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Myocardial Steatosis and Left Ventricular Function in Type 2 Diabetes Mellitus : Assessed with Magnetic Resonance Imaging and Spectroscopy

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Myocardial Steatosis and Left Ventricular Function in Type 2 Diabetes Mellitus

Assessed with Magnetic Resonance Imaging and Spectroscopy

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Myocardial Steatosis and Left Ventricular Function in Type 2 Diabetes Mellitus

Assessed with Magnetic Resonance Imaging and Spectroscopy

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General introduction and outline

The global epidemic of type 2 diabetes mellitus (T2DM) is strongly associated with the increasing prevalence of obesity due to changing lifestyles, such as the consumption of more calorie-rich food and a sedentary lifestyle. T2DM is a major risk factor for cardiovascular disease, in particular coronary artery disease and congestive heart failure, and for early death. However, even in the absence of coronary artery disease or hypertension, cardiac functional and structural changes occur in asymptomatic subjects with T2DM.^{1,2} These abnormalities have been ascribed to diabetic cardiomyopathy. Although the pathophysiology underlying diabetic cardiomyopathy is complex and the exact mechanisms of disease have not been completely clarified, micro-angiopathy, autonomic neuropathy, increased collagen accumulation, and metabolic abnormalities have been proposed.

Increasing evidence is emerging indicating that lipid oversupply to cardiomyocytes, which may lead to lipotoxic injury, plays a role in the development of diabetic cardiomyopathy.³⁻⁵ Increased fluxes of non-esterified fatty acids arising from the disproportionate amount of insulin resistant (visceral) adipose tissue lead to excessive delivery and uptake of non-esterified fatty acids by the heart. This uptake of non-esterified fatty acids exceeds the oxidative capacity of the organ giving rise to accumulation of myocardial triglycerides. Neutral triglycerides are probably inert and harmless to cells and may initially even provide a protective buffer by diverting fatty acids from deleterious pathways.⁶ Eventually, however, excessive triglyceride stores enter a continuous cycle of hydrolysis and fatty acid re-esterification, yielding cardiotoxic intermediates, such as ceramide and diacylglycerol, which seems to be an important route leading to myocardial dysfunction, at least in animal models. Evidence exists that accumulation of these fatty acid intermediates causes mitochondrial dysfunction and reactive oxygen species, leading to myocardial dysfunction either directly through cell-damage and apoptosis or indirectly through the induction of inflammatory cascades.⁷⁻¹⁰

Some of the earliest descriptions of fatty degeneration of myocytes originate from the 19th century.^{11,12} However, it took more than a century until lipid cardiomyopathy was identified in the hypertrophied hearts of obese mice.¹³ Thereafter, the deleterious effect of fatty acids on myocytes has been well documented in animal models. In ZDF fa/fa rats, evidence of increased non- β -oxidative fatty acid metabolism in the myocardium is reflected by elevations in myocardial triglyceride and ceramide content¹⁰ and by increased myocardial oxidative stress.¹⁴ Furthermore, there is echocardiographic evidence of reduced myocardial contractility attributed to loss of functioning myocytes through apoptosis. Therapeutic interventions aiming at reduction of myocardial triglyceride accumulation due to disturbed fatty acid metabolism have been shown to have beneficial effects on myocardial function.¹⁰

Despite the evidence mentioned above that fatty acids and ceramide may damage cardiomyocytes, myocardial lipotoxicity is not currently recognized as a clinical entity.

Nonetheless, a reduction in the number of cardiomyocytes through apoptosis is a recognized cause of heart failure,¹⁵ and lipotoxicity could well be an important cause of apoptosis.^{10,16} Furthermore, in explanted hearts of obese and T2DM patients with end-stage heart failure, lipid staining was a common finding.⁴

Magnetic resonance is one of the most versatile imaging modalities that allows assessment of myocardial morphology, biochemistry and function on the same instrument. Developments in proton magnetic resonance spectroscopy (¹H-MRS) and magnetic resonance imaging (MRI), enable us to investigate myocardial triglyceride accumulation and myocardial function non-invasively. Since the introduction of human myocardial ¹H-MRS, it has been recognized as a promising tool for in vivo assessment of intracellular triglyceride content.¹⁷ Major problems concerning myocardial and respiratory motions which greatly influence spectral quality and reproducibility were solved by applying a combination of cardiac and respiratory triggering.¹⁸ ¹H-MRS can thus provide important contributions to the elucidation of the role of fat accumulation in the human heart in health and disease. In addition, MRI is an established tool to evaluate cardiac function. Systolic function can reproducibly be calculated by assessing myocardial volumes. In addition, flow velocity encoded MRI across the mitral valve gives insight in left ventricular filling dynamics, representing diastolic function.

OUTLINE OF THIS THESIS

The purpose of the studies described in this thesis is to provide more insight into the influence of myocardial triglyceride accumulation on left ventricular function in healthy volunteers and in patients with type 2 diabetes mellitus.

In **chapter 2**, cardiovascular metabolic MR techniques such as ¹H-MRS are discussed and some examples of their clinical use are shown. In **chapter 3**, reproducibility of the assessment of myocardial triglyceride content by ¹H-MRS with the use of respiratory motion compensation based on navigator echoes is evaluated. **Chapter 4** evaluates the influence of a short-term very low calorie diet on myocardial and hepatic triglyceride accumulation and on myocardial function in healthy subjects. In **chapter 5**, the findings of chapter 4 are extended by assessing the influence of complete caloric restriction on myocardial and hepatic triglyceride accumulation and on myocardial function. To further elucidate the influence on ectopic triglyceride depositions of different dietary calorie and fat intake, **chapter 6** describes the adaptations of the liver and the heart to a high-fat, high-energy diet in healthy volunteers. **Chapter 7** uses MR imaging and spectroscopy to study the association between physiological ageing, myocardial triglyceride content, and heart function.

In **chapter 8**, the influence of adding acipimox to a very low calorie diet in patients with T2DM is investigated. In **chapter 9**, cardiovascular function in patients with uncomplicated T2DM is compared to age- and body mass index matched healthy subjects. **Chapter 10** describes myocardial triglyceride accumulation and function in the diabetic heart, and finally, **chapter 11** evaluates the effects of pioglitazone treatment in patients with uncomplicated T2DM on myocardial metabolism and function.

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Technical evaluation

PART I

Cardiovascular molecular magnetic resonance imaging

HJ Lamb

RW van der Meer

A de Roos

JJ Bax

Eur J Nucl Med Mol Imaging 2007;34:S99-S104

ABSTRACT

Background

Cardiovascular molecular imaging is a rapidly evolving field of research, aiming to image and quantify molecular and cellular targets in vivo. MR imaging has some inherent properties that make it very suitable for cardiovascular molecular imaging. Until now, only a limited number of studies have been published on cardiovascular molecular imaging using MR imaging.

Review

In the current review, MR techniques that have already shown potential are discussed. Metabolic MR imaging can be performed by ^{31}P -MR spectroscopy, ^{23}Na -MR spectroscopy and ^1H -MR spectroscopy; some examples are shown. Furthermore, a concise overview is given of several aspecific and specific contrast agents for cardiovascular molecular MR imaging, such as gadolinium-based contrast agents, iron oxide MR contrast agents and fibrin-targeted MR contrast agents.

Conclusion

We expect that in the next decade currently promising MR molecular imaging agents will be introduced into the clinical arena to guide diagnosis and therapy of cardiovascular disease.

Conventional imaging modalities, including magnetic resonance (MR), are primarily based on anatomical, functional or metabolic properties to study (patho)physiology. Molecular imaging is a rapidly evolving field of research, aiming to image and quantify molecular and cellular targets in vivo. Molecular imaging can be applied to a wide range of scientific and clinical fields of interest. One of the most promising applications of molecular imaging is in the field of cardiovascular imaging. Imaging of cardiac anatomy, dimensions and function has some limitations concerning, for example, prediction of therapy outcome. Addition of specific information on, for instance, plaque composition and total plaque burden can be very helpful in guiding therapy.

Imaging of molecular processes is desirable because cardiovascular disease may be detected earlier, risk stratification may be more accurate, monitoring of innovative therapies may be improved, or a more accurate prognosis may be provided.¹

MR imaging has some inherent properties that make it very suitable for cardiovascular molecular imaging. The interaction between inherent tissue properties and specific contrast agents may lead to more specific clinical conclusions and prediction of therapy outcome. Thereby, cardiovascular molecular MR imaging may help in diagnosing cardiovascular disease, and in deciding whether expected beneficial effects of (invasive) therapy counterbalance the risk of complications of therapy.

A conventional approach to molecular MR imaging concerns MR spectroscopy (MRS). Furthermore, there are two main innovative contrast agents that may be used clinically soon: (1) iron oxide MR contrast agents and (2) fibrin-targeted MR contrast agents.

MAGNETIC RESONANCE SPECTROSCOPY

MR spectroscopy allows non-invasive characterization of myocardial metabolism. In principle, MRS is a pure form of molecular 'imaging' technique. Clinically, several nuclei allow non-invasive MRS of the heart. Initially, human MRS research was focused on the ^{31}P nucleus to study high-energy phosphate metabolism. An example concerning the effects of type 2 diabetes mellitus on myocardial high-energy phosphate metabolism is shown in Figure 2.1.² Another interesting new application of ^{31}P -MRS was published by Smith et al.,³ who measured myocardial creatine kinase (CK) metabolite concentrations and adenosine triphosphate (ATP) synthesis through CK, the primary energy reserve of the heart, to test the hypothesis that ATP flux through CK is impaired in patients with left ventricular hypertrophy (LVH) and chronic heart failure. It turned out that myocardial ATP levels were normal, but creatine phosphate levels were 35% lower in LVH patients than in normal subjects. Furthermore, the myocardial CK rate constant was normal in LVH, but halved in patients with LVH combined with chronic heart failure. Thereby, they could show that it is not the relative or absolute CK metabolite pool size but rather the kinetics

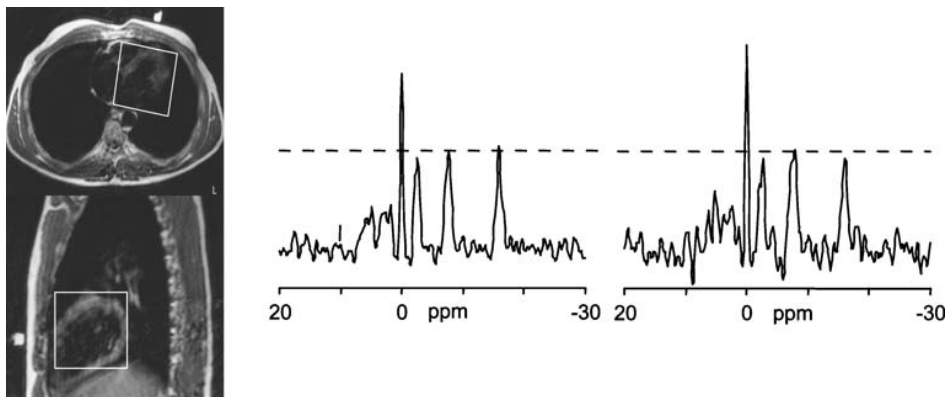


Figure 2.1

Left panel: planscan of the volume of interest on transverse and sagittal spin-echo MR images. Right panel: phosphorus-31 magnetic resonance spectroscopy (^{31}P -MRS) obtained at rest from the anterior left ventricular wall in a patient with type 2 diabetes mellitus (left) and a healthy subject (right). Note the decreased signal amplitude of phosphocreatine in the patient with type 2 diabetes mellitus. Courtesy of Diamant et al.²

of ATP turnover through CK that distinguishes failing from non-failing hypertrophic hearts. These observations support the hypothesis that a deficit in myofibrillar energy delivery contributes to chronic heart failure pathophysiology in human LVH. The same technique was applied by Weiss et al. to study ATP flux through CK in the normal, stressed and failing human heart.⁴ The latter studies are elegant examples of the capability of MR to measure non-invasively the concentration of metabolites and even the rate constant of enzyme turnover.

Another interesting application of metabolic imaging is ^{23}Na -MR imaging, as shown by Jansen et al. They applied this innovative spectroscopic imaging technique as a diagnostic modality for early detection of myocardial ischemia and viability in a rat model.⁵ They tested whether ^{23}Na -MR imaging can be used to assess viability after low-flow ischemia. ^{23}Na -MR chemical shift imaging was alternated with ^{23}Na -MR spectroscopy. ^{23}Na -image-intensity increased with increasing severity of ischemia. ^{23}Na -image intensity at end low-flow ischemia was well correlated with CK release during reperfusion, as well as with infarct size. Therefore, their study indicates that ^{23}Na -MR imaging is a promising tool for the assessment of myocardial viability. Ouwerkerk et al. applied ^{23}Na -MR imaging to measure cardiac tissue sodium concentrations in the human myocardium.⁶ They used a surface coil at 1.5T MR to non-invasively quantify regional myocardial sodium concentrations in the left ventricular (LV) free wall, septum and adipose tissue. Their ^{23}Na -MR imaging results were consistent with prior invasive measurements on biopsy and autopsy specimens.

In the past, ^1H -MRS of the myocardium was first applied to non-invasively study creatine depletion in non-viable infarcted myocardium.⁷ Total creatine was measured in the pos-

terior and anterior left ventricle and septum, and was significantly lower in regions of infarction than in non-infarcted regions of myocardium in patients or in the myocardium of healthy controls. Therefore, they showed for the first time that spatially localized ^1H -MRS can be used to measure total creatine non-invasively throughout the human heart. The detection of regional creatine depletion may provide a metabolic means to distinguish healthy from infarcted non-viable myocardium. Szczepaniak et al. used ^1H -MRS to measure myocardial triglyceride content.⁸ Studies in rat tissue ex vivo and in healthy humans in vivo provided evidence that ^1H -MRS constitutes a reproducible technique for the measurement of myocardial triglyceride content. Increased myocardial triglyceride content was accompanied by elevated LV mass and suppressed septal wall thickening as measured by cardiac imaging. More recently, ^1H -MRS of the human myocardium was improved by implementing the respiratory navigator technique to monitor diaphragmatic motion, and thereby correct data acquisition prospectively for breathing motion (Figure 2.2). First results from our institution show improved reproducibility of human cardiac ^1H -MRS measurements when using the respiratory navigator technique, as compared with conventional continuous breathing. Respiratory navigator gated ^1H -MRS was recently applied in an experimental setting in our institution to evaluate the effects of a very low

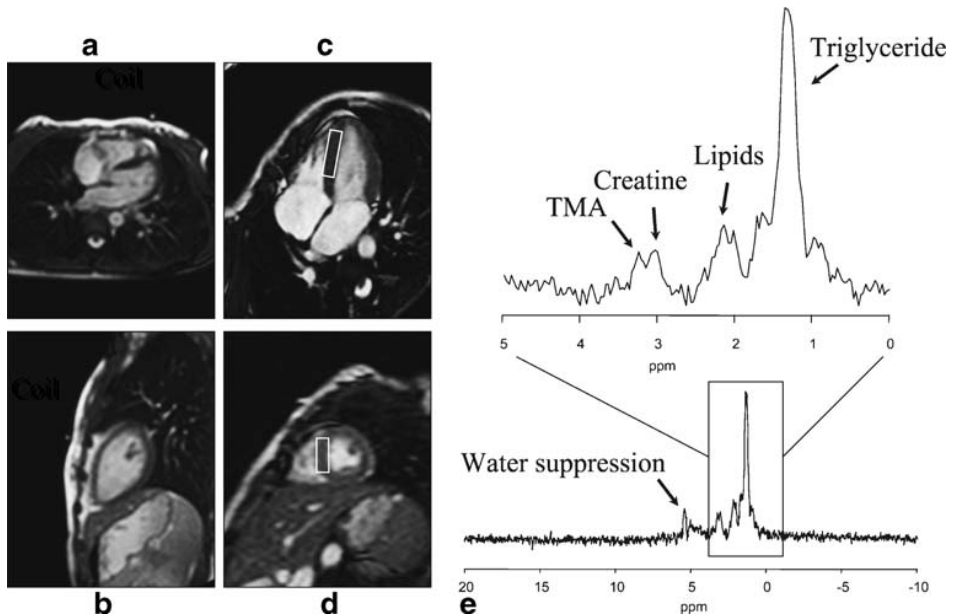


Figure 2.2

The surface coil was positioned just below the mitral valve level of the heart (a, b). Spectroscopic volume localization in the interventricular septum on four-chamber (c) and short-axis (d) views. Special care was taken to avoid contamination from epicardial fat. Panel e shows a typical water-suppressed ^1H -MR spectrum of myocardial tissue located in the interventricular septum. Peak heights are in arbitrary units.

calorie diet on myocardial triglyceride content. First results show that after a short-term very low calorie diet, there is an increase in intramyocardial triglyceride content.

GADOLINIUM-BASED CONTRAST AGENTS

Gadolinium-based contrast agents can be applied to study regional myocardial perfusion. After a rapid intravenous contrast injection, there is marked signal enhancement first in the RV cavity, then in the LV cavity, and subsequently in the LV myocardium.⁹ The peak signal intensity is related to the concentration of the contrast agent in the local tissue and is directly proportional to the coronary blood flow. Perfusion MR at rest and after infusion of pharmacological agents (adenosine and persantine) have been compared with standard methods (angiography or radionuclide scintigraphy) and demonstrated reasonable sensitivity (67–83%) and specificity (75–100%).⁹

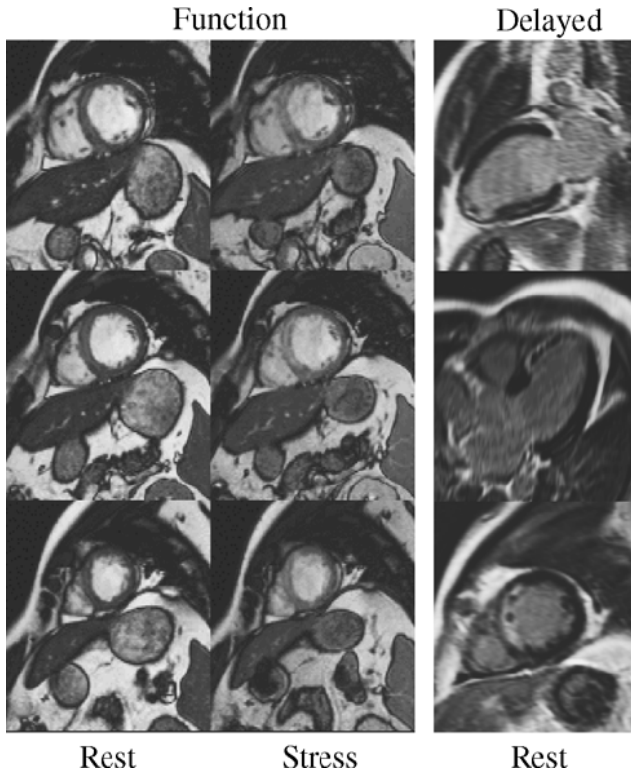
Currently, multiple MR imaging techniques are available to assess myocardial viability. Cardiovascular MR imaging can be used to assess end-diastolic wall thickness and contractile function at rest.¹⁰ Segments with an end-diastolic wall thickness < 6 mm most likely represent transmural scar formation, and contractile function will not improve after myocardial revascularisation. Dobutamine MR imaging can be used to evaluate contractile reserve, in a similar manner to dobutamine echocardiography. Gadolinium contrast-enhanced MR imaging allows for detection of the extent and transmural extent of scar tissue (Figure 2.3).¹¹ Recently reported sensitivity and specificity are in the range of 74% and 82% respectively.

