge from future studies building on our results can lead to more insights in cardiac metabolism, leading to the development of new drugs for the treatment of diastolic dysfunction, thereby reducing the risk for heart failure in diabetes and prediabetes.

Other researchers will be informed about the results described in this thesis through publications in scientific journals and presentations at national and international conferences. Results will also be shared on websites, social media and participant information events, thereby informing the people at risk for the development of diastolic dysfunction due to (pre)diabetes.

Summary

The prevalence of type 2 diabetes mellitus (T2DM) is strongly increasing and this leads to severe comorbidities and early mortality in western society. Worse, in prediabetes health risks are already apparent. This is due to the higher glucose concentrations (but still within the non-diabetic range) which affect the whole-body metabolism. In combination with a reduced oxidative capacity, this leads to a low fat oxidation in the fasted state and in the face of a high availability of free fatty acids, this favours the accretion of ectopic fat accumulation in muscle, heart and liver.

Data from clinical studies on cardiac metabolism in prediabetes is scarce, though current literature on the changes in cardiac metabolism in prediabetes are reviewed in **chapter 2**. The available data in obesity and T2DM, as well as animal studies however, do support the notion that metabolic changes in prediabetes might contribute to the development of diastolic dysfunction in humans. In the prediabetic state, an increased cardiac fat accumulation has been found, which was accompanied by an increased uptake and oxidation of free fatty acids (FFA). Although a vastly decreased glucose uptake, glucose oxidation, and a declined mitochondrial function have been shown in T2DM, the few studies in prediabetes show conflicting results and the contribution of insulin resistance and mitochondrial inefficiency to the development of cardiac dysfunction remains unclear.

Chapter 3 revealed that even in the absence of signs of pre-diabetes on a whole body level, an increase in the adipose tissue surrounding the heart (pericardial fat) is associated with a decline in diastolic function. Analysis of a large cohort consisting of 254 healthy patients with normal cardiac function, showed that pericardial fat independently of age, BMI, and sex, is associated with the four diastolic ultrasound parameters which are decisive in the evaluation of diastolic function. A potential underlying mechanism of this may be that an increase of the fat mass around the myocardium may compress the heart, leading to a limited distensibility in the diastole and fibrosis as seen in cardiac remodelling, and thus, may lead to diastolic dysfunction. This study adds to the growing body of research that explores possible mechanisms in the development of diastolic failure. However, the exact causality of this effect and the relationship with fibrosis remains to be determined.

To gain more insight in the mitochondrial function in prediabetes, research with a non-invasive *in vivo* method is needed. As oxidative phosphorylation is the major contributor to ATP synthesis, the cardiac energy status (determined as the ratio of phosphocreatine (PCr) over adenosine triphosphate (ATP)) which is measured with 31P-Magnetic Resonance Spectroscopy (31P-MRS) might be a reflection of cardiac mitochondrial function. To investigate the correlation between the non-invasive *in vivo* PCr/ATP ratio with the invasive *ex vivo* mitochondrial function, we enrolled thirty-eight patients scheduled for open heart surgery in our study described in **chapter 4**. The *ex vivo* assessment of mitochondrial function using high-resolution respirometry on tissue specimens from the right atrial appendage revealed no correlation with PCr/ATP ratio in the left ventricle. Thus, our results do not support the use of cardiac energy status (PCr/ATP) as a surrogate marker of mitochondrial function in the heart, however, the value of cardiac energy status remains indisputable in many cardiac pathologies as it has been shown to be of prognostic value in heart failure (1). The dissociation of the two parameters in the present study suggests that mitochondrial function is not the only determinant of cardiac energy status.

Although cardiac energy status does not directly correlate with cardiac mitochondrial function in **chapter 4**, cardiac PCr/ATP ratios were shown to be negatively correlated with the fasting plasma free fatty acid (FFA) concentrations (2, 3). As in prediabetes FFA in the morning are elevated, a lowered PCr/ATP ratio in prediabetes may be expected, as a hallmark of metabolic changes in the heart in the prediabetic state. Indeed, **chapter 5** shows that the PCr/ATP ratio in our insulin resistant prediabetic volunteers is lower in the morning, when compared to healthy overweight and obese controls. This suggests that the myocardial energy status is decreased already in prediabetes when glucose concentration is still normal, which is an important finding since PCr/ATP ratio is proven to be a predictive value for CVD morbidity and mortality (1) and hence is an important marker of cardiovascular health.