Based on as yet unpublished scientific developments, it is expected that gadolinium-based delayed enhancement of the vessel wall may become reality. This MR imaging technique may allow fast total body screening for total plaque burden, an important predictor for morbidity and mortality.

In general, gadolinium-based contrast agents are not perfectly suited for molecular imaging because of the inherent high threshold of detectability. Therefore, new contrast agents are under development to potentiate the effect of distortion of the magnetic field.

IRON OXIDE MAGNETIC RESONANCE CONTRAST AGENTS

Superparamagnetic iron oxide (SPIO) particles can be detected at micromolar concentrations of iron, and offer sufficient sensitivity for MR imaging.¹² SPIO-based cellular imaging has become an established technique to label and detect cells of interest. Imaging of macrophage activity was the initial and is still the most significant application, with several products either approved for or in clinical trials.¹² Another exciting application of

**Figure 2.3**

Left panel: short-axis MR images at rest and during dobutamine stress. Note the lack of improvement in myocardial wall motion in the anteroseptal region when dobutamine stress is applied. Right panel: delayed gadolinium contrast-enhanced MR images in two-chamber, four-chamber and short-axis views. Note the almost transmural delayed enhancement of the anteroseptal/apical region, corresponding to the region without contractile response due to dobutamine stress in the left panel. The anteroseptal region is considered as 'non-viable' myocardial tissue.

SPIOs is labeling of myocardial stem cells. In a swine model for myocardial infarction, magnetically labeled stem cells were injected in the infarcted myocardial region (Figure 2.4). Using delayed contrast-enhanced MR imaging, the infarcted area can be identified with high accuracy. New technical developments may even allow specific delivery of magnetically labelled therapeutics to the infarcted myocardial region.¹² Combined with measurements of myocardial function, MR imaging seems an excellent modality for planning, delivery and follow-up of myocardial stem cells as therapy for ischemic heart disease.

Another promising application of SPIO MR imaging is visualisation of vessel wall inflammation. SPIOs are 'digested' by macrophages, involved in inflammatory processes. Imaging of the SPIO-induced magnetic inhomogeneities allows for imaging of inflamma-

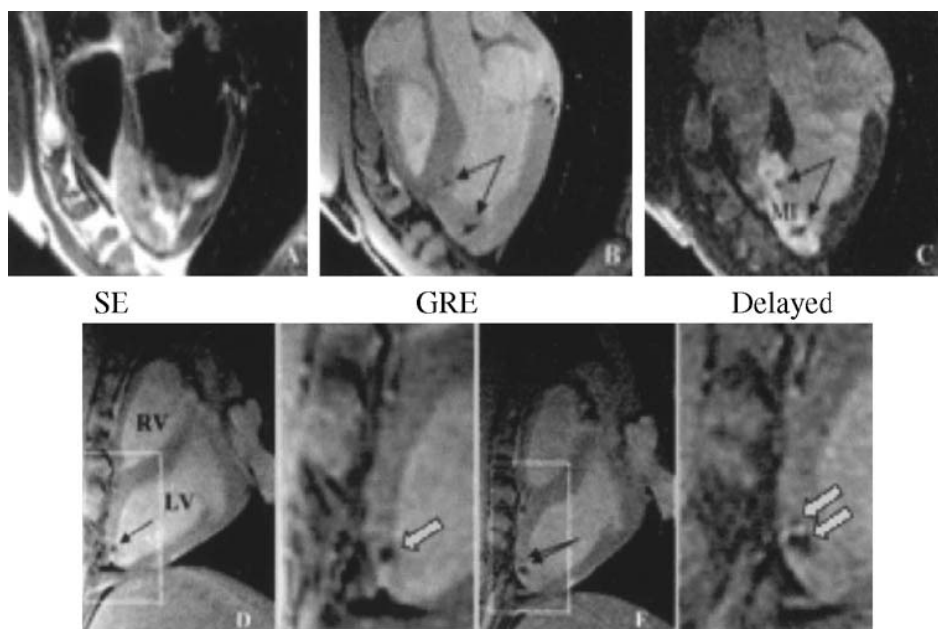


Figure 2.4

Detection of delivery and migration of Feridex-labelled myocardial stem cells in a swine model. Hypointense lesions in spin-echo (SE), gradient-echo (GRE) and delayed-enhanced (Delayed) MR imaging (upper panel) of injection sites (arrows) within 24 hours of intramyocardial injection. Cells were injected in the myocardial infarct (MI). Long-axis MR images (lower panel) show hypointense lesions (arrows) caused by labelled myocardial stem cells acquired within 24 hours and 1 week.

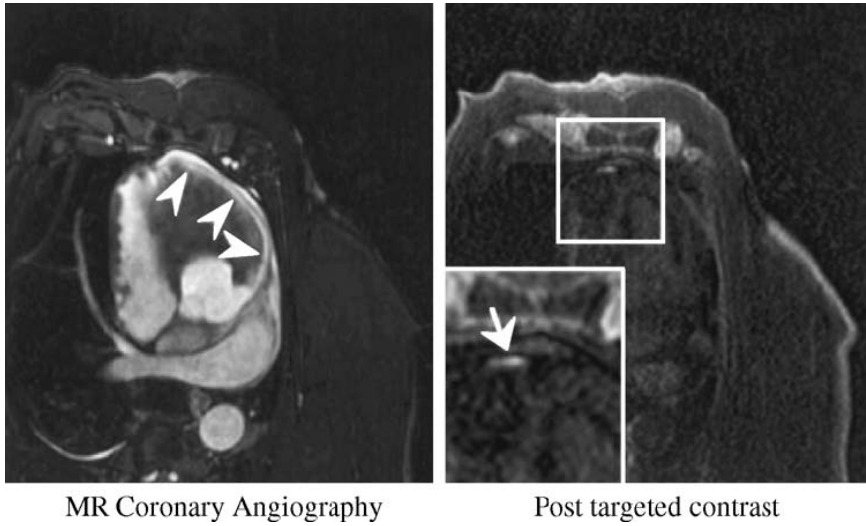
LV = left ventricle; RV = right ventricle. Courtesy of Bulte and Kraitchman.¹²

tion. Such an approach is currently only available in a research setting; it is, however, expected that these contrast agents will become available for clinical application soon.

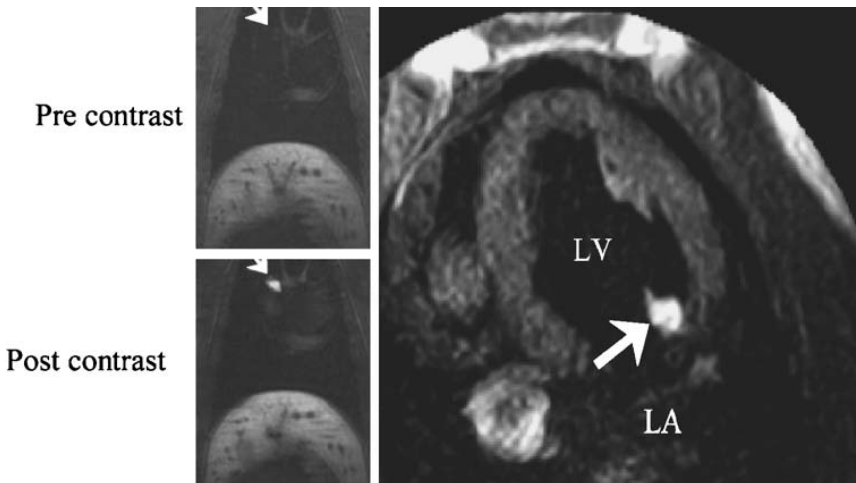
FIBRIN-TARGETED MAGNETIC RESONANCE CONTRAST AGENTS

Exciting recent developments allow selective and non-invasive molecular MR imaging of thrombus.¹³ The principle of the contrast agent is that it is targeted to fibrin. In an elegant study by Spuentrup et al., a swine model was used to test the innovative fibrin-targeted MR imaging contrast agent, which can be administered intravenously.¹⁴ The imaging protocol consisted of coronary MR angiography to demonstrate the lumen of a coronary artery, combined with molecular thrombus MR imaging. Thereby, anatomical information can be linked to specific information of vessel wall components. In an area of focal coronary artery stenosis, intraluminal thrombus could be detected (Figure 2.5).

The same contrast agent can be applied to detect, for example, right atrial thrombus, a potential source of more distal emboli (Figure 2.6). An atrial clot could be visualized eas-

**Figure 2.5**

Coronary thrombus visualization with a fibrin-targeted molecular MR imaging contrast agent. Left panel: double-oblique white blood coronary MR angiography (multiplanar reconstruction) demonstrating the lumen of the left anterior descending artery with bright signal (arrowheads). Right panel: double-oblique MR images after administration of the fibrin-targeted contrast agent (multiplanar reconstruction, same orientation). Note the increased signal (arrow) in the left anterior descending coronary artery, corresponding to thrombus. Courtesy of Spuentrup et al.¹⁴

**Figure 2.6**

Molecular MR imaging of atrial clot in a swine model. The left panel shows pre-contrast (upper) and post-contrast (lower) coronal images. Note the presence of high MR signal in the area of the right atrium, indicating an atrial clot. The right panel shows increased MR signal in the left atrium (LA), corresponding to a left atrial clot (arrow). These clots are potential sources for more distal emboli. LV = left ventricle. Courtesy of Spuentrup et al.¹⁴

ily with this molecular MR imaging technique, by intravenous administration of the fibrin-targeted contrast agent. Furthermore, MR clot imaging can be combined with functional imaging of the heart in the same imaging session. An even more exciting application of this fibrin-targeted contrast agent is detection of pulmonary emboli (Figure 2.7).

The above-described applications of molecular MR imaging may be especially suitable for fast screening for cardiovascular disease in an emergency setting. Patients presenting with chest pain in the emergency room can be studied by MR imaging to confirm or rule out ischemic heart disease or pulmonary embolus. Molecular MR imaging using fibrin-targeted contrast agents allows selective visualization of acute coronary, cardiac and pulmonary thrombi. Additional functional cardiac imaging can help determine the functional effects of detected thrombi.

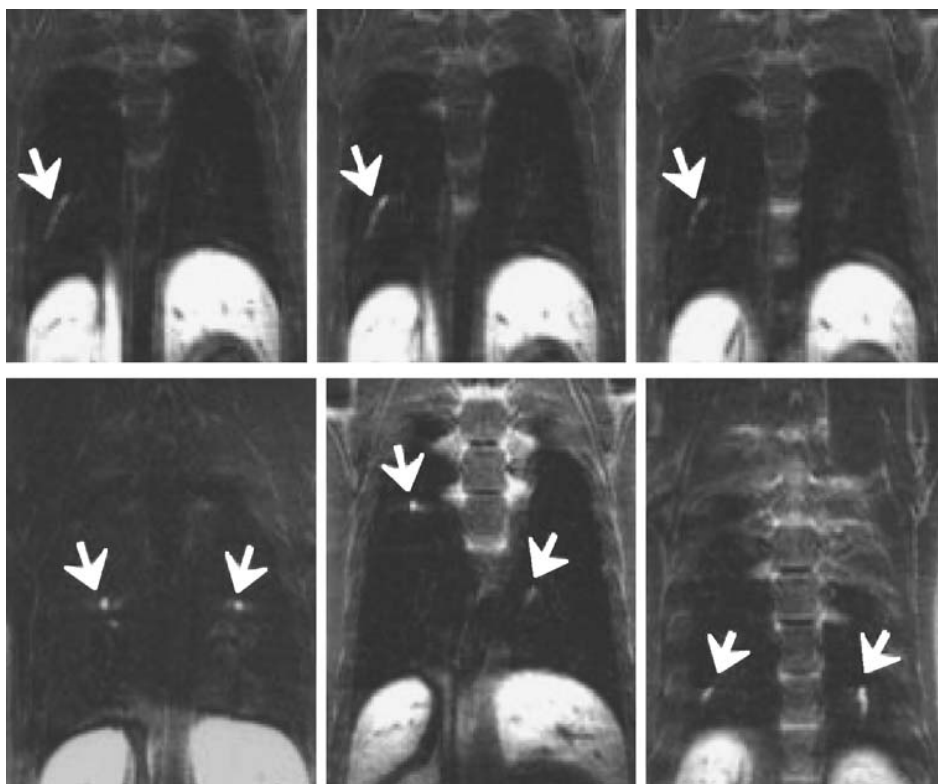


Figure 2.7

Examples of molecular MR imaging of pulmonary embolus. Two examples are shown, each consisting of three adjacent coronal slices (horizontal). The MR imaging technique is such that signal from surrounding blood pool and lung parenchyma is suppressed. The upper row shows pulmonary embolus (arrow) in the right lower lobe. The lower panel shows bilateral pulmonary emboli (arrows) after intravenous administration of fibrin-targeted MR imaging contrast agent. Courtesy of Spuentrup et al.¹⁴

CONCLUSION

Molecular MR imaging is an exciting and rapidly evolving new area of cardiovascular imaging. MR imaging seems very suitable for molecular imaging, although many technical difficulties have to be overcome. The main current limitation is the low sensitivity of MR imaging to detect small changes in magnet homogeneity. We expect that in the next decade, currently promising MR molecular imaging agents will be introduced into the clinical arena to guide diagnosis and therapy of cardiovascular disease.

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Metabolic imaging of myocardial triglyceride content: reproducibility of ¹H-magnetic resonance spectroscopy with respiratory navigator gating in volunteers

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ABSTRACT

Background

The purpose of this study was to prospectively compare spectral resolution and reproducibility of hydrogen 1 (^1H) magnetic resonance (MR) spectroscopy, with and without respiratory motion compensation based on navigator echoes, in the assessment of myocardial triglyceride content in the human heart.

Methods

In 20 volunteers (14 men, 6 women; mean age $\pm$ standard error, 31 ± 2.8 years [range, 19 – 60 years]; body mass index, 19 – 30 kg/m²) without history of cardiovascular disease, ^1H -MR spectroscopy of the myocardium was performed at rest, with and without respiratory motion compensation.

Results

Unsuppressed water signal linewidth changed from 11.9 to 10.7 Hz ($P < 0.001$) with the use of the navigator, which indicated better spectral resolution. The navigator improved the intraclass correlation coefficient for the assessment of myocardial triglyceride content from 0.32 to 0.81.

Conclusion

Respiratory motion correction is essential for reproducible assessment of myocardial triglycerides.

Proton (hydrogen 1 [^1H]) magnetic resonance (MR) spectroscopy is a promising tool for metabolic imaging to assess triglyceride content of myocardial tissue in humans.¹⁻³ Findings from rat studies have shown that there is a negative correlation between myocardial triglyceride content and heart function, while treatment with insulin-sensitizing drugs reduced myocardial triglyceride deposition and reversed contractile dysfunction in lipotoxic heart disease in obese Zucker rats.^{4,6} These findings suggest that intramyocardial triglyceride accumulation is deleterious to the heart.⁷ Furthermore, myocardial triglyceride content may be a marker of myocardial viability after coronary occlusion due to enhanced esterification and/ or reduced oxidation of fatty acids in ischemically insulted but viable myocardium.⁸

Motion artifacts from cardiac and respiratory motion have a negative effect on the reliability of myocardial ^1H -MR spectroscopy. Motion of the heart relative to the volume of interest may lead to reduced spectral resolution and contamination of the ^1H -MR spectrum by, for example, epicardial fat. In addition, respiratory motion may negatively influence ^1H -MR spectral resolution by preventing optimal shimming and water suppression.

Several methods for respiratory gating have been proposed to improve repeatability and spectral resolution at ^1H -MR spectroscopy.^{2,3,9} Recently, respiratory navigator gating and volume tracking for double-triggered cardiac ^1H -MR spectroscopy became available.¹⁰ However, the influence of respiratory navigator gating on spectral resolution and on the reproducibility of myocardial triglyceride measurements is unknown.

Therefore, the purpose of our study was to prospectively compare spectral resolution and reproducibility of ^1H -MR spectroscopy, with and without respiratory motion compensation based on navigator echoes, to assess myocardial triglycerides in the human heart.

METHODS

One of the authors (MS) is an employee of Philips Medical Systems (Cleveland, Ohio). This author provided technical and intellectual input to the study. The authors who were not employed by Philips Medical Systems had full control of the inclusion of the data and information that might have presented a conflict of interest for this author.

In 20 volunteers (14 men, 6 women; mean age $\pm$ standard error, 31 ± 2.8 years [range, 19–60 years]; body mass index, $19\text{--}30 \text{ kg/m}^2$) without a history of cardiovascular disease, ^1H -MR spectroscopy of the myocardium was performed at rest. Furthermore, one healthy male subject (age, 22 years; body mass index, 23 kg/m^2) underwent ^1H -MR spectroscopy before and after a very low calorie diet. This healthy subject had no history or clinical evidence of cardiovascular disease, diabetes, or any other chronic disease (screening visit consisted of a medical history, physical examination, electrocardiography (ECG), and screening laboratory tests such as fasting plasma glucose and lipid levels

and an oral glucose tolerance test). The medical ethical committee of our institution (Leiden University Medical Center, Leiden, the Netherlands) approved our study protocol, and all participants gave informed consent.

Study design

ECG-triggered ^1H -MR spectroscopy was performed twice during one session with the same parameters, without changing the position of the voxel, both with and without the use of respiratory navigator gating and volume tracking. Thereafter, the volunteer was removed from the imager. After 5 minutes, the volunteer was repositioned in the magnet bore, and ECG-triggered ^1H -MR spectroscopy was repeated with and without respiratory navigator gating and volume tracking after completing all preparation phases (see below). No marking of coil position on the chest wall of subjects or other efforts to minimize variability were performed.

To test the ability of respiratory navigator gated ^1H -MR spectroscopy to demonstrate changes in myocardial triglyceride content after metabolic interventions, ^1H -MR spectroscopy was performed in one volunteer before and after a 3-day very low calorie diet. The very low calorie diet consisted of 471 kilocalories, 50.2 g of carbohydrates, and 6.9 g of fat (0.94 g saturated fat, Modifast Intensive; Nutrition & Santé Benelux, Breda, the Netherlands) per day. The volunteer was instructed to remain fasted for 4 hours prior to both ^1H -MR spectroscopy examinations.

Magnetic resonance technique

Cardiac MR examinations were performed at 1.5T (Gyrosan ACS/ NT15; Philips, Best, the Netherlands). A 17 cm diameter circular surface coil, with a vitamin A capsule in the center for visualization of the coil center on survey images, was positioned on the chest wall. Gradient-echo survey images were acquired to verify location of the ^1H -MR spectroscopy surface coil. When necessary, the coil was repositioned to place the coil center just below the mitral valve level of the heart (Figure 3.1). Once the coil was at the correct position, ECG-triggered MR imaging was performed to acquire multiphase gradient-echo images (repetition time ms/ echo time ms, 3.5/ 1.75; 35–40 heart phases) in the four-chamber and short-axis views to image the interventricular septum and to determine the time point of end-systole (Figure 3.1).

Magnetic resonance spectroscopy technique

ECG-triggered cardiac ^1H -MR spectra were obtained from the interventricular septum with subjects in the supine position. The body coil was used for radiofrequency transmission, and a 17 cm diameter circular surface coil was used for signal reception. An 8 ml voxel ($4 \times 2 \times 1$ cm) was positioned in the interventricular septum on the four-chamber and short-axis images at end-systole, thereby avoiding contamination from epicardial fat

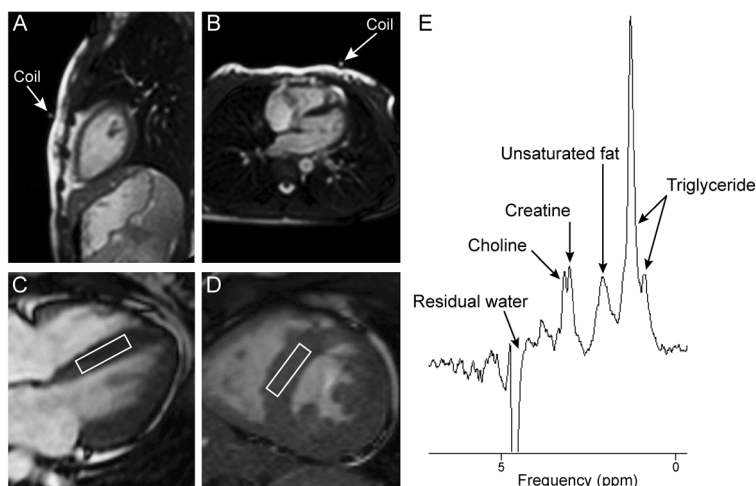


Figure 3.1

Images show coil position and spectroscopic volume. The surface coil was positioned just below the mitral valve level of the heart on *A*, sagittal and *B*, transverse balanced steady-state free precession MR images. Spectroscopic volume localization in the interventricular septum on *C*, four-chamber and *D*, short-axis views (ECG-triggered balanced steady-state free precession MR images) is demonstrated. Care was taken to avoid contamination from epicardial fat. *E*, Typical water-suppressed ^1H spectrum of myocardial tissue located in the interventricular septum. Peak heights are in arbitrary units.

(Figure 3.1). A section-selective 90° pulse, followed by two section-selective refocusing pulses (a point resolved spectroscopy sequence) was used to acquire single-voxel MR spectroscopic data.¹ Spectra were acquired at end-systole, with an echo time of 26 ms and a repetition time of at least 3000 ms; 1024 data points were collected by using 1000-Hz spectral width and 128 signals acquired. The repetition time of 3000 ms was chosen to approach complete relaxation of the triglyceride signals. A pencil beam navigator was positioned on the lung-liver interface of the right hemidiaphragm (Figure 3.2) for respiratory motion gating and tracking¹⁰⁻¹² by one of the authors (RWvdM). A two-dimensional spatially selective radiofrequency pulse for pencil beam shaped excitation was used. A pencil beam with a diameter of 25 mm and a length of 80 mm was selected. Respiratory navigator gated spectroscopic data were accepted during data acquisition when the diaphragm-lung interface was within a predefined acceptance window of 5 mm around end-expiration. Motion tracking was used to compensate for any residual translational shifts of the diaphragm-lung interface within the predefined acceptance window. The assumed scale factor between diaphragmatic motion and cardiac motion in the feet-to-head direction was 0.6.¹³

Automatic center frequency determination, gradient shimming, transmit power, receiver gain optimization, and water suppression were performed by using respiratory navigator gating and tracking. Without changing any parameter, a spectrum without water

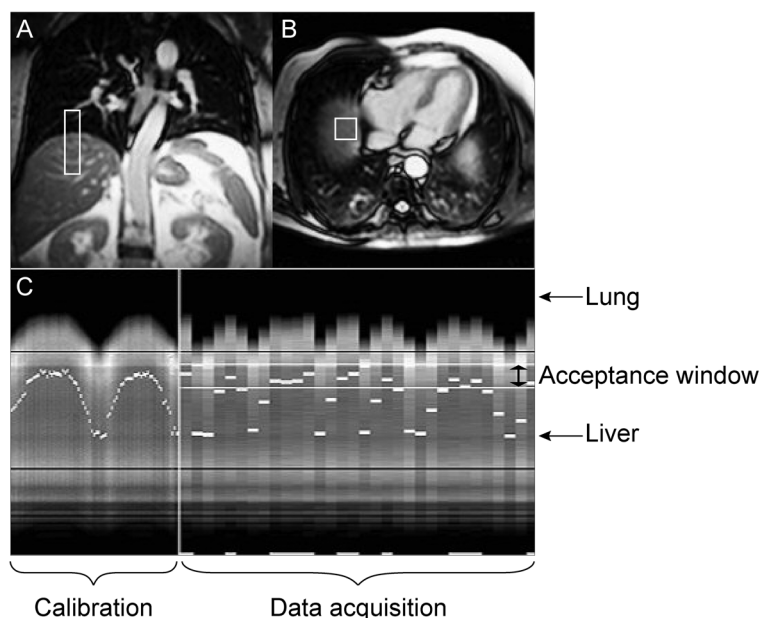


Figure 3.2

Images show position of pencil beam on right hemidiaphragm. A, coronal and B, transverse balanced steady-state free precession MR images show positioning of pencil beam on right hemidiaphragm. C, White dots (left) represent the automatically traced position of diaphragm (pencil beam excitation pulse is applied in foot-head direction). Two respiratory cycles are used for calibrations (smooth white line); thereafter, during data acquisition, navigator samples are taken with lower temporal resolution (white points, right). White horizontal lines indicate acceptance window (end-expiration, 5 mm); whenever the detected motion state of the diaphragm is within the window, the spectroscopic measurement is accepted.

suppression was obtained, with a repetition time of 10 seconds (to approach complete relaxation of the water signal) and 4 signals acquired, to be used as an internal reference (see next section). Total acquisition time for both a water-suppressed and a water-unsuppressed spectrum, including (re)positioning of the patient, shimming, and parameter adjustment for water suppression, was on average 25 minutes. Assessment of a single, water-suppressed spectrum with 128 signals acquired took approximately 10 minutes depending on the respiratory cycle of the volunteer and on the acceptance rate of the respiratory navigator.

Spectral quantification

All ^1H -MR spectroscopic data were fitted in the time domain, directly on free induction decays by using Java-based MR user-interface software (jMRUI version 2.2; A. van den Boogaart, Katholieke Universiteit Leuven, Leuven, Belgium)¹⁴ in consensus by two authors (RWvdM and HJL, with 2 and 15 years of experience in myocardial MR imaging, re-

spectively). The Hankel-Lanczos filter (single-variable decomposition method) was used to remove residual water signal from spectra acquired with water suppression. Myocardial triglyceride signal amplitudes were analyzed automatically by using the Advanced Magnetic Resonance, or AMARES, fitting algorithm within the jMRUI software.¹⁵ The AMARES fitting algorithm within jMRUI also provides the standard deviation of the amplitude (one time the Cramer-Rao standard deviation [CRSD]), which can be used as a measure of the accuracy of the fitted signal amplitude, reflecting the signal-to-noise ratio.¹⁶ The CRSD of the lipid signal was divided by the lipid signal amplitude, yielding a relative CRSD, which is inversely related to the signal-to-noise ratio. Resonance frequency estimates for intramyocardial lipids were described with the assumption of Gaussian line shapes at 0.9, 1.3, and 2.1 ppm. (In keeping with the approach of Torriani et al.,¹⁷ we summed the amplitudes of lipid resonances at 0.9 and 1.3 ppm for triglyceride quantification for statistical analysis). Prior knowledge was incorporated into the fitting algorithm by using previously published criteria.¹⁸⁻²⁰ Fixed frequencies for triglyceride peaks were used, and linewidths and amplitudes were unconstrained. The zero-order phase correction was estimated by using the AMARES algorithm, and the first-order phase correction was fixed to 0.13 ms. The water signal from spectra without water-suppression obtained from the same voxel was used as internal reference for relative quantification of triglyceride resonances. The water signal peak at 4.7 ppm was quantified and the linewidth (full width at half maximum) was calculated by using a Lorentzian line shape in the AMARES algorithm. The percentage of myocardial triglyceride content relative to water was calculated as the signal amplitude of triglyceride divided by the signal amplitude of water, and multiplied by 100.

Statistical analysis

To compare reproducibility of percentage of myocardial triglyceride content with and without respiratory navigator gating and volume tracking, the intraclass correlation coefficients were calculated by using a mixed-effects analysis of variance (with patients as random factor) for both conditions separately. Furthermore, the coefficients of variance were calculated for both conditions separately. Moreover, Bland-Altman plots were constructed. Statistical significance of differences was assessed by using two-tailed paired T-tests, and $P < 0.05$ was considered to indicate a significant difference. Mean values $\pm$ standard errors are given. Statistical analyses were performed by using statistical software (version 12.0; SPSS, Chicago, IL).

RESULTS

The full width at half maximum value of the unsuppressed water signal changed from 11.9 ± 0.4 Hz to 10.7 ± 0.44 Hz (all data pooled, $P < 0.001$), without and with respiratory navigator gating, respectively. A decrease was observed in the calculated mean myocardial triglyceride percentage with use of respiratory navigator gating compared with myocardial triglyceride percentage assessed without use of the navigator (Table 3.1). In all acquisition conditions, the CRSD was less than 1% of the lipids signal amplitude.

Bland-Altman plots of the observed percentage of myocardial triglyceride without and with respiratory navigator showed smaller limits of agreement (mean ± 2 standard deviations) when respiratory navigator is used, indicating improved reproducibility (Figure 3.3). Without use of the respiratory navigator, the intraclass correlation coefficient was 0.32 (95% confidence interval: -0.14, 0.66; $P = 0.08$), and this coefficient improved to 0.81 (95% confidence interval: 0.58, 0.92; $P < 0.001$) with use of navigator gating and volume tracking. Furthermore, the coefficient of variation of the assessment of myocardial triglyceride percentage without use of the navigator was 14.5% higher than with the use of the navigator (32.4% vs. 17.9%).

The very low calorie diet induced an 83% increase in myocardial triglyceride content compared with baseline percentage of triglyceride (1.1% and 0.6% triglyceride content, respectively [Figure 3.4]) in one volunteer.

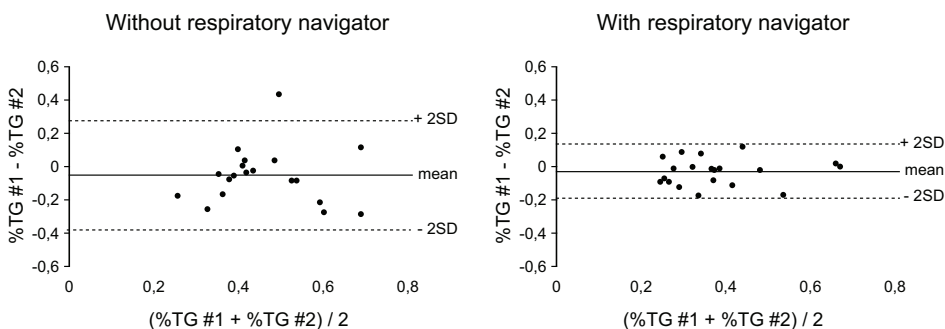


Figure 3.3

Bland-Altman plot of metabolic imaging of myocardial triglyceride content without (left) and with (right) respiratory navigator gating and volume tracking. Without respiratory navigator gating, the mean inter-acquisition difference ($\pm$ standard error) of the percentage of triglycerides is -0.05 ± 0.04 % ($P = 0.20$); with respiratory navigator gating, the difference of the two acquisitions in the same subjects is -0.03 ± 0.02 % ($P = 0.11$). No trends are observed. Limits of agreement (mean ± 2 standard deviations [2SD]) are smaller when respiratory navigator is used, indicating improved reproducibility.

%TG = myocardial triglyceride percentage relative to unsuppressed water; #1 and #2 = acquisitions 1 and 2.

Table 3.1 Reproducibility of human myocardial triglyceride content

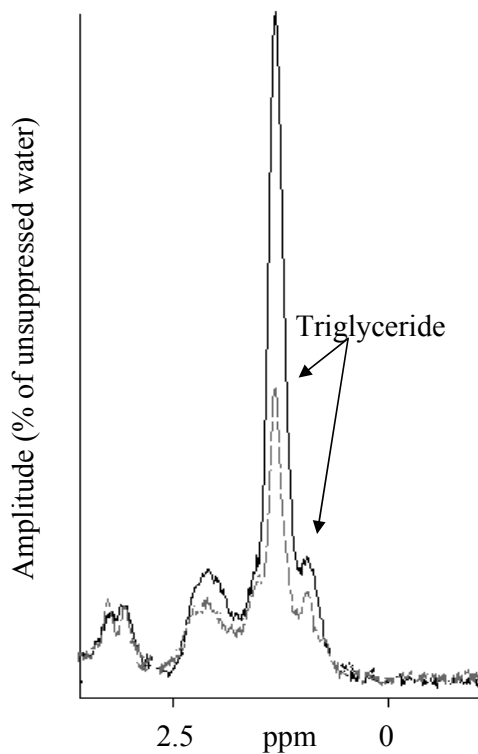
	Without Navigator		With Navigator	
	%TG*	rCRSD†	%TG*	rCRSD†
All data (n = 40)	0.46 ± 0.02	0.80% ± 0.07	0.38 ± 0.02‡	0.92% ± 0.09
Acquisition #1 (n = 20)	0.43 ± 0.03	0.86% ± 0.12	0.37 ± 0.03‡	0.95% ± 0.12
Acquisition #2 (n = 20)	0.48 ± 0.03	0.74% ± 0.09	0.40 ± 0.03‡	0.89% ± 0.12

Values are mean ± standard error.

* %TG = percentages of myocardial triglycerides relative to the water signal.

† rCRSD = Cramer Rao standard deviation relative to the triglyceride signal amplitude. rCRSD was used as a representative for the signal-to-noise ratio.

‡ %TG without navigator is significantly different compared to %TG with navigator (two-tailed paired T-tests, $P < 0.05$).

**Figure 3.4**

Water-suppressed spectra from metabolic imaging show effect of a short-term very low calorie diet on triglyceride content in a healthy volunteer. Peak height is relative to the water signal in a reference spectrum without water suppression. Myocardial triglyceride peak height increased nearly twofold after a 3-day very low calorie diet. Since this diet was tested in only one subject, results cannot be taken as a proved finding.

Dashed line = baseline; solid line = very low calorie diet.

DISCUSSION

In our study, reproducibility of metabolic imaging findings by using ECG-triggered proton MR spectroscopy to assess myocardial triglyceride accumulation was assessed with and without the use of respiratory navigator echo-based motion compensation. Spectral resolution (defined by means of the linewidth of the unsuppressed water signal), which is a measure of spectroscopic quality, increased significantly ($P < 0.001$) with use of respiratory motion compensation. Furthermore, reproducibility of the assessment of myocardial triglyceride content was improved when respiratory navigator gating was applied.

Respiratory motion causes a relative displacement of the acquisition volume in relation to the position of the human heart. Thereby, respiratory motion may hamper shimming and water suppression. In our study, the full width at half maximum values of the unsuppressed water signal decreased significantly with use of the respiratory navigator compared with acquisitions without respiratory navigator gating and tracking. The observed values in our study of full width at half maximum with use of the respiratory navigator technique correspond to values reported for the tibialis anterior muscle¹⁷ and are lower than previously published values for myocardial ^1H -MR spectroscopy.¹ Therefore, application of respiratory navigator gating and tracking improves spectral resolution for metabolic imaging of myocardial triglycerides of the human heart.

The mean percentages of myocardial triglyceride, assessed with and without respiratory motion compensation, were in accordance with previously published data from other studies,⁹ but with respiratory navigator, the percentage myocardial triglyceride was lower than the acquired values in our study without use of respiratory motion compensation. The observed percentages of triglyceride are scattered over a relatively large range for all acquisition conditions. In all acquisition conditions, the CRSDs were less than 1% of the signal amplitude, and thus spectral noise was considered to have a negligible contribution to the uncertainty of our measurements. Therefore we assume that the observed range in myocardial triglyceride percentages reflects differences in measurement conditions (i.e. presence or absence of navigator gating).

The observed higher percentage of myocardial triglyceride without application of respiratory motion compensation is probably caused by contamination of epicardial fat. The contamination is most likely caused by the relative displacement of the acquisition volume in myocardial tissue, due to respiratory motion causing contamination from outside the selected voxel and thereby to an increase in the apparent percentage of myocardial triglyceride.

Bland-Altman analysis showed improved agreement in myocardial triglyceride assessment with use of respiratory navigator gating and tracking. No comparable data could be found in previous reports. In addition, with use of the respiratory navigator, reproducibility of myocardial triglyceride assessment expressed as the intraclass correlation

coefficient and the coefficient of variation improved significantly. In our study, use of respiratory navigator gating and tracking improved the intraclass correlation coefficient from 0.32 to 0.81 and decreased the coefficient of variation from 32.4% to 17.9% for assessment of myocardial triglycerides. A coefficient of variation of 17.9% for the assessment of myocardial triglycerides with use of respiratory motion compensation is in concordance with results of previous studies in which various other methods were used for cardiac and respiratory motion correction to increase spectroscopic quality.^{2,3} Szczepaniak et al. showed a coefficient of variation for MR spectroscopic determination of myocardial triglycerides of 17%, with use of a pressure belt for respiratory gating,³ while others reported a coefficient of variation of 13% for triglyceride determination by using double triggering based on the ECG signal.²

In our study, an increase in myocardial triglyceride content was found after a short-term very low calorie diet in a healthy subject. Although this test was performed in only one volunteer, and thus is not representative of a proved finding, the result corresponds to the findings of Reingold et al.⁹ The clinical interpretation of the above-mentioned finding needs to be established in a larger cohort study. This clinical example suggests that metabolic imaging of myocardial triglyceride content may be a useful new tool for monitoring effects of dietary and/or medical interventions in metabolic and cardiac disorders, such as metabolic syndrome, diabetes, and myocardial lipotoxicity. Furthermore, metabolic imaging of myocardial triglyceride content may provide new (patho)physiologic insights of myocardial triglyceride handling, also in relation to global and regional cardiac function.