Chapter 6 focusses on the importance of PPARα in cardiac metabolism. Results from animal studies suggest that PPARα agonists can have metabolically beneficial effects, counteracting the negative effects of overweight, however, human data is largely lacking. We evaluated the effects of the PPARα agonist ciprofibrate on cardiac metabolism in 10 male prediabetic patients in a randomized cross-over trail. Five weeks of treatment with the PPARα ligand ciprofibrate decreases insulin-stimulated glucose uptake in the liver, with a similar tendency in the heart.

An increase in liver fat was not associated with a decline in whole body insulin sensitivity, nor with reduced cardiac function parameters or cardiac energy status. Our results are in line with the expectation that PPARα treatment results in a stimulation of fatty acid metabolism, affecting fatty acid uptake as well as oxidation. The induced metabolic changes in the prediabetic individual (e.g. increase in liver fat) were not associated with the typically seen negative effects of liver fat accumulation on whole body insulin sensitivity. These results suggest a stimulatory effect on fat metabolism by the PPARα agonist ciprofibrate in prediabetic humans, possibly more pronounced in prediabetics with a higher hepatic lipid content, without deterioration of whole-body glucose metabolism.

Overall, the studies described in this thesis investigated cardiac metabolism in prediabetes using advanced imaging methodologies, specifically focusing on cardiac mitochondrial function and dynamic glucose uptake. From our studies it appears that in prediabetes the cardiac energy status is decreased. Furthermore, cardiac function may already be hampered before the onset of prediabetes, here the accumulation of pericardial fat may be important which often increases together with BMI. Influencing the PPARα pathway on systemic level seems to affect both liver and heart metabolism, however, the long-term effects of such changes on metabolic health are still unknown. To investigate cardiac metabolism in more detail in humans, it is necessary to develop new non-invasive imaging methodologies. This will be instrumental in gaining more knowledge about the metabolic changes in obesity and prediabetes and may guide us towards novel targets in the prevention and treatment of diastolic dysfunction in (pre)diabetes.

Samenvatting

De prevalentie van type 2 diabetes mellitus (T2DM) neemt sterk toe en dit leidt in de westerse landen tot ernstige ziekten en vroegtijdig overlijden. De bijhorende gezondheidsrisico's zijn zelfs reeds in prediabetes aanwezig, doordat het verhoogde bloedsuiker (weliswaar nog steeds binnen het niet-diabetische bereik) het metabolisme (stofwisseling) reeds beïnvloedt. In combinatie met een verminderde oxidatieve capaciteit leidt dit tot een lage vetoxidatie (verbranding) in nuchtere toestand. De hierbij grote beschikbaarheid van vrije vetzuren bevordert de ophoping van ectopisch vet in spieren, hart en lever.

Gegevens uit klinisch onderzoek naar het hartmetabolisme bij mensen met prediabetes zijn schaars, de huidige literatuur over de veranderingen in het hartmetabolisme bij prediabetes wordt in **hoofdstuk 2** besproken. Zowel deze literatuur als data uit dierstudies ondersteunen het vermoeden dat metabole veranderingen reeds bij prediabetes optreden en zo bijdragen aan het ontstaan van diastole dysfunctie. Zo gaat de verhoogde vetstapeling in het hart bij prediabetes gepaard met een verhoogde opname en oxidatie van vrije vetzuren. Het effect van de bekende insuline resistent bij prediabetes op het cardiale glucose metabolisme en hartfunctie is vooralsnog onduidelijk, al leidt het in T2DM tot een sterk verminderde opname en oxidatie van glucose met daarbij ook een verminderde mitochondriale functie.

Hoofdstuk 3 onthulde dat zelfs in een gezonde populatie een toename van de vetopslag rondom het hart is geassocieerd met een afname van de diastole hartfunctie. Analyse van een groot cohort bestaande uit 254 gezonde patiënten zonder prediabetes en met een normale hartfunctie liet zien dat dit vet rondom het hart onafhankelijk van leeftijd, BMI en geslacht is geassocieerd met de vier belangrijkste diastole echo parameters. De verhoogde vetopslag rondom het hart leidt hierbij mogelijk tot diastole disfunctie doordat enerzijds de hartspier wordt samengedrukt waardoor het nog maar beperkt kan uitrekken in de diastole, en anderzijds het fibrose in de hand kan werken dat bekend is van cardiale remodellering. Hoewel de precieze causaliteit van deze toegenomen vetopslag in relatie tot fibrose nog moet worden vastgesteld, draagt onze cohort studie bij aan het ontrafelen van de mogelijke mechanismen die kunnen bijdragen aan het ontstaan van diastolisch hartfalen.