Our study has some limitations: First, ¹H-MR spectroscopy was performed in healthy volunteers only. A patient who is experiencing any sort of stress due to a medical condition is possibly less cooperative with a longer acquisition time caused by the respiratory motion compensation. We think, however, that a clinical cardiac MR imaging examination time of approximately 25 minutes to acquire a cardiac spectrum is not any different from other clinical cardiac MR imaging applications. The more reliable results of respiratory motion compensated spectroscopy compared with non-respiratory motion compensated spectroscopy warrants the extra time investment.

Second, the use of cardiac ¹H-MR spectroscopy currently has only limited clinical relevance. However, it is potentially a very useful tool in cardiac metabolic studies for example, in the evaluation of diet and therapy effects.

Third, ¹H-MR spectra were obtained in the myocardial septum only. The use of ¹H-MR spectroscopy in other regions of the heart was not demonstrated. We think that the myocardial septum is the most favorable region for acquiring cardiac spectra in generalized disorders that affect the myocardium, such as diabetes mellitus. Motion in the myocardial septum is limited, and the myocardial septum is far from the free walls and from the pericardial fat, which could contaminate the spectra. However, more work needs to

be done to develop a reliable MR spectroscopy technique for the lateral walls of the heart, which could be of interest in assessing myocardial lipid accumulation in localized disorders such as myocardial infarction.

In conclusion, respiratory navigator gated and ECG-triggered ^1H -MR spectroscopy of the human heart to assess myocardial triglyceride content showed substantially better spectral resolution and reproducibility than ECG-triggered ^1H -MR spectroscopy without respiratory motion correction. Therefore, we believe that respiratory motion correction is essential for reproducible metabolic imaging of myocardial triglyceride content of the human heart.

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**Effects of myocardial
triglyceride accumulation
in the healthy population**

PART II

Short-term caloric restriction induces accumulation of myocardial triglycerides and decreases left ventricular diastolic function in healthy subjects

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ABSTRACT

Background

Diabetes and obesity are associated with increased plasma non-esterified fatty acid (NEFA) levels, myocardial triglyceride accumulation, and myocardial dysfunction. Because a very low calorie diet (VLCD) also increases plasma NEFA levels, we studied the effect of a VLCD on myocardial triglyceride content and cardiac function in healthy subjects.

Methods

Fourteen healthy non-obese men underwent ^1H -magnetic resonance spectroscopy (MRS) to determine myocardial and hepatic triglyceride content, ^{31}P -MRS to assess myocardial high-energy phosphate (HEP) metabolism (phosphocreatine/ATP), and magnetic resonance imaging of myocardial function at baseline and after a 3-day VLCD.

Results

After the dietary intervention, plasma NEFA levels increased compared with those at baseline (from 0.5 ± 0.1 to 1.1 ± 0.1 mmol/l, $P < 0.05$). Concomitantly, myocardial triglyceride content increased by approximately 55% compared with that at baseline (from 0.38 ± 0.05 to $0.59 \pm 0.06\%$, $P < 0.05$), whereas hepatic triglyceride content decreased by approximately 32% (from 2.2 ± 0.5 to $1.5 \pm 0.4\%$, $P < 0.05$). The VLCD did not change myocardial phosphocreatine-to-ATP ratio (2.33 ± 0.15 vs. 2.33 ± 0.08 , $P < 0.05$) or systolic function. Interestingly, deceleration of the early diastolic flow across the mitral valve decreased after the VLCD (from 3.37 ± 0.20 to 2.91 ± 0.16 ml/s² $\times 10^{-3}$, $P < 0.05$). This decrease in diastolic function was significantly correlated with the increase in myocardial triglyceride content.

Conclusion

Short-term VLCD induces accumulation of myocardial triglycerides. In addition, VLCD decreases left ventricular diastolic function, without alterations in myocardial HEP metabolism. This study documents diet-dependent physiological variations in myocardial triglyceride content and diastolic function in healthy subjects.

In diabetes and obesity, plasma non-esterified fatty acid (NEFA) levels are elevated because of excessive lipolysis in adipose tissue.¹ In animal models of type 2 diabetes mellitus and obesity, excessive plasma NEFA levels result in accumulation of myocardial triglycerides.^{2,3} In these models, triglyceride accumulation in cardiomyocytes is directly related to cardiac dysfunction^{4,6} and an increased susceptibility for cardiac ischemia.⁷ This so-called “myocardial lipotoxicity” is due to complex mechanisms, most likely involving intermediates of NEFA metabolism and oxidative stress.^{2,6,8} Interestingly, in animal models, therapeutic interventions aimed at reducing myocardial triglyceride accumulation reversed myocardial dysfunction.⁶ In addition to contributing to myocardial lipotoxicity, increased plasma NEFA levels may affect myocardial high-energy phosphate (HEP) metabolism.⁹

Myocardial triglyceride accumulation has been demonstrated *ex vivo* in myocardial tissue of type 2 diabetic patients¹⁰ and patients with heart failure.¹¹ Recently, myocardial ¹H-magnetic resonance spectroscopy (MRS) has been developed and validated to measure myocardial triglyceride content in humans *in vivo*.^{12,13} Using this technique, a relation between body mass index (BMI) and myocardial triglyceride content was suggested.^{12,14} However, dynamic changes in myocardial triglyceride content and myocardial function have not been documented within subjects. Because short-term exposure to a very low calorie diet (VLCD) increases plasma NEFA levels,¹⁵ we hypothesized that this dietary intervention might be a model to study the flexibility of myocardial triglyceride content and myocardial function in healthy subjects.

Therefore, the purpose of the present study was to determine the effect of a VLCD on myocardial triglyceride content and cardiac function in healthy subjects, using ¹H-MRS, ³¹P-MRS, and cardiac magnetic resonance imaging (MRI). Each subject was studied twice, before and after 3 days of VLCD. ³¹P-MRS was used to assess myocardial HEP metabolism. Cardiac MRI was used to assess myocardial function in detail. Furthermore, hepatic triglyceride content was assessed concomitantly using ¹H-MRS to study the tissue-specific effects of a VLCD.

METHODS

Fourteen healthy men participated in this study, which was approved by the local ethics committee. All volunteers provided written informed consent. Subjects were included if they were aged >18 years and had no known acute or chronic disease based on history, physical examination, standard laboratory tests (blood counts, fasting blood glucose, lipids, serum creatinine, alanine aminotransferase, aspartate aminotransferase, and electrocardiogram [ECG]). Exclusion criteria included drug treatment, smoking, sub-

stance abuse, hypertension, or impaired glucose tolerance (as determined by a 75-g oral glucose tolerance test ¹⁶).

Subjects underwent MR scanning in the afternoon on two different occasions. Before both visits, they were instructed to follow one of two different dietary regimes for 3 days before the measurements. In the first regime, each subject used his normal diet, and this dietary condition was used for the collection of baseline data. During the second regime, subjects consumed a VLCD consisting of 471 kcal, 50.2 g carbohydrates, and 6.9 g fat (0.94 g saturated fat; Modifast Intensive, Nutrition & Santé Benelux, Breda, Netherlands) per day. The low fat content was used to induce a physiological elevation of plasma NEFA levels. Subjects were instructed to maintain a sufficient fluid intake (>1.5 liter daily). Use of alcohol was not allowed during the 3-day diets. The last meal of each diet was consumed 4 hours before venous blood sampling and subsequent cardiac MRI and MRS measurements. Furthermore, MRS of the liver was performed to study the extra-cardiac effects of the VLCD. The effect of the VLCD on the study parameters was compared with the data obtained after the reference diet.

Proton magnetic resonance spectroscopy

All MRI/ MRS studies were performed with the use of a 1.5T whole-body MR scanner (Gyroscan ACS/ NT15; Philips, Best, Netherlands) with subjects in supine position at rest. Myocardial ¹H-MR spectra were obtained from the interventricular septum. The body coil was used for radiofrequency transmission, and a 17 cm diameter circular surface coil was used for signal reception.

An 8 ml voxel was positioned in the interventricular septum on four-chamber and short-axis images in end-systole, carefully avoiding contamination from epicardial fat (Figure 4.1). A point resolved, spatially localized spectroscopic pulse sequence was used

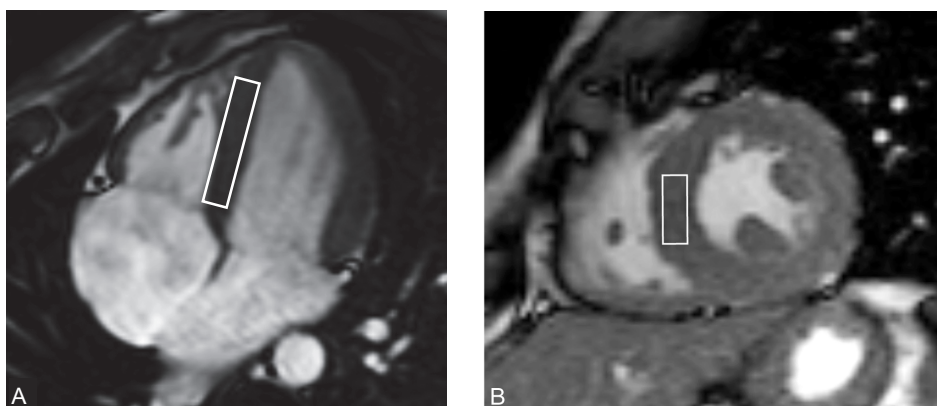


Figure 4.1

Myocardial voxel localization for ¹H-MRS. Voxel position in four-chamber (A) and short-axis (B) views.

to acquire single-voxel MR spectroscopic data.¹⁷ Spectroscopic data acquisition was double triggered using ECG-triggering and respiratory navigator echoes to minimize breathing influences.^{13,18} Spectra were acquired at end-systole, with an echo time of 26 ms and a repetition time of at least 3000 ms. A total of 1024 data points were collected using a 1000-Hz spectral width and averaged over 128 acquisitions. Without changing any parameter, spectra without water suppression with a repetition time of 10 seconds and 4 averages were obtained to be used as an internal standard. ¹H-MRS of the liver was performed with an 8 ml voxel positioned in the liver, avoiding gross vascular structures and adipose tissue depots. The 12th thoracic vertebra was used as a landmark to ensure the same position of the voxel during both visits. Spectra were obtained using the same parameters as described above. Sixty-four averages were collected with water suppression.

All ¹H-MR spectroscopic data were fitted using Java-based MR user interface (jMRUI) software (version 2.2 [developed by A. van den Boogaart, Katholieke Universiteit Leuven, Leuven, Belgium]).¹⁹ Spectra were analyzed in the time domain directly on free-induction decays. For spectra acquired with water suppression, the Hankel-Lanczos filter was used to remove residual water signal, using the single-variable decomposition method. Myocardial triglyceride signals were analyzed using the Advanced Magnetic Resonance (AMARES) fitting algorithm within jMRUI.²⁰ Resonance frequency estimates for intramyocardial lipids were described with the assumption of Gaussian line shapes at 0.9, 1.3, and 2.1 ppm (only data from the peaks at 0.9 and 1.3 ppm were summated and used on statistical analysis²¹). Prior knowledge was incorporated into the fitting algorithm by using previously published criteria.²²⁻²⁴ The zero-order phase correction was estimated by using the AMARES algorithm, and the first-order phase correction was fixed to 0.13 ms. The water signal from spectra without water suppression obtained from the same voxel was used as an internal reference for relative quantification of lipid resonances. The water signal peak at 4.7 ppm was quantified using a Lorentzian line shape and analyzed using the AMARES algorithm. Myocardial and hepatic triglyceride content was calculated as a percentage relative to water: triglyceride/water × 100.

Furthermore, peak estimates of the creatine signals of the heart spectrum at 3.0 ppm were derived from the water-suppressed spectrum using jMRUI, and the triglyceride-to-creatine ratio and the percentage of creatine (creatine/water × 100) were calculated.

Phosphorus magnetic resonance spectroscopy

A 100 mm-diameter surface coil was used to acquire ECG-triggered ³¹P-MR spectra of the left ventricular (LV) anterior wall with subjects in the supine position. Volumes of interest were selected by image-guided spectroscopy with 3D-ISIS. Shimming was performed automatically, and tuning and matching of the ³¹P surface coil was performed manually. Technical details of data acquisition and spectral quantification were similar as

previously described.²⁵ Shortly, spectroscopic volume size was typically $7 \times 7 \times 7$ cm. Acquisitions were based on 192 averaged free-induction decays, and total acquisition time was 10 min. ^{31}P -MR spectra were quantified automatically in the time domain using prior spectroscopic knowledge and were corrected for partial saturation effects and for the ATP contribution from blood in the cardiac chambers. The phosphocreatine-to-ATP ratios of the spectra were calculated and used as a parameter representing myocardial HEP metabolism.²⁶

Left ventricular function

All images were analyzed quantitatively using dedicated software (FLOW or MASS; Medis, Leiden, Netherlands). The entire heart was imaged in short-axis orientation using ECG-gated breath-holds, with a sensitivity-encoding balanced steady-state free precession sequence. Imaging parameters included the following: echo time = 1.7 ms, repetition time = 3.4 ms, flip angle = 35° , slice thickness = 10 mm with a gap of 0 mm, field of view = 400×400 mm, and reconstructed matrix size = 256×256 . LV ejection fraction was assessed for the determination of LV systolic function. Furthermore, an ECG-gated gradient-echo sequence with velocity encoding (Venc) was performed to measure blood flow across the mitral valve for the determination of LV diastolic function. Imaging parameters included the following: echo time = 5 ms, repetition time = 14 ms, flip angle = 20° , slice thickness = 8 mm, field of view = 350×350 mm, matrix size = 256×256 , Venc = 100 cm/s, and scan percentage = 80%. Early diastolic filling, mean deceleration of the early diastolic flow across the mitral valve, and an estimation of LV filling pressures (E/E_a)²⁷ were used as parameters of LV diastolic function. During MRI, blood pressure and heart rate were measured.

Assays

Plasma glucose and triglycerides were measured on a fully automated P800 analyzer (Roche, Almere, Netherlands) and insulin on an Immulite 2500 random-access analyzer with a chemoluminescence immunoassay (DPC, Los Angeles, CA). Coefficients of variation (CV) were < 2% for glucose and triglycerides and < 5% for insulin. Leptin and adiponectin were measured with radioimmunoassays from Linco Research (St. Charles, MO). For leptin, the CV varied from 3.0 to 5.1% and the sensitivity was $0.5 \mu\text{g/l}$; for adiponectin, these data were 6.3 to 8.1%, with a sensitivity of $1 \mu\text{g/l}$. Plasma NEFA were measured by using a commercial kit (NEFA-C; Wako Chemicals, Neuss, Germany).

Statistical analysis

Statistical analysis was performed using SPSS for windows (version 12.0; SPSS, Chicago, IL). Data are expressed as means $\pm$ standard error (SE). Between-group differences

were calculated using a two-tailed dependent sample T-test. Pearson r values were used for correlations. Significance was assumed at $P < 0.05$ (two-tailed).

RESULTS

^1H -MRS and myocardial function were successfully assessed in all 14 subjects. In nine subjects, ^{31}P -MRS was successfully completed at both occasions. In the other five subjects, ^{31}P -MRS data at baseline or after the VLCD could not be assessed because of time constraints or technical problems. Mean age of the studied subjects was 25 ± 2 years. Characteristics at baseline and after the VLCD are shown in Table 4.1. All subjects performed exercise (walking, running, and/ or biking) regularly (ranges 3 - 5 hours weekly), but none of the subjects engaged in high-performance sports.

Table 4.1 Characteristics of the study group at baseline and after a very low calorie diet

	Baseline	Very low calorie diet
Body mass index (kg/m^2)	23.6 ± 0.7	$23.2 \pm 0.7^*$
Systolic blood pressure (mmHg)	123 ± 4	118 ± 3
Diastolic blood pressure (mmHg)	66 ± 2	$62 \pm 2^*$
Heart rate (beats/min)	60 ± 2	61 ± 3
Plasma glucose (mmol/l)	4.90 ± 0.09	$4.26 \pm 0.10^*$
Plasma insulin (mU/l)	9.14 ± 1.27	7.9 ± 1.16
Plasma triglycerides (mmol/l)	1.29 ± 0.09	$0.82 \pm 0.07^*$
Plasma non-esterified fatty acids (mmol/l)	0.5 ± 0.1	$1.1 \pm 0.1^*$
Plasma leptin ($\mu\text{g}/\text{l}$)	2.99 ± 0.49	$1.41 \pm 0.20^*$
Plasma adiponectin (mg/l)	7.81 ± 0.84	6.79 ± 0.61

Values are mean $\pm$ standard error.

* $P < 0.05$ compared to baseline (paired T-test).

Myocardial and hepatic spectroscopy

Typical myocardial ^1H - and ^{31}P -MR spectra at baseline and after the VLCD of the same subject are shown in Figure 4.2. After the VLCD, myocardial triglyceride content as well as the myocardial triglyceride-to-creatine ratio were increased compared with those at baseline (from 0.38 ± 0.05 to 0.59 ± 0.06 and from 3.11 ± 0.39 to 5.42 ± 0.71 , respectively; $P < 0.05$), whereas the myocardial percentage of creatine did not change (Figure 4.3). The VLCD did not change the myocardial phosphocreatine-to-ATP ratio compared with baseline values (2.33 ± 0.15 vs. 2.33 ± 0.08 , $P < 0.05$).

Hepatic triglyceride content decreased during the VLCD compared with that at baseline (from 2.2 ± 0.5 to $1.5 \pm 0.4\%$, $P < 0.05$).

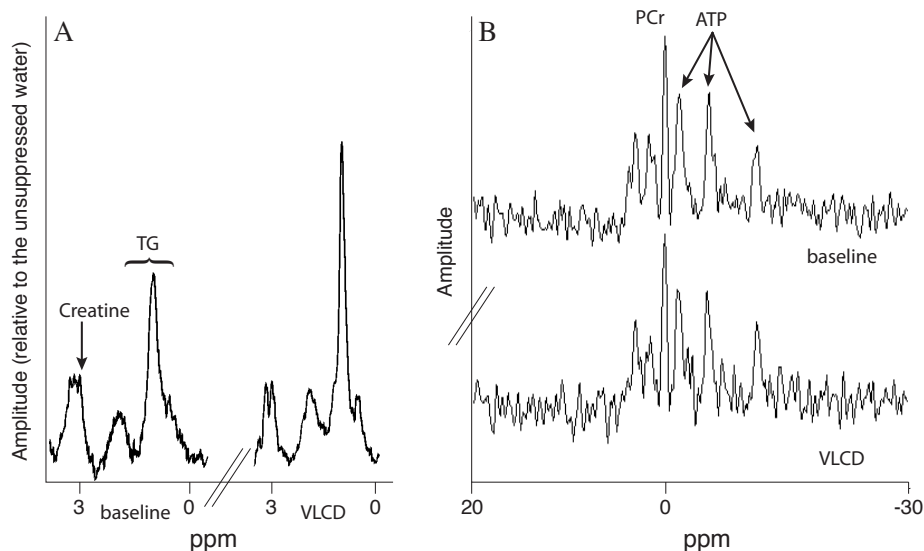


Figure 4.2
 Typical ^1H - and ^{31}P -MR spectra at baseline and after dietary intervention. A, ^1H -MR spectra at baseline and after a VLCD diet are displayed. Note the increase of the triglyceride signal amplitude at 0.9 and 1.3 ppm after the VLCD without a change in the creatine signal amplitude at 3.0 ppm. B, ^{31}P -MR spectra of one volunteer at baseline and after a VLCD are displayed. Note an unchanged phosphocreatine (PCr) and ATP signal.
 TG = triglyceride; VLCD = very low calorie diet.

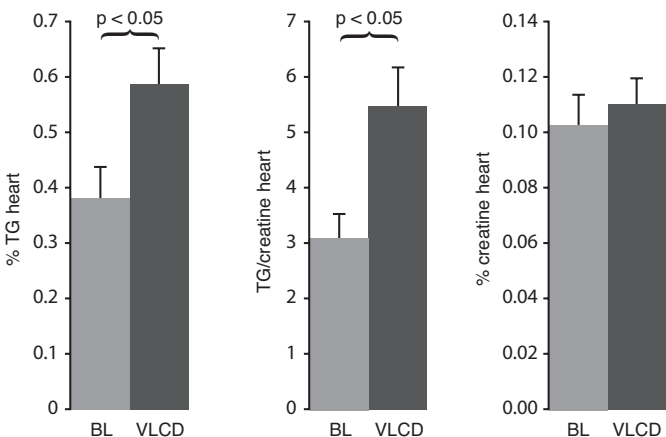


Figure 4.3
 Influence of a short-term VLCD on myocardial triglyceride and creatine content. A VLCD increased the percentage of myocardial triglycerides and the triglyceride-to-creatine ratio without changing the creatine-to-water ratio. Therefore, the increase in myocardial percentage of triglycerides by magnetic resonance spectroscopy is the effect of an increase of myocardial triglycerides rather than of decreased myocardial water content.
 BL = baseline; TG = triglyceride; VLCD = very low calorie diet.

Myocardial function

LV systolic function, represented by the ejection fraction, did not change (60 ± 1 vs. $60 \pm 1\%$, $P > 0.05$) after the VLCD. In contrast, the mean deceleration of the early diastolic flow across the mitral valve decreased after the VLCD compared with baseline values (from 3.37 ± 0.20 to 2.91 ± 0.16 $\text{ml/s}^2 \times 10^{-3}$, $P < 0.05$). This decrease in mean deceleration of the early diastolic flow across the mitral valve after the VLCD was significantly correlated with the increase in myocardial triglyceride content after the VLCD (Figure 4.4). Furthermore, there was no statistically significant change in LV filling pressures between the VLCD and baseline ($E/E_a = 10.0 \pm 1.3$ vs. 9.3 ± 0.7 , $P > 0.05$).

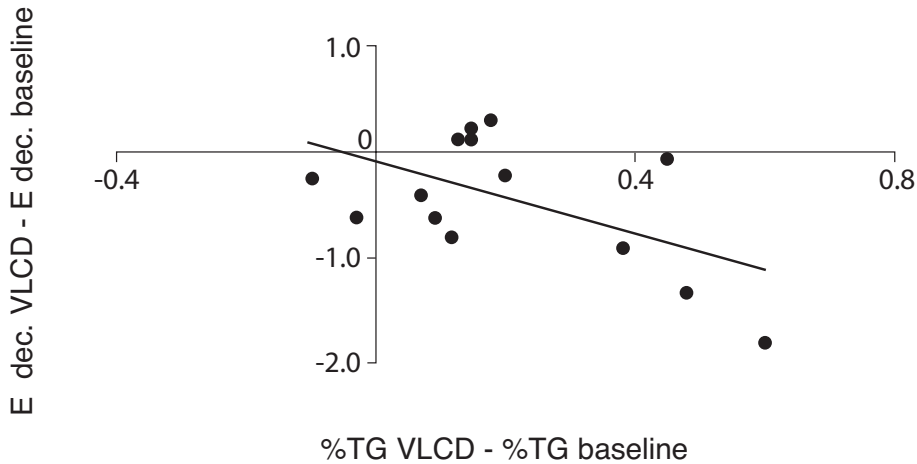


Figure 4.4

Correlation between diastolic function and myocardial triglycerides. The decrease in LV diastolic function after the VLCD was significantly correlated with the increase in myocardial triglyceride content after the VLCD (Pearson $r = -0.55$, $P < 0.05$).

%TG = myocardial percentage of triglycerides; E dec = deceleration of early diastolic flow across the mitral valve; VLCD = very low calorie diet.

DISCUSSION

This study shows that in healthy subjects, a short-term consumption of a VLCD increases myocardial triglyceride content and concomitantly decreases LV diastolic function without changing myocardial HEP metabolism. Moreover, this study shows that short-term caloric restriction exerts differential tissue-specific effects on triglyceride content in liver and myocardium. These observations stress the physiological flexibility of ectopic triglyceride pools.

Under normal conditions, myocardial energy is mainly derived from NEFA.¹ However, the rates of uptake and oxidation of NEFA in cardiomyocytes are not tightly coupled.

When NEFA are taken up in excess of fatty acid oxidation, myocardial triglyceride content increases. Apparently, myocardial fatty acid uptake is increased in relation to myocardial fatty acid oxidation during a VLCD.

Several animal models of type 2 diabetes mellitus and obesity demonstrated that excessive plasma NEFA levels result in accumulation of myocardial triglycerides.^{2,3} However, these diabetic models lead to very different metabolic changes compared with the physiologically increased NEFA concentrations seen in healthy subjects as a result of caloric restriction. Since very little is known about the flexibility of myocardial triglyceride content and myocardial function in healthy subjects in reaction to increased NEFA levels, in our study a VLCD was used as a model for a short-term physiological increase of plasma NEFA levels. The present findings of an increase in myocardial triglyceride content after a VLCD are in concordance with the findings of Reingold et al.,²⁸ who showed increased myocardial triglyceride content after 48 hours of fasting. Both conditions are associated with increased plasma NEFA levels.

Since the myocardial triglyceride content measured by MRS is expressed relative to water, the VLCD-induced increase in myocardial triglyceride content may also be explained by a decrease of myocardial water content. Therefore, the myocardial triglyceride-to-creatine and creatine-to-water ratios were assessed additionally. The increased myocardial triglyceride-to-creatine ratio in the presence of the unchanged creatine-to-water ratio in our study supports our conclusion that the diet-related increase in myocardial triglyceride content assessed by MRS is due to increased myocardial triglyceride accumulation rather than decreased myocardial water content.

The increase in myocardial triglyceride content can be derived from plasma NEFA and/or plasma triglycerides. The heart is especially effective in removal of circulating triglycerides.^{29,30} Moreover, heart lipoprotein lipase activity increases during fasting.³¹ In contrast, however, VLCD decreased plasma triglyceride levels, whereas plasma NEFA levels increased in our study. Therefore, it remains unclear to what extent the VLCD has altered the relative contribution of plasma NEFA versus plasma triglycerides to myocardial triglyceride stores.

In addition to increasing myocardial triglyceride content, the short-term VLCD intervention was associated with altered myocardial function. Although myocardial systolic function and heart rate were not changed after a VLCD in our study, a significant impact on diastolic function was observed. The deceleration of the early filling phase of the left ventricle decreased significantly after the VLCD. Transmitral filling patterns can be influenced by LV filling pressure and myocardial relaxation capacity. Although we observed a change in diastolic blood pressures, tissue MRI²⁷ showed no diet-induced changes in estimated LV filling pressures. We therefore hypothesize that changed relaxation of the left ventricle accounts for the observed change in the transmitral filling pattern. The mechanism(s) responsible for the change in diastolic function during a VLCD cannot be derived from

the present data. Short-term caloric restriction in mice causes remodeling of myocardial membranes through the activation of phospholipases.³² Altered membrane structure in a fatty acid based metabolic system may lead to changes in calcium homeostasis^{33,34} and thereby to altered LV diastolic function.³⁵ Therefore, altered calcium uptake might be involved in the mechanisms causing the decreased diastolic function observed during VLCD. Another explanation for decreased myocardial diastolic function after the VLCD might be the lower plasma glucose levels and the higher plasma NEFA levels. As a consequence, the heart becomes relatively more reliable on NEFA than on plasma glucose for its fuel supply. Carbohydrate oxidation, however, has potential salutary effects on myocardial function and efficiency.^{36,37}

Based on the present data, we cannot implicate myocardial triglyceride accumulation as the mediator of decreased myocardial diastolic function. During a VLCD, many hormonal, metabolic, and biophysical changes occur within the myocardium that will impact myocardial function. Nonetheless, the increase in myocardial triglyceride content is a reflection of these changes within the myocardium during a VLCD, which significantly correlated with the decrease in deceleration of the early diastolic flow across the mitral valve.

In the present study, the short-term VLCD did not affect myocardial HEP metabolism. This confirms findings in previous animal studies, in which an increase in myocardial triglyceride content did not cause a significant decrease in HEP status.³⁸ A possible explanation for the preserved myocardial HEP metabolism is that, in these healthy young men, myocardial ATP demand remains unchanged after a VLCD. A disturbance in the HEP metabolism might only be present when the heart is additionally stressed, e.g., by adenosine/ exercise testing or ischemia.

In parallel to the increase in myocardial triglyceride, hepatic triglyceride content decreased after the short-term VLCD. Westerbacka et al. previously reported similar findings on the effects of dietary interventions on hepatic triglyceride content.³⁹ In their study, decreased dietary fat content in obese women reduced hepatic fat content within 2 weeks without changing plasma NEFA levels. This could be an indication that dietary fat is an important direct source of fatty acids for the liver separate from NEFA. The decrease in hepatic fat after the VLCD in lean healthy subjects in our study, where plasma NEFA levels were increased after the VLCD, points in the same direction. However, obese subjects have a different metabolic profile than lean subjects and might therefore have shown other reactions to the VLCD. We think that further studies need to be conducted to evaluate the influence of different metabolic profiles on reactions to dietary fat content. The opposite changes in myocardial and hepatic triglyceride content indicate differential, organ-specific mechanisms underlying tissue-specific distribution of plasma triglycerides and/ or fatty acids among non-adipose organs, at least with respect to the liver and the heart. Unfortunately, the underlying mechanisms cannot be derived from the methods used in our study.

In conclusion, short-term VLCD induces accumulation of myocardial triglycerides. In addition, VLCD decreases LV diastolic function without alterations in myocardial HEP metabolism. This study documents diet-dependent, physiological variations in myocardial triglyceride content and diastolic function in healthy subjects.

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Progressive caloric restriction induces dose-dependent changes in myocardial triglyceride content and diastolic function in healthy men

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ABSTRACT

Background

In animal experiments, high plasma concentrations of non-esterified fatty acids (NEFA) are associated with increased triglyceride stores in liver and heart, and impaired cardiac function. In humans, caloric restriction increases plasma NEFA levels. The purpose of this study was to assess the effects of progressive caloric restriction on myocardial and hepatic triglyceride content and myocardial function.

Methods

Ten lean healthy men were included in this prospective intervention study. Three-day partial (471 kcal/day) and complete starvation was performed. Plasma levels of NEFA, myocardial and hepatic triglyceride content, and myocardial function were calculated.

Results

Plasma NEFA increased from 0.6 ± 0.4 to 1.2 ± 0.4 and to 1.9 ± 0.7 mmol/l, after partial and complete starvation, respectively ($P < 0.001$). Myocardial triglyceride content increased from 0.35 ± 0.14 to 0.59 ± 0.27 , and $1.26 \pm 0.49\%$, respectively ($P < 0.01$). The ratio between the early diastole and atrial contraction decreased from 2.2 ± 0.4 to 2.1 ± 0.4 ($P = 0.7$) and 1.8 ± 0.4 , respectively ($P < 0.01$), and diastolic early deceleration decreased from 3.4 ± 0.7 to 2.9 ± 0.5 and 2.8 ± 0.9 ml/s² × 10⁻³, respectively ($P < 0.05$). Hepatic triglyceride content decreased after partial starvation (from 2.23 ± 2.24 to $1.43 \pm 1.33\%$; $P < 0.05$) but did not change upon complete starvation.

Conclusion

Progressive caloric restriction induces a dose-dependent increase in myocardial triglyceride content and a dose-dependent decrease in diastolic function in lean healthy men. Hepatic triglyceride content showed a differential response to progressive caloric restriction, indicating that redistribution of endogenous triglyceride stores is tissue-specific.

Almost all endogenous triglycerides are stored in adipose tissue to accommodate discrepancies between whole body fat uptake and fat oxidation. However, a very small proportion is stored in non-adipose tissues like the heart,¹ the liver,² and skeletal muscle,³ especially in obesity and type 2 diabetes mellitus. There are indications that this storage of triglycerides in non-adipose tissues is not merely an inert phenomenon but is associated with more or less subtle physiological changes in organ-specific functioning.⁴⁻⁸ In animal models there is an inverse relation between myocardial triglyceride content and myocardial function. For example, myocardial lipid accumulation is associated with a decrease in left ventricular systolic function in obese Zucker rats, and treatment with thiazolidinediones reduces myocardial triglyceride content and improves left ventricular function.⁸ The underlying mechanisms of the decrease in left ventricular function are complex and are related to effects of fatty acid (FA) derivatives, like fatty acyl-coenzyme A, ceramides, and diacylglycerol.^{4,5,7}

High plasma concentrations of non-esterified fatty acids (NEFA) may result in excessive FA uptake in non-adipose tissues, such as the liver and heart, which may affect normal organ function.^{7,8} However, in humans the relation between myocardial triglyceride accumulation and myocardial function was difficult to study by non-invasive methods because measurement of myocardial triglyceride content is challenging due to artifacts induced by cardiac and respiratory motion. Recently, proton magnetic resonance spectroscopy (¹H-MRS) of the heart was developed that enables the measurement of myocardial triglyceride content in humans in vivo.^{1,10-13} Using this method, Reingold et al.¹⁴ documented that fasting for 48 hours increases plasma NEFA levels and myocardial triglyceride content in healthy subjects, whereas myocardial triglyceride content did not change after a single high-fat meal.

In another, cross-sectional study, Kankaanpää et al.¹² showed that increased levels of plasma NEFA in obese subjects correlate positively with myocardial triglyceride content and inversely with myocardial function. However, both studies did not address the relation between myocardial function in relation to myocardial triglyceride content within the same subjects. In a recent study, we documented that the use of a very low calorie diet increases plasma NEFA and myocardial triglyceride content, and was associated with a decrease in myocardial diastolic function.¹⁵ Therefore, it appears that myocardial triglyceride content is not fixed but varies within the same subject according to physiological conditions. It is yet unknown whether our recent findings of myocardial flexibility can be extrapolated when caloric restriction is progressively increased.

Therefore, the aim of the present study was to extend the conditions of partial caloric restriction to complete caloric restriction, i.e. complete starvation. For this purpose we compared baseline observations with those obtained after 3-day partial starvation (471 kcal/day) and after 3-day complete starvation with respect to plasma levels of NEFA, myocardial triglyceride content, myocardial function, and hepatic triglyceride content.

METHODS

Subjects

There were 10 non smoking, healthy men included in this study (age; mean $\pm$ SD: 23.7 $\pm$ 4.7 years, range 20.8 – 36.0 years; body mass index [BMI]: 23.6 $\pm$ 0.9 kg/m²). Women were excluded because the hormonal status or contraceptive use may affect lipid metabolism.¹⁶ The study population was partly based on a previous cohort.¹⁵ In each subject, medical history was obtained, and physical examination was performed. An electrocardiogram (ECG) was made during the first visit. Subjects with any aberrations on the ECG were excluded. In addition, a 75-g oral glucose tolerance test to exclude subjects suffering from diabetes mellitus was performed.¹⁷ Other exclusion criteria were: obesity (BMI > 30 kg/m²); liver disease (increased plasma levels of alanine aminotransferase, aspartate aminotransferase, and/or γ -glutamyl transferase $\pm$ 2 SD above the reference value of our institution); renal disease (defined by plasma creatinine levels $\pm$ 2 SD above the reference value of our institution); use of any medication; and a history of (congenital) heart disease. Specifically, subjects with prior or present coronary artery disease (based on medical history) or hypertension (defined as sitting systolic blood pressure > 130 mmHg and/ or diastolic blood pressure > 85 mmHg) were excluded. Written informed consent was obtained from all participants before the study. The local ethics committee approved the study.

Study design

Baseline measurements were made, while subjects followed a normal diet but abstained from alcohol for 3 days (mean intake 2065 kcal/day). Subjects were admitted 4 hours after the last meal for measurement of plasma concentrations of glucose, insulin, and lipids, and for evaluation by magnetic resonance imaging (MRI) and MRS. The second measurement was performed after a 3-day period of partial caloric restriction (471 kcal/day; Modifast Intensive, Nutrition & , Santé Benelux, Breda, the Netherlands). The third measurement was performed after a 3-day period of complete starvation (0 kcal/day, only water was allowed), after which subjects were again admitted for blood sampling and MRI/ MRS evaluation. Plasma concentrations of NEFA and insulin were used to assess study compliance.¹⁸ Between all study occasions, a washout period with a minimum of 14 days was acquired,¹⁹ and the sequence of the second and third measurement was determined by balanced assignment.