Om meer inzicht te krijgen in de mitochondriale functie bij prediabetes is onderzoek met een niet-invasieve *in vivo* methode nodig. Aangezien oxidatieve fosforylering de belangrijkste bijdrage levert aan de ATP synthese, zou de cardiale energie status (bepaald als de verhouding tussen fosfocreatine (PCr) en adenosinetrifosfaat (ATP)) die wordt gemeten met ^{31}P-Magnetic Resonance Spectroscopy (^{31}P-MRS) een weerspiegeling kunnen zijn van de cardiale mitochondriale functie. Om de correlatie tussen de niet-invasieve *in vivo* PCr/ATP ratio en de invasieve *ex vivo* mitochondriale functie te onderzoeken, onderzochten wij in **hoofdstuk 4** achtendertig patiënten die gepland waren voor een open hart operatie. Wij vonden geen correlatie tussen de cardiale energie status (PCr/ATP ratio) in het linker ventrikel (*in vivo*) en de mitochondriële functie gemeten met behulp van hoge-resolutie respirometrie op de weefselmonsters van het rechter hartoortje (*ex vivo*). Onze resultaten tonen dus dat de cardiale energie status (PCr/ATP) geen surrogaat marker is van de mitochondriale functie in het hart. Desalniettemin blijft de cardiale energie status van belang in veel cardiale ziektebeelden vanwege de prognostische waarde ervan bij hartfalen (1). De dissociatie van de twee parameters suggereert dat de mitochondriale functie niet de enige determinant is van de cardiale energie status.

Ondanks dat de cardiale energie status niet correleert met de cardiale mitochondriale functie in **hoofdstuk 4**, is aangetoond dat de cardiale PCr/ATP ratio's wel negatief correleren met de nuchtere plasma vrije vetzuur (FFA) concentraties (2,3). Aangezien bij prediabetes de FFA in de ochtend verhoogd zijn, kan daarom een verlaagde PCr/ATP ratio bij prediabetes in de ochtend worden verwacht, al is dat nog niet beschreven in de literatuur. **Hoofdstuk 5** laat inderdaad zien dat de PCr/ATP ratio in onze vrijwilligers met prediabetes in de ochtend lager is vergeleken met gezonde controles met overgewicht en obesitas. Dit suggereert dat de myocardiale energiestatus al bij prediabetes verlaagd is (zelfs wanneer de glucoseconcentratie nog normaal is) en dat er dus al in prediabetes metabole veranderingen zijn in het hart. Dit is een belangrijke bevinding omdat PCr/ATP ratio een belangrijke marker is voor cardiovasculaire gezondheid gezien zijn voorspellende waarde voor de morbiditeit en mortaliteit van cardiovasculaire ziekten (43).

Hoofdstuk 6 richt zich op het belang van PPARα werkingsmechanisme binnen het cardiale metabolisme. Resultaten van dierstudies suggereren dat PPARα agonisten metabool gunstige effecten kunnen hebben mede door de negatieve effecten van overgewicht tegen te gaan, maar gegevens over mensen ontbreken grotendeels. Daarom onderzochten wij de effecten van de

PPARα agonist ciprofibraat op het hartmetabolisme bij 10 mannelijke vrijwilligers met overgewicht en prediabetes in een gerandomiseerd cross-over onderzoek. Wij vonden dat vijf weken behandeling met de PPARα agonist ciprofibraat de insuline-gestimuleerde glucose-opname in de lever verminderde, met een vergelijkbare tendens in het hart. Een toename van levervet was niet geassocieerd met een afname van de insulinegevoeligheid van het gehele lichaam, noch met verminderde hartfunctie parameters of een verminderde cardiale energiestatus. Onze resultaten komen overeen met de verwachting dat PPARα-behandeling leidt tot een stimulering van het vetzuurmetabolisme, waarbij zowel de vetzuuropname als de oxidatie worden beïnvloed. De geïnduceerde metabole veranderingen in het prediabetische individu (bv. toename van levervet) gingen niet gepaard met de typisch gevonden negatieve effecten van toename van het levervet op de insulinegevoeligheid van het hele lichaam. Deze resultaten suggereren een stimulerend effect op het vetmetabolisme door de PPARα agonist ciprofibraat bij mensen met prediabetes, mogelijk meer uitgesproken bij prediabetici met een hoger gehalte aan levervet, zonder verslechtering van de insuline sensitiviteit van het hele lichaam.