¹H-magnetic resonance spectroscopy of liver and heart

All MRI/ MRS studies were performed with the use of a 1.5T whole-body MR scanner (Gyrosan ACS/ NT15; Philips, Best, Netherlands) with subjects in supine position at rest. Localized single-voxel (2 $\times$ 2 $\times$ 2 cm for the liver and 2 $\times$ 4 $\times$ 1 cm for the heart)

spectra were recorded using a body coil for radiofrequency transmission and a surface coil (17 cm diameter) for signal receiving. For the heart, the spectral volume was placed in the interventricular septum on four-chamber and short-axis images at end-systole, avoiding contamination with epicardial fat (Figure 5.1). Data collection was double triggered using ECG-triggering and navigator echoes for compensation of respiratory motion as described earlier.¹³ For the liver, voxel sites were matched at both study occasions, carefully avoiding gross vascular structures. To detect the weak lipid signals, water-suppressed spectra with 128 averages for the heart and 64 for the liver were collected. Spectral parameters were: a repetition time (TR) of at least 3000 ms, echo time (TE) = 26 ms, and a total of 1024 data points were collected using a 1000-Hz spectral width. In the same voxel, using the same parameters except for a TR of 10 seconds, unsuppressed spectra with 4 averages were collected. Spectra were analyzed in the time domain, using Java-based MR user interface software (version 2.2²⁰) and prior knowledge files, as described earlier.¹³ Peak estimates of lipid resonances of myocardial triglycerides at 1.3 parts per million (ppm) and 0.9 ppm were summed and calculated as a percentage of the unsuppressed water signal (triglyceride content, $\text{triglyceride}/\text{water} \times 100$).

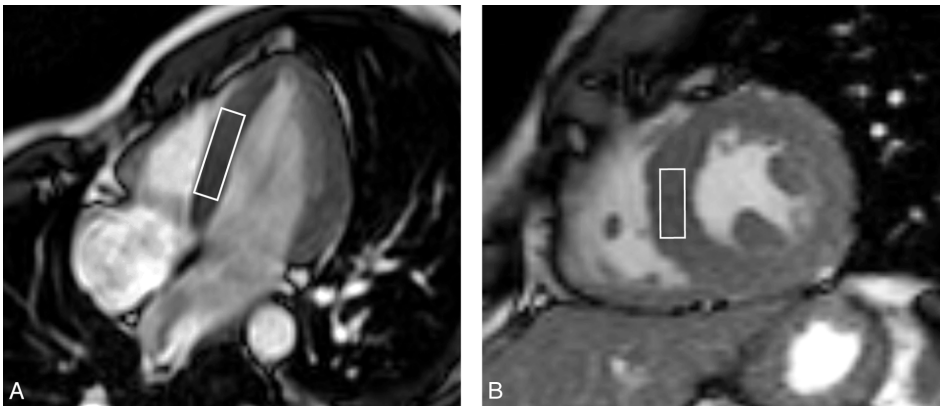


Figure 5.1

Myocardial spectroscopic volume. Localization of the myocardial spectral voxel in the four-chamber (A) and short-axis views (B).

Magnetic resonance imaging of the heart

Imaging of the heart was performed using a body coil for radiofrequency transmission and a five-element synergy coil for signal receiving. To assess systolic function, the heart was imaged from apex to base with 12–14 imaging levels (dependent on the heart size) in short-axis view using an ECG-triggered, sensitivity-encoding balanced steady-state free precession sequence. Imaging parameters were: field of view = 400 × 400 mm, matrix size = 256 × 256, slice thickness = 10 mm, slice gap = 0 mm, flip angle = 35°,

TE = 1.7 ms, and TR = 3.4 ms. Temporal resolution was 25–39 ms. End-diastolic and end-systolic images were identified on all slices, and endocardial contours were drawn using MASS post-processing software (Medis, Leiden, the Netherlands) as described previously.²¹ Left ventricular ejection fraction (LVEF) was calculated for the assessment of systolic function. Furthermore, an ECG-gated gradient-echo sequence with velocity encoding (Venc) was performed to measure blood flow across the mitral valve for the determination of left ventricular diastolic function.^{22,23} Imaging parameters included the following: TE = 5 ms, TR = 1.4 ms, flip angle = 20°, slice thickness = 8 mm, field of view = 350 × 350 mm, matrix size = 256 × 256, Venc = 100 cm/s, and scan percentage = 80%. Flow velocities in early diastole (E) and at atrial contraction (A) were measured, and their peak flow ratio was calculated (E/A ratio) using the FLOW analytical software package (Medis, Leiden, the Netherlands) by defining a region of interest on the modulus images in all cardiac phases. Furthermore, the mean deceleration of the E wave and an estimation of left ventricular filling pressures (E/Ea)²⁴ were measured. All spectroscopic and functional analyses were performed by an experienced observer, blinded to the interventions. During MRI, blood pressure and heart rate were measured twice with an automatic device (Dinamap DPC100X; Freiburg, Germany) and averaged for analysis.

Assays

Plasma glucose, total cholesterol, and triglycerides were measured on a fully automated P800 analyzer (Roche, Almere, the Netherlands) and plasma insulin on a Immulite 2500 random access analyzer with a chemoluminescence immunoassay (Diagnostic Products Corp., Los Angeles, CA). Coefficients of variation were less than 2% for plasma glucose, total cholesterol, and triglycerides, and less than 5% for insulin. Plasma NEFA were measured using a commercial kit (FFA-C; Wako Chemicals, Neuss, Germany).

Statistical analysis

All statistical analyses were performed using SPSS (version 12.0; SPSS, Chicago, IL). Statistical comparisons among the three physiological conditions were made by repeated measures ANOVA. Pearson *r* values were used for correlation analysis. Data are shown as mean ± standard deviation (SD). *P* < 0.05 (two-tailed) was considered significant. Based on a previous report, we expected a decrease in diastolic early deceleration. Therefore, *P* < 0.05 (one-tailed) was considered significant for this parameter.¹⁵

RESULTS

Metabolic effects of progressive caloric restriction

Subject characteristics at baseline, after partial starvation, and after complete starvation are shown in Table 5.1. Postabsorptive plasma glucose levels decreased from 5.0 ± 0.3 mmol/l at baseline to 4.3 ± 0.4 mmol/l after partial ($P = 0.001$) and to 3.9 ± 0.5 mmol/l after complete starvation ($P < 0.001$). This was associated with a dose-dependent decrease in plasma insulin levels. Simultaneously, plasma concentrations of NEFA increased dose-dependently from 0.6 ± 0.4 to 1.2 ± 0.4 mmol/l after partial ($P < 0.001$) and to 1.9 ± 0.7 mmol/l after complete starvation ($P < 0.001$). Plasma triglyceride levels decreased after partial starvation (from 1.3 ± 0.4 to 0.9 ± 0.3 mmol/l ($P = 0.009$)) but did not change upon complete starvation ($P = 0.677$). Total cholesterol increased from 5.0 ± 1.3 mmol/l at baseline to 5.1 ± 1.4 mmol/l after partial ($P = 0.810$) and to 5.9 ± 1.8 mmol/l after complete starvation ($P = 0.005$).

Table 5.1 Metabolic response to partial and complete starvation

Plasma concentrations	Baseline	Partial starvation	Complete starvation
Glucose (mmol/l)	5.0 ± 0.3	$4.3 \pm 0.4^*$	$3.9 \pm 0.5^*$
Insulin (mU/l)	10.1 ± 5.3	8.0 ± 3.7	$3.0 \pm 1.8^*$
Non-esterified fatty acids (mmol/l)	0.6 ± 0.4	$1.2 \pm 0.4^\dagger$	$1.9 \pm 0.7^\dagger$
Triglycerides (mmol/l)	1.3 ± 0.4	$0.9 \pm 0.3^*$	1.3 ± 0.6
Total cholesterol (mmol/l)	5.0 ± 1.3	5.1 ± 1.4	$5.9 \pm 1.8^*$

Values are mean $\pm$ standard deviation. Blood samples were collected 4 hours after the last meal.

* $P < 0.01$ and $^\dagger P < 0.001$ vs. baseline.

Effects of progressive caloric restriction on myocardial and hepatic triglyceride content

Myocardial triglyceride content increased dose dependently from $0.35 \pm 0.14\%$ at baseline to $0.59 \pm 0.27\%$ after partial ($P = 0.006$) and to $1.26 \pm 0.49\%$ after complete starvation ($P < 0.001$; Figure 5.2). Hepatic triglyceride content correlated with BMI at baseline ($r = 0.67$; $P = 0.033$). Hepatic triglyceride content significantly decreased after partial starvation (from 2.24 ± 2.24 to $1.43 \pm 1.33\%$; $P = 0.031$), whereas it did not change after complete starvation ($2.54 \pm 2.53\%$; $P = 0.378$; Figure 5.3).

Effects of progressive caloric restriction on myocardial function

Systolic and diastolic blood pressure, heart rate, and myocardial LVEF did not change significantly during/ after partial and complete starvation, compared with baseline (Table 5.2). Furthermore, estimated left ventricular filling pressures were unchanged after partial (8.8 ± 3.8 ; $P = 0.742$) and complete starvation (8.2 ± 2.5 ; $P = 0.299$) compared with baseline (9.3 ± 2.6). Diastolic E/A ratio decreased dose dependently from $2.2 \pm$

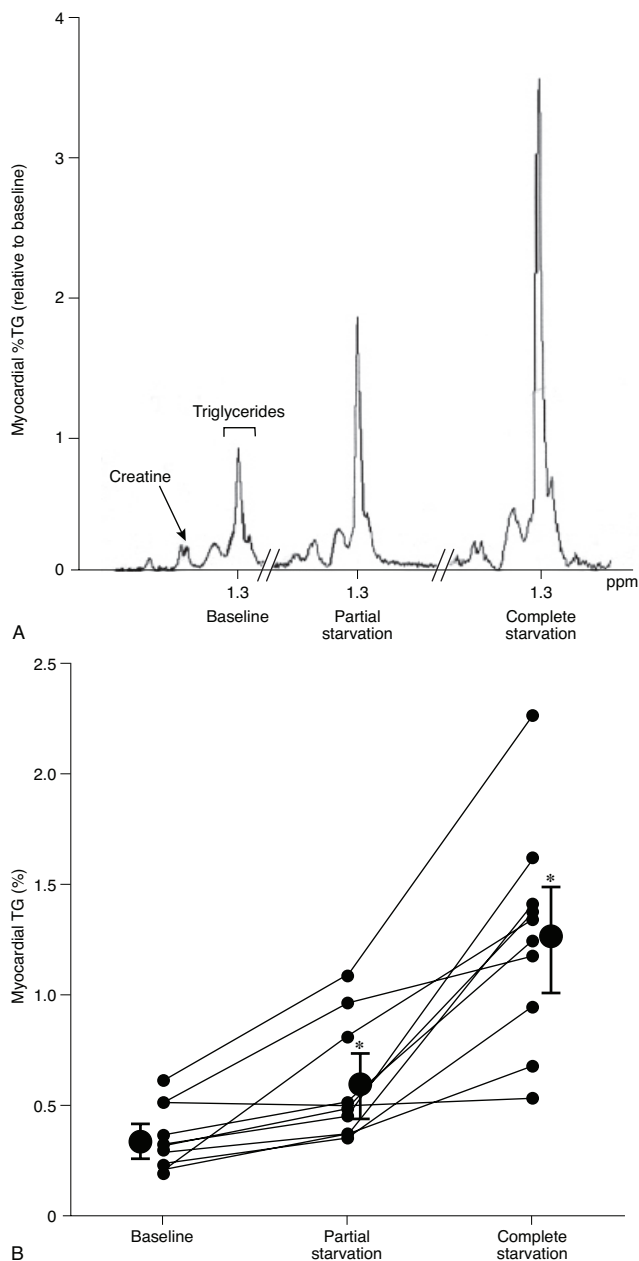


Figure 5.2

Myocardial triglyceride content at baseline and after partial and complete starvation. Typical proton spectra of myocardial triglyceride content of one subject at baseline and after partial and complete starvation scaled relative to baseline (A) and individual changes in myocardial triglyceride content upon complete starvation ($n = 10$ [B]). Vertical lines represent mean $\pm$ standard deviation.

TG = triglyceride.

* $P < 0.01$ vs. baseline.

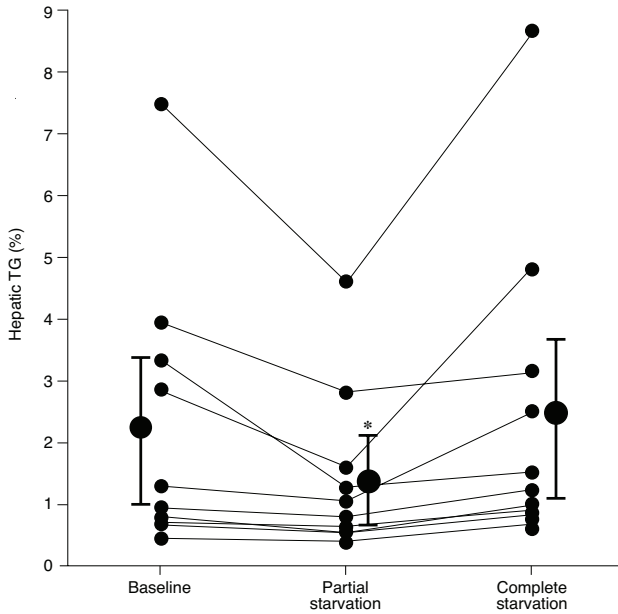


Figure 5.3

Hepatic triglyceride content at baseline, and after partial and complete starvation. Individual changes in hepatic triglyceride content upon complete starvation ($n = 10$). Vertical lines represent mean $\pm$ standard deviation.

* $P < 0.05$ vs. baseline.

0.4 at baseline to 2.1 ± 0.4 after partial starvation ($P = 0.687$) and to 1.8 ± 0.4 after complete starvation ($P = 0.005$). E deceleration decreased dose dependently from $3.4 \pm 0.7 \text{ ml/s}^2 \times 10^{-3}$ at baseline to $2.9 \pm 0.5 \text{ ml/s}^2 \times 10^{-3}$ after partial ($P = 0.036$) and to $2.8 \pm 0.9 \text{ ml/s}^2 \times 10^{-3}$ after complete starvation ($P = 0.032$).

Table 5.2 Effects of progressive caloric restriction on myocardial function

	Baseline	Partial starvation	Complete starvation
Systolic blood pressure (mmHg)	120 ± 10	118 ± 9	122 ± 12
Diastolic blood pressure (mmHg)	64 ± 7	62 ± 7	61 ± 5
Heart rate (bpm)	62 ± 13	59 ± 10	65 ± 10
Left ventricular ejection fraction (%)	60 ± 4	59 ± 4	60 ± 6
E/A	2.2 ± 0.4	2.1 ± 0.4	$1.8 \pm 0.4^*$
E deceleration ($\text{ml/s}^2 \times 10^{-3}$)	3.4 ± 0.7	$2.9 \pm 0.5^\dagger$	$2.8 \pm 0.9^\dagger$

Values are mean $\pm$ standard deviation.

* $P < 0.01$, $^\dagger P < 0.05$ vs. baseline.

DISCUSSION

This study demonstrates that progressive caloric restriction increases myocardial triglyceride content in lean healthy men. This increase is paralleled by decreased diastolic myocardial function. In addition, the results document a dose-dependent effect between the degree of caloric restriction and the myocardial effects. These observations point to physiological variations in myocardial triglyceride content and diastolic function. The effect of caloric restriction on redistribution of endogenous triglyceride stores is tissue-specific because we demonstrated differential effects of partial and complete starvation on liver triglyceride content.

Different degrees of starvation were associated with a considerable increase in plasma NEFA levels, in accordance with previous observations.^{25,26} These increased NEFA levels reflect increased lipolysis of triglyceride content in adipose tissue. Apparently, during starvation myocardial FA uptake exceeds the requirements of myocardial FA oxidation, resulting in increased triglyceride stores. Moreover, progressive caloric restriction has dose-dependent effects on myocardial triglyceride accumulation and myocardial function. However, a causal relationship between myocardial triglyceride content and myocardial function cannot be derived from the present data.

Our data are supported by animal experiments. In those studies excessive exposure of the myocardium to plasma NEFA is accompanied by increased storage of myocardial triglycerides, resulting in the production of fatty acid intermediates, and ultimately in deteriorations in myocardial function.^{8,27,28} Accordingly, it has been suggested that in obese subjects, subclinical diastolic dysfunction is due to changes in myocardial metabolism.²⁹⁻³² Kankaanpää et al. reported that alterations in left ventricular function in moderate obese subjects are associated with increased myocardial triglyceride content, compared with lean subjects.¹² Moreover, Szczepaniak et al. showed increased myocardial triglyceride content in overweight and obese subjects, which was accompanied by increased left ventricular mass.¹ In accordance with our study, Reingold et al. documented that short-term fasting leads to myocardial triglyceride accumulation, although they did not document effects on myocardial function.¹⁴ The current results, documenting dose-dependent effects of caloric restriction on levels of plasma NEFA, myocardial triglyceride content, and diastolic function, extend these findings and support the general concept that increased myocardial triglyceride content is associated with decreased myocardial function.³³ Alternatively, starvation profoundly alters endogenous metabolic regulation and other, yet undefined, metabolic effects than merely increased levels of plasma NEFA and myocardial triglyceride content, which may be involved in explaining the reduction in myocardial diastolic function. For example, caloric restriction might change calcium homeostasis in the myocardium,³⁴ which affects myocardial diastolic function.³⁵

Transmitral flow velocities are load dependent and can be affected by changes in intravascular volume. However, estimated left ventricular filling pressures were unchanged upon progressive caloric restriction. Therefore, we believe that the observed change in transmitral flow patterns results from a change in the relaxation of the left ventricle. Caloric restriction enhances adipose tissue lipolysis, reflected in increased levels of plasma NEFA, due to reduced insulin levels. Similar to our results in the heart, others found corresponding results of increased triglyceride content of skeletal muscle after fasting.^{19,25,26} Starvation affects more parameters of lipid metabolism because plasma NEFA stimulate the hepatic production of very low density lipoprotein (VLDL), which is an important supplier for triglycerides to the heart.^{36,37} Plasma NEFA levels also increase during starvation and most likely will contribute to increased myocardial triglyceride levels. However, the relative contribution of albumin-bound fatty acids vs. fatty acids derived from VLDL-triglyceride to myocardial triglyceride stores during caloric restriction cannot be derived from the present data.

We found a correlation between hepatic fat content and BMI, in accordance with previous observations.^{2,38} However, despite the increase in the flux of plasma NEFA to the liver, considering the increased plasma NEFA levels, hepatic triglyceride content was decreased after partial starvation but was unchanged after complete starvation. In line with our results, Westerbacka et al. previously documented that a low fat diet in moderately obese women decreases hepatic triglyceride content.⁹ Because hepatic triglyceride content is tightly regulated by the balance of hepatic fatty acid uptake, hepatic fatty acid oxidation, and output of VLDL-triglyceride particles, it is possible that this hepatic balance between fatty acid uptake and triglyceride output is differentially affected by partial and complete starvation. Nonetheless, our data indicate that progressive caloric restriction differentially affects tissue-specific stores of triglycerides in heart and liver, and show that myocardial triglyceride content and myocardial function vary depending on nutritional conditions, at least with respect to progressive degrees of starvation. Additional studies are required to elucidate to which extent these results can be extrapolated to clinically relevant conditions like type 2 diabetes mellitus and obesity.

In conclusion, progressive caloric restriction induces a dose-dependent increase in myocardial triglyceride content and a dose-dependent decrease in diastolic function in lean healthy men. Hepatic triglyceride content showed a differential response to progressive caloric restriction, indicating that redistribution of endogenous triglyceride stores is tissue-specific, at least in lean healthy men.

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Effects of short-term high-fat, high-energy diet on hepatic and myocardial triglyceride content in healthy men

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ABSTRACT

Background

An association has been suggested between elevated plasma non-esterified fatty acid (NEFA) levels, myocardial triglyceride accumulation, and myocardial function. Therefore, our objective was to investigate the effects of an elevation of plasma NEFA by a high-fat, high-energy (HFHE) diet on hepatic and myocardial triglyceride accumulation, and on myocardial function.

Methods

There were 15 healthy males (mean $\pm$ SD age: 25.0 ± 6.6 years) subjected to a 3-day HFHE diet consisting of their regular diet, supplemented with 800 ml cream (280 g fat) every day. ^1H -magnetic resonance spectroscopy was performed for assessing hepatic and myocardial triglycerides. Furthermore, left ventricular function was assessed using magnetic resonance imaging.

Results

The HFHE diet increased hepatic triglycerides compared with baseline (from 2.01 ± 1.79 to $4.26 \pm 2.78\%$; $P < 0.001$) in parallel to plasma triglycerides and NEFA. Myocardial triglycerides did not change (0.38 ± 0.18 vs. $0.40 \pm 0.12\%$; $P = 0.7$). The HFHE diet did not change myocardial systolic function. Diastolic function, assessed by dividing the maximum flow across the mitral valve of the early diastolic filling phase by the maximum flow of the atrial contraction (early filling phase to atrial contraction ratio), decreased compared with baseline (from 2.11 ± 0.39 to 1.89 ± 0.33 ; $P = 0.031$). This difference was no longer significant after adjustment for heart rate ($P = 0.12$).

Conclusion

Short-term HFHE diet in healthy males results in major increases in plasma triglycerides and NEFA concentrations and hepatic triglycerides, whereas it does not influence myocardial triglycerides or myocardial function. These observations indicate differential, tissue-specific distribution of triglycerides and/ or fatty acids among non-adipose organs during HFHE diet.

Dietary triglycerides are absorbed for more than 95% by the gut. After absorption, these triglycerides can either be oxidized or stored in adipose tissue. A minimal part of these dietary triglycerides may be stored in non-adipose tissue, such as the pancreas, liver, and myocardium. Storage of triglycerides in non-adipose tissues is very tightly regulated, and disruption of this regulation is associated with functional and structural changes. In humans, high-fat (HF) diets rapidly increase plasma triglycerides and non-esterified fatty acid (NEFA) levels, increase hepatic triglyceride content, and cause insulin resistance.¹ Short-term HF diets also increase intramyocellular triglyceride content in skeletal muscle accompanied by molecular adaptations that favor fat storage in muscle rather than oxidation.²

In some conditions, the myocardium can also accumulate triglycerides. This increase in myocardial triglyceride content may be of pathophysiological relevance. Patients suffering from type 2 diabetes mellitus show increased myocardial triglyceride content,³ and healthy volunteers, who were fed a very low calorie diet for 3 days, showed increased plasma NEFA levels and accumulated myocardial triglycerides. The increase in myocardial triglycerides was associated with alterations in myocardial function.⁴ In addition, in an animal model, a HF diet for 7 weeks causes cardiac steatosis and myocardial dysfunction.⁵

Increased plasma NEFA levels are also associated with abnormal myocardial energy metabolism. In patients with type 2 diabetes mellitus, myocardial high-energy phosphate (HEP) metabolism was significantly impaired.⁶ Furthermore, obese men with preserved systolic and diastolic function showed abnormal myocardial HEP metabolism, which was associated with insulin resistance.⁷

Proton (¹H)-magnetic resonance spectroscopy (MRS), phosphorus (³¹P)-MRS, and magnetic resonance imaging (MRI) are imaging tools, perfectly capable of assessing hepatic and myocardial triglyceride content, myocardial HEP metabolism, and myocardial function non-invasively.⁸⁻¹¹

In humans, a single HF containing meal had no influence on myocardial triglyceride content and on hepatic triglyceride content.^{12,13} This one-meal intervention might have been too subtle to initiate myocardial and hepatic triglyceride accumulation. The effect of a prolonged disturbance of plasma lipids on myocardial triglyceride accumulation remains to be investigated.

Therefore, the goal of the present study was to investigate the effect of a 3-day high-fat, high-energy diet on hepatic and myocardial triglyceride accumulation, myocardial HEP metabolism, and on myocardial function in healthy subjects using MRS and MRI.

METHODS

Subjects

There were 15 healthy men who volunteered to participate in this study that was approved by the local ethics committee. Only males were included because the hormonal status or use of contraceptives may affect lipid metabolism in women. Given the well-documented effects of estrogens on lipid metabolism (including plasma lipid levels, adipose tissue) and the gender differences in expression of certain cell-surface receptors/transporters of fatty acids,^{14,15} we decided to exclude women at this stage to avoid the possible confounding influences of potential fluctuation in lipid metabolism in women on hepatic and myocardial triglyceride accumulation. All volunteers signed written informed consent. Subjects were included if they met the following criteria: 1) age older than 18 years; and 2) no known acute or chronic disease based on history, physical examination, and standard laboratory tests (blood counts, serum creatine, alanine aminotransferase, aspartate aminotransferase, and electrocardiogram [ECG]). Exclusion criteria included treatment with drugs, smoking, substance abuse, hypertension, or impaired glucose tolerance (as determined by a 75-g oral glucose tolerance test).¹⁶ All subjects performed exercise (walking, running, biking) regularly (range 3–5 hours weekly), but none of the subjects engaged in high-performance sports.

Study design

Subjects underwent magnetic resonance (MR) scanning in the afternoon at two different occasions. Before both visits, they were instructed to follow different dietary regimes for 3 days before the measurements. The use of alcohol was not allowed during the 3-day diets. In the first regime, each subject used his normal diet. Mean intake was approximately 2100 kcal/day. The calories were approximately divided as follows: carbohydrates 40%, fat 35%, and protein 25%.

This reference diet was used for the collection of baseline data. The last meal was consumed 4 hours before venous blood samples and data collection. During the second regime, the subjects were placed on a 3-day hypercaloric diet characterized by high-fat, high-energy (HFHE) content. The HFHE diet consisted of the same intake as the reference diet, complemented with 800 ml cream every day. The cream added 2632 kcal/day (carbohydrates 3.5%, fat 94%, and protein 2.5%). Therefore, during the HFHE diet, total energy intake was approximately 4732 kcal/day with the calories divided as: carbohydrates 20%, fat 69%, and protein 11%. The last 200 ml cream was taken 4 hours before data collection. The HFHE content was used to induce an elevation of plasma NEFA and triglyceride levels. At each visit, after venous blood collection, MRI and MRS of the heart and liver were performed.

Proton magnetic resonance spectroscopy

All MRI/ MRS studies were performed using a 1.5T whole-body MR scanner (Gyrosan ACS/ NT15; Philips, Best, the Netherlands) with subjects in the supine position at rest. Cardiac ^1H -MR spectra were obtained from the interventricular septum as described before.⁹ The body coil was used for radiofrequency transmission, and a 17 cm diameter circular surface coil was used for signal reception.

A point-resolved spectroscopy sequence was used to acquire single voxel MR spectroscopic data from an 8 ml voxel, located in the interventricular septum (Figure 6.1). Spectra were acquired at end-systole, with an echo time (TE) of 26 ms and a repetition time (TR) of at least 3000 ms. A total of 1024 data points was collected using a 1000-Hz spectral width and averaged over 128 acquisitions. The spectroscopic data acquisition was ECG-triggered, and respiratory gating based on navigator echoes was applied to minimize breathing influences.⁹ Without changing any parameter, spectra without water suppression with a TR of 10 seconds and 4 averages were obtained, to be used as an internal standard.

^1H -MRS of the liver was performed with an 8 ml voxel positioned in the liver, avoiding gross vascular structures and adipose tissue depots. The 12th thoracic vertebra was used as a landmark to ensure the same position of the voxel during both visits. Spectra

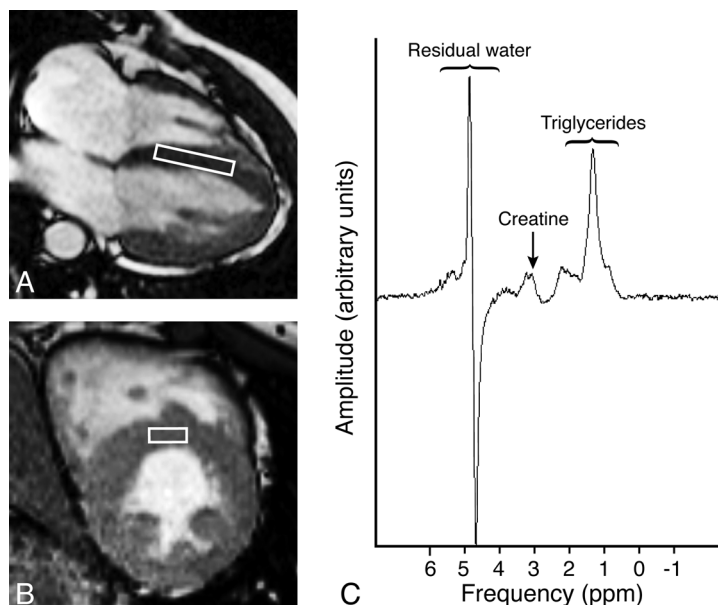


Figure 6.1

Myocardial voxel localization for ^1H -MRS.

Voxel position in four-chamber (A) and short-axis (B) views. An 8 ml voxel was positioned in the interventricular septum in end-systole. In panel C, a typical water-suppressed spectrum is demonstrated.

were obtained without respiratory motion compensation using the same parameters as described previously. Only 64 averages were collected with water suppression.

All ^1H -MR spectroscopic data were fitted using Java-based MR user interface software (jMRUI version 2.2; developed by A. van den Boogaart, Katholieke Universiteit Leuven, Leuven, Belgium)¹⁷ as described before.⁹ Resonance frequency estimates for intramyocardial lipids were described with the assumption of gaussian line shapes at 0.9, 1.3, and 2.1 parts per million (ppm; only data from the peaks at 0.9 and 1.3 ppm were summated and used on statistical analysis¹⁸). Prior knowledge was incorporated into the fitting algorithm using previously published criteria.^{19,21} The water signal from spectra without water suppression obtained from the same voxel was used as an internal reference for relative quantification of lipid resonances. The water signal peak at 4.7 ppm was quantified using a Lorentzian line shape and analyzed using the AMARES algorithm. The percentage of myocardial and hepatic triglyceride signals relative to the water signal was calculated as: (signal amplitude of triglycerides)/(signal amplitude of water) $\times$ 100 (percent triglyceride uncorrected for T2 decay times of the studied metabolites).

Phosphorus magnetic resonance spectroscopy

A 10 cm diameter surface coil was used to acquire ECG-triggered ^{31}P -MR spectra of the left ventricular (LV) anterior wall with subjects in the supine position. Volumes of interest were selected by image-guided spectroscopy with three-dimensional image selected in vivo spectroscopy. Shimming was performed automatically, and tuning and matching of the ^{31}P -surface coil were performed manually. Technical details of data acquisition and spectral quantification were similar as described before.¹¹ Shortly, spectroscopic volume size was typically $7 \times 7 \times 7$ cm. Acquisitions were based on 192 averaged free induction decays, and total acquisition time was 10 min. ^{31}P -MR spectra were quantified automatically in the time domain using prior spectroscopic knowledge, and were corrected for partial saturation effects and for the ATP contribution from blood in the cardiac chambers. The phosphocreatine (PCr)/ATP ratios of the spectra were calculated and used as a parameter representing myocardial HEP metabolism.²²

Magnetic resonance imaging

The entire heart was imaged in short-axis orientation using ECG-gated breath-holds with a sensitivity encoding balanced steady-state free precession sequence. Imaging parameters included the following: TE = 1.7 ms, TR = 3.4 ms, flip angle = 35° , slice thickness = 10 mm with a gap of 0 mm, field of view = 400×400 mm, and reconstructed matrix size = 256×256 . The temporal resolution was 25–39 ms. All images were analyzed quantitatively using dedicated software (MASS; Medis, Leiden, the Netherlands). LV ejection fractions were assessed as measures of LV systolic function. Furthermore, an ECG-gated gradient-echo sequence with velocity encoding (Venc) was performed to

measure blood flow across the mitral valve for the determination of LV diastolic function. Imaging parameters included the following: TE = 4.8 ms, TR = 14 ms, flip angle = 20°, slice thickness = 8 mm, field of view = 350 × 350 mm, matrix size × 256 × 256, Venc = 100 cm/s, and scan percentage = 80%. Analysis was performed using dedicated software (FLOW; Medis). The early filling phase (E) and the atrial contraction (A) were analyzed, and the ratio of the maximal flow rate of E and the maximal flow rate of A (E/A) were calculated. In addition, the peak deceleration gradient of E was assessed. Furthermore, LV filling pressures (E/Ea) were estimated.²³ During MRI, blood pressure and heart rate were measured.

Assays

Plasma glucose and triglycerides were measured by a fully automated P800 analyzer (Roche, Almere, the Netherlands) and insulin using an Immulite 2500 random access analyzer with a chemoluminescence immunoassay (Diagnostic Products Corp., Los Angeles, CA). Coefficients of variation were less than 2% for glucose and triglycerides, and less than 5% for insulin. The homeostasis model of assessment index was calculated as (glucose × insulin)/22.5. Plasma NEFA were measured using a commercial kit (NEFA-C; Wako Chemicals, Neuss, Germany). C-reactive protein (CRP) was determined with a us-CRP ELISA (Diagnostic Systems Laboratories, Inc., Webster, TX). The sensitivity was 1.6 µg/l, and the interassay coefficients of variation ranged from 3–5%.

Statistical analysis

Statistical analysis was performed with SPSS for windows (version 12.0; SPSS, Chicago, IL). Data are expressed as mean ± standard deviation (SD). The two study conditions were compared by a two-tailed paired-samples T-test. The linear mixed model was used for correcting within-subject differences when necessary. Significance was assumed when $P < 0.05$.

RESULTS

Clinical and biochemical characteristics

All participants completed the protocol uneventfully. The mean age of the studied subjects was 25.0 ± 6.6 years. Characteristics of the studied subjects at baseline and after the HFHE diet are shown in Table 6.1.

After the HFHE diet, postprandial plasma insulin levels increased significantly (from 9.1 ± 4.6 to 21.4 ± 8.8 mU/l; $P < 0.001$), as did plasma triglycerides (from 1.3 ± 0.4 to 2.9 ± 1.1 mmol/l; $P < 0.001$) and plasma NEFA (from 0.54 ± 0.29 to 0.92 ± 0.33

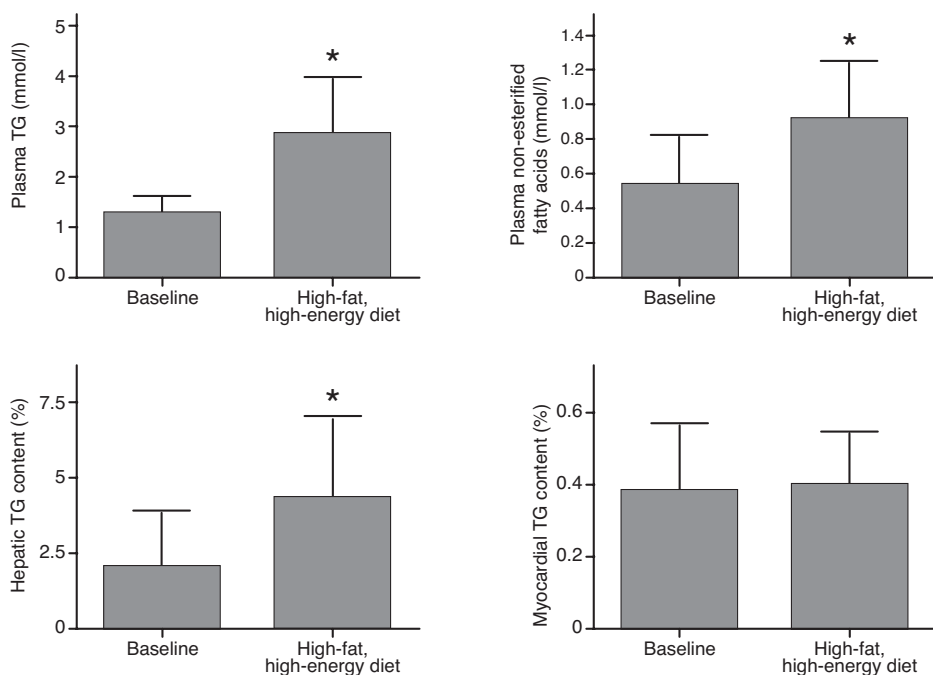
Table 6.1 Clinical and biochemical characteristics

	Baseline	High-fat, high-energy diet	P-value
Body mass index (kg/m ²)	23.4 ± 2.5	23.6 ± 2.5	0.098
Systolic blood pressure (mmHg)	123 ± 13	125 ± 13	0.673
Diastolic blood pressure (mmHg)	67 ± 8	64 ± 8	0.179
Heart rate (beats/min)	60 ± 9	69 ± 11	0.008
Plasma glucose (mmol/l)	4.9 ± 0.3	5.0 ± 0.4	0.356
Plasma insulin (mU/l)	9.1 ± 4.6	21.4 ± 8.8	< 0.001
HOMA index	2.0 ± 1.2	4.9 ± 2.3	0.001
Plasma triglycerides (mmol/l)	1.3 ± 0.4	2.9 ± 1.1	< 0.001
Plasma non-esterified fatty acids (mmol/l)	0.54 ± 0.29	0.92 ± 0.33	0.002
Plasma alanine aminotransferase (mmol/l)	25 ± 16	28 ± 13	0.769
Plasma aspartate aminotransferase (mmol/l)	33 ± 10	33 ± 7	0.250
Gamma glutamyl transferase (mmol/l)	20 ± 8	20 ± 6	0.849
Ultra-sensitive C-reactive protein (mg/l)	3.6 ± 4.8	1.4 ± 1.1	0.074

Values are mean ± standard deviation.