In het algemeen hebben de in dit proefschrift beschreven onderzoeken met behulp van geavanceerde beeldvormende technieken het hartmetabolisme bij prediabetes onderzocht, waarbij de nadruk specifiek lag op de mitochondriale functie van het hart en de dynamische opname van glucose. Uit onze studies blijkt dat bij prediabetes de cardiale energiestatus verlaagd is. Bovendien kan de hartfunctie al vóór het begin van prediabetes belemmerd zijn, waarbij de toename van vet rondom het hart (die vaak samen met de BMI toeneemt) een belangrijke rol kan spelen. Beïnvloeding van het vetmetabolisme via PPARα op systemisch niveau lijkt zowel het lever- als het hartmetabolisme te beïnvloeden, maar de lange termijn effecten van dergelijke veranderingen op de metabole gezondheid zijn nog onbekend. Om het hartmetabolisme bij de mens gedetailleerder te onderzoeken, is het noodzakelijk nieuwe niet-invasieve beeldvormende technieken te ontwikkelen. Dit zal bijdragen tot het verkrijgen van meer kennis over de metabole veranderingen bij obesitas en prediabetes, en kan ons leiden naar nieuwe aangrijpingspunten voor de preventie en behandeling van diastole disfunctie bij (pre)diabetes.

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About the author

Vera de Wit was born on July 28th 1991 in Berg en Terblijt, the Netherlands. After graduating high school at the Porta Mosana College in Maastricht, she started Technical Health Engineering at Technical University Eindhoven to gain more insight into the organization of healthcare.

When she was able to start medical training, this change of career path felt like a dream come true. After obtaining her Bachelor's degree in Medicine in 2013, she continued the Master's program Medicine at Maastricht University. The internships in The Netherlands, Germany and Ecuador sparked her interest in internal medicine and cardiology. This also motivated her to perform the scientific internship at The Maastricht Study, where her research focused on the role of glucose metabolism status in the development of white matter hyperintensities (abnormalities) in the brain.

After obtaining the Medical Doctor degree, she was given the opportunity to do further metabolic research within the Diabetes and Metabolism Research Group at Maastricht University. As of September 2016, she started her PhD under supervision of prof. dr. Patrick Schrauwen, dr. Vera Schrauwen-Hinderling and dr. Tineke van de Weijer. During her PhD she focussed on human (intervention) studies aiming to further understand cardiac metabolism in people with (pre)diabetes. In this human research she applied both invasive techniques, such as hyperinsulinemic euglycemic clamps and biopsies, and non-invasive techniques such as Magnetic Resonance Spectroscopy and Positron Emission Tomography.

She found it challenging that she not only got the chance to learn these techniques, but also to learn project management from A to Z. She was allowed to propose research ideas, develop them further, and discuss them with other experts in the field. She enjoyed working together and found it very important to involve other disciplines in this process. She was also medically responsible for a number of projects and biopsies within the research group from other PhD students and also enthusiastically taught medical students for their regular curriculum.

In addition, she devoted herself to linking the group's scientific research with society. She set up different social media platforms to communicate findings from the research group in order to inform and create awareness of science-related topics, and herewith indirectly highlighting the importance of participating in scientific research. In addition she initiated also science inreach by restarting cross-departmental research meetings within the research school NUTRIM. She also raised money for the Dutch diabetes fund, a foundation that supported her own research.

Following the end of her PhD project in 2021, Vera returned to practicing medicine starting as a resident (ANIOS) at Geriatric Medicine, Department of Internal Medicine at Zuyderland hospital in The Netherlands. Here she felt grateful to contribute to the care of seriously ill COVID patients during the pandemic, and she experienced the impact of a comorbidity such as diabetes on their prognosis.

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