P-values were calculated using two tailed paired-samples T-tests.

HOMA = homeostasis model assessment.

**Figure 6.2**

Lipid response to a high-fat, high-energy diet.

Hepatic and myocardial triglyceride content are relative to the hepatic and myocardial water signals.

* $P < 0.05$, bars represent mean + standard deviation.

TG = triglycerides.

mmol/l; $P = 0.002$) levels (Figure 6.2). Plasma glucose levels remained unchanged (4.9 ± 0.3 vs. 5.0 ± 0.4 mmol/l).

Magnetic resonance spectroscopy

After the HFHE diet, ^1H -MRS revealed a significant increase in hepatic triglyceride content compared with baseline (4.26 ± 2.78 vs. 2.01 ± 1.79 ; $P < 0.001$; Figure 6.2). Typical hepatic ^1H -MR spectra of one volunteer before and after the HFHE diet are shown

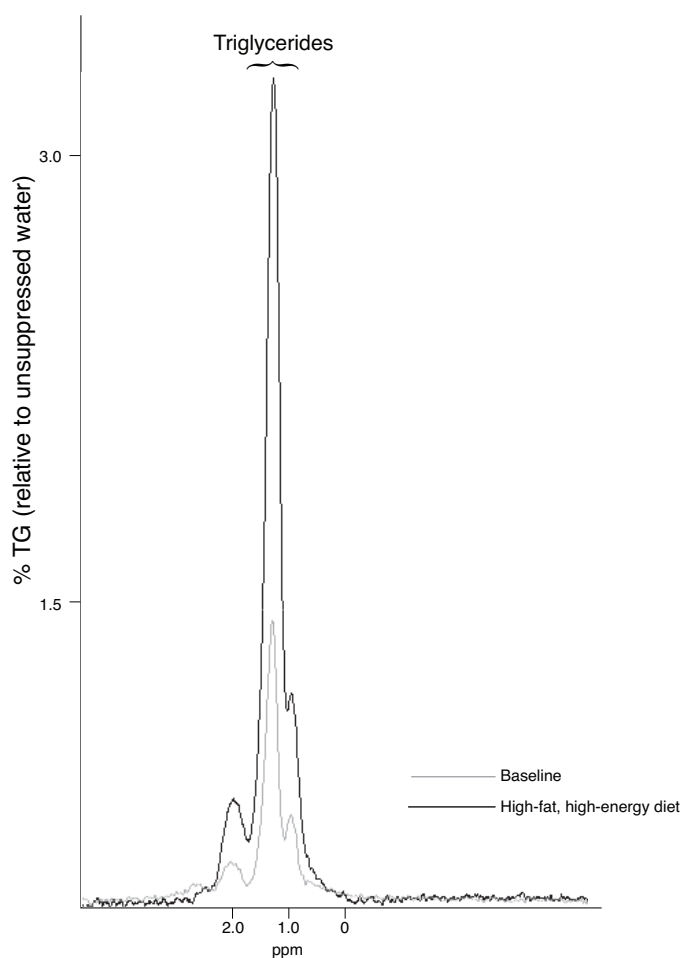


Figure 6.3

Typical ^1H -MRS of the liver of one subject before and after high-fat, high-energy diet. Only the triglyceride region is displayed. Note the marked increase in hepatic triglycerides after the high-fat, high-energy diet.

%TG is the amount of triglycerides relative to water $\times 100$.

in Figure 6.3. No significant difference in myocardial triglyceride content was detected after the HFHE diet compared with baseline (0.40 ± 0.12 vs. $0.38 \pm 0.18\%$; $P = 0.7$; Figure 6.2).

In eight subjects, ^{31}P -MRS was successfully completed on both study occasions. In the other seven subjects, ^{31}P -MRS data at baseline or after the HFHE diet could not successfully be completed due to technical problems or insufficient spectral quality (relative Cramer-Rao SD > 20% ¹¹). After the HFHE diet, the myocardial PCr/ATP ratio remained unchanged (2.37 ± 0.51 vs. 2.35 ± 0.46 ; $P = 0.95$).

Myocardial function by magnetic resonance imaging

The parameters of myocardial function are shown in Table 6.2. The HFHE diet did not affect LV systolic function. Myocardial workload, represented by the rate pressure product (RPP; RPP = heart rate $\times$ systolic blood pressure) was significantly increased after the HFHE diet compared with baseline (from 7312 ± 1354 to 8563 ± 1867 mmHg $\times$ beats per minute (bpm); $P = 0.02$). The HFHE diet decreased the E/A ratio, a measure of diastolic function, significantly compared with baseline (from 2.11 ± 0.39 to 1.89 ± 0.33 ; $P = 0.031$) and increased heart rate significantly from 60 ± 9 to 69 ± 11 bpm ($P = 0.008$). After adjustment for heart rate, there were no significant differences in E/A ratios between the two diets ($P = 0.12$).

Table 6.2 The effects of a high-fat, high-energy diet on metabolic and left ventricular functional parameters

	Baseline	High-fat, high-energy diet	P-value
Triglyceride content liver (%)	2.01 ± 1.79	4.26 ± 2.78	0.001
Triglyceride content heart (%)	0.38 ± 0.18	0.40 ± 0.12	0.696
PCr/ATP	2.37 ± 0.51	2.35 ± 0.46	0.945
Ejection fraction (%)	60 ± 4	62 ± 5	0.100
Rate pressure product (mmHg $\times$ bpm)	7312 ± 1354	8563 ± 1867	0.023
E deceleration peak (ml/s ² $\times 10^{-3}$)	5.0 ± 1.0	5.1 ± 1.2	0.668
E/Ea	8.8 ± 2.0	9.1 ± 4.0	0.659
E peak filling rate (ml/s)	614 ± 89	630 ± 125	0.529
A peak filling rate (ml/s)	299 ± 64	340 ± 75	0.024
E/A	2.11 ± 0.39	$1.89 \pm 0.33^*$	0.031*

Values are mean $\pm$ standard deviation.

P-values were calculated using two tailed paired-samples T-tests.

E = early diastolic filling phase; A = atrial contraction; E/A = ratio of maximal left ventricular early peak filling rate and the maximal left ventricular atrial peak filling rate; E/Ea = estimation of LV filling pressures.

* Adjusted for heart rate, there was no significant difference in E/A between the two diets.

DISCUSSION

This study shows that in males, a short-term intervention with a hypercaloric, high-fat diet increases postprandial plasma NEFA and triglyceride concentrations considerably, which is associated with a more than 2-fold increase in hepatic triglyceride content. In contrast, this HFHE diet has no acute effects on myocardial triglyceride content, myocardial HEP metabolism, or myocardial function, despite the increased supply of NEFA and triglycerides to the heart. These observations stress the short-term physiological and tissue-specific flexibility of ectopic triglyceride pools.

Increased plasma NEFA and triglyceride levels after the 3-day hypercaloric HF diet, indicating good dietary compliance of the volunteers, were associated with a more than 2-fold increase in hepatic triglyceride content. Westerbacka et al.²⁴ previously reported similar findings on the effects of dietary interventions on hepatic triglyceride content in women. The liver acts as a buffer for excessive postprandial flux of NEFA and triglycerides.²⁵ The current observation indicates that these hepatic triglyceride stores already expand during very short-term HFHE diets. Based on the unchanged plasma levels of liver enzymes and CRP, short-term hepatic triglyceride accumulation did not contribute to overt indications for hepatic cellular damage or steatohepatitis. Previously published data showed that hepatic liver steatosis is associated with insulin resistance.²⁶ Our study supports these findings. However, because our study only involves a short-term exposure to an unphysiological HFHE diet, we cannot simply extrapolate the findings of our study to the longer term.

The HFHE diet was also associated with increased plasma insulin levels. Insulin promotes the synthesis and storage of triglycerides in the liver, and inhibits the release of very low-density lipoprotein-triglyceride into the circulation.²⁷ In addition, insulin increases the expression or activity of enzymes that catalyze lipid synthesis, whereas insulin inhibits the activity or expression of those that catalyze degradation. Many of these processes require an insulin induced increase of the transcription factor sterol-regulatory element-binding protein-1c,²⁸ which, in the liver, is increased by a HF diet.²⁹

In contrast to the accumulation of hepatic triglycerides, myocardial triglyceride content remained unchanged after the HFHE diet. Apparently, increased plasma NEFA and triglyceride levels after short-term consumption of a HFHE diet do not change the inter-relationship between myocardial NEFA and triglyceride uptake and oxidation in the healthy human heart. This absence of effects of a HFHE diet on myocardial triglyceride content is also in contrast to the response of skeletal muscles, which have accumulated triglycerides under HF feeding conditions.^{1,2} We expected a similar response of the myocardium to a HFHE diet based on these reports. Apparently, increased plasma NEFA and triglyceride levels are not a determinant of excessive myocardial fatty acid uptake, in excess of fatty acid oxidation during short-term HFHE diets in healthy male volunteers.

This might be explained by the increased RPP in our study after the HFHE diet. Because plasma glucose concentrations were constant, and plasma NEFA and triglyceride levels increased, the increased cardiac workload probably led to an increase in cardiac lipid oxidation rates^{30,31} that compensated for the increased lipid availability resulting in no net change in myocardial triglyceride content. In the conditions of our study, carbohydrate intake was not changed. It has been suggested³² that in healthy, non-diabetic human subjects, dietary induced intramyocellular triglyceride accumulation and NEFA oxidation in healthy humans may be influenced by dietary carbohydrate intake, plasma glucose availability,³³ and muscular glycogen stores.³⁴ We cannot exclude the possibility that HF diets with decreased carbohydrate content may have resulted in changed myocardial triglyceride content.

Chronically elevated plasma NEFA levels in patients with type 2 diabetes mellitus are associated with altered myocardial HEP metabolism.⁶ Increased fatty acid availability in these patients results in increased NEFA uptake in the mitochondria, which decreases the amount of ATP produced per molecule of oxygen consumed in the mitochondrial electron transport chain.³⁵ In the present study, the short-term HFHE diet and the associated increase in plasma NEFA levels did not affect myocardial HEP metabolism. In our opinion these findings are in line with the unchanged myocardial triglyceride content that may indicate no dysregulation of mitochondrial substrate handling.

We used MRI to study the impact of a HFHE diet-induced increase of plasma NEFA levels on LV function. Chronically elevated levels of plasma NEFA have been associated with decreased diastolic function in obesity.³⁶ In the present study, short-term elevated plasma NEFA levels as a consequence of a HFHE diet did not affect myocardial systolic and diastolic function. Although there was a slight decrease in the diastolic E/A ratio after the HFHE diet, mainly caused by an increase in A peak flow rate, this change in the E/A ratio was accompanied by an increased heart rate, which is a well-known postprandial alteration, especially during HF feeding.³⁷⁻³⁹ An elevated heart rate accounts for an increased preload of the left ventricle, which influences LV filling velocities. Adjusted for heart rate, the E/A ratios before and after the HFHE diet were not significantly different, indicating no change in LV diastolic function.

Caloric restriction also increases plasma NEFA levels, which is accompanied by decreased plasma glucose and unchanged plasma insulin levels. This leads to myocardial triglyceride accumulation, associated with decreased myocardial function.⁴ Increased plasma NEFA levels after the HFHE diet were accompanied by unchanged plasma glucose and increased plasma insulin levels. After a HFHE diet, there are no changes in myocardial triglyceride content or myocardial function. Apparently, increased plasma NEFA levels after caloric restriction or HFHE diets are associated with different metabolic states, and, therefore, influence myocardial triglycerides and function differently. These findings are in line with the hypothesis that there might be an association between

myocardial triglyceride accumulation and diastolic function. Most likely, myocardial triglyceride stores themselves are inert but, rather, are a reflection of increased intracellular concentrations of fatty acid intermediates that alter myocellular structure and function by complex molecular mechanisms.⁴⁰ Further studies need to be conducted to unravel this hypothesis.

Some potential limitations of this study should be addressed. First, excluding women from the study and the narrow age range used in this study limit the generalizability of the present study. Further studies need to be initiated to extend the present finding to subjects from both genders and different ages.

Second, data on myocardial lipid uptake and oxidation rates would extend our findings. However, to approximate myocardial lipid uptake and oxidation rates, positron emission tomography using palmitate tracers should be performed, which is a complicated technique.

Finally, only half the volunteers completed ³¹P-MRS measurements, and, therefore, sample size for this parameter is limited and should be interpreted with caution.

In conclusion, a short-term HFHE diet in healthy males results in major increases in plasma triglycerides and NEFA concentrations and hepatic fat content, whereas it does not influence myocardial triglyceride content or myocardial function. These observations indicate differential, tissue-specific distribution of triglycerides and/ or fatty acids among non-adipose organs during a HFHE diet.

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The ageing male heart: myocardial triglyceride content as independent predictor of diastolic function

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ABSTRACT

Background

In animal models of obesity and diabetes mellitus, myocardial triglyceride accumulation is associated with decreased myocardial function. In the physiologically ageing heart, myocardial triglyceride accumulation may also occur due to reduced myocardial fatty acid oxidation. The role of myocardial triglyceride in the ageing human heart is unknown. Therefore, the purpose of our study was to evaluate the effects of ageing on myocardial triglyceride content, and to determine the association between myocardial triglyceride content and heart function.

Methods

^1H -magnetic resonance spectroscopy and magnetic resonance imaging of the heart were performed in 43 healthy male subjects. Mean age (range) of the subjects was 44 (20–66) years. Body mass index (BMI), blood pressure, and biochemical markers were determined.

Results

Age correlated significantly to myocardial triglyceride content ($r = 0.57$, $P < 0.05$) independently of BMI. Furthermore, myocardial triglyceride content correlated negatively with left ventricular diastolic function (represented by E/A ratio, $r = -0.68$, $P < 0.05$). Multivariable analysis indicated myocardial triglyceride content as independent predictor ($P < 0.05$) of the age related decrease in diastolic heart function.

Conclusion

Myocardial triglyceride content increases in the physiologically ageing male heart and is associated with the age-related decline in diastolic function, independent of BMI, blood pressure, and biochemical blood markers.

The risk of cardiovascular complications is increased in patients with type 2 diabetes mellitus.¹ Despite commendable efforts to understand the pathophysiology of these co-morbidities, the exact mechanism of disease remains unknown. It is suggested that myocardial lipotoxicity plays a role in developing diabetic cardiomyopathy.² Recently, McGavock et al.³ demonstrated increased myocardial triglyceride content in patients with diabetes. In animal models of obesity and diabetes mellitus, myocardial triglyceride accumulation has been demonstrated to be associated with decreased myocardial function.^{2,4}

In humans, physiological ageing is a significant risk factor for developing obesity or type 2 diabetes mellitus⁵ and is associated with a variety of complex changes in cardiovascular function.^{6,7} Ageing leads to a decline in diastolic left ventricular function, and increased cardiovascular stiffness partly caused by a decrease in vascular endothelial-dependent dilatation and increased endothelial oxidative stress.^{6,8}

Furthermore, in the ageing heart, myocardial fatty acid oxidation is reduced.⁹ This may affect the balance between fatty acid oxidation and storage of non-esterified fatty acids (NEFA) in the form of triglycerides in cardiomyocytes. It is postulated that NEFA taken up from the blood plasma by myocytes in excess of oxidative requirements will be stored as triglycerides, resulting in triglyceride accumulation.¹⁰ Most likely, myocardial triglyceride stores themselves are inert, but rather are a reflection of increased intracellular concentrations of fatty acid intermediates that alter myocellular structure and function by complex molecular mechanisms.^{10,11} Age-related physiological triglyceride accumulation in the heart may become important by increasing susceptibility to lipotoxicity in otherwise healthy individuals. However, the relation between ageing, myocardial triglyceride content, and myocardial function has not been addressed in humans.

It has been shown that visceral fat content and hepatic triglyceride content are associated with myocardial triglyceride content.³ These sites of extra-cardiac lipid accumulation are fat depots which are increased in the older population and correlate with body mass index (BMI) and are of pathophysiological interest since they are indicators of metabolic disturbances.¹²⁻¹⁵

¹H-magnetic resonance spectroscopy (MRS) enables to measure myocardial and hepatic triglyceride content in humans non-invasively.^{13,16,17} Furthermore, magnetic resonance imaging (MRI) is a reproducible tool for assessing cardiovascular function^{18,19} and visceral adipose tissue.²⁰ Accordingly, the purpose of the present study was to evaluate the association between ageing and myocardial triglyceride content, and to determine the effect of myocardial triglyceride content on heart function using MRS and MRI techniques. In addition, hepatic and visceral fat depositions were studied.

METHODS

Study subjects

Volunteers for this study that was approved by the local ethics committee were recruited by advertisements in local papers. Only males were included, because the hormonal status or use of contraceptives may affect lipid metabolism in women. Given the well documented effects of estrogens on lipid metabolism (including plasma lipid levels, adipose tissue) and the gender differences in expression of certain cell surface receptors/transporters of fatty acids,^{21,22} we decided to exclude women at this stage to avoid possible confounding influences of potential fluctuation in lipid metabolism in women on hepatic and myocardial triglyceride accumulation.

Overall selection criteria were: age >18 years, no known acute or chronic disease based on history and physical examination and standard laboratory tests (blood counts, fasting plasma glucose, lipids, creatinine, alanine aminotransferase, aspartate aminotransferase); a normal electrocardiogram; no signs or symptoms of (previous) cardiovascular disease. Exclusion criteria included drug treatment, smoking, substance abuse, or hypertension. Furthermore, a 75-g oral glucose tolerance test²³ was conducted to exclude diabetes mellitus. Moreover, homeostasis model assessment (HOMA) index was calculated as fasting plasma glucose $\times$ fasting plasma insulin/22.5.²⁴

Based on two-tailed sample size calculation (G*Power version 3.0.8, Universität Kiel, Germany: one correlation: difference from constant—one sample case) indicated that with a zero-hypothesis = no correlation between age and myocardial triglyceride content, and as alternative hypothesis = a correlation of at least 0.5, when having a power of 90% and an α -level of 0.05 we had to include 37 subjects. Technical difficulties which would result in loss of data were anticipated by small oversampling. Therefore, we aimed to include more than 40 subjects. We used the same assumptions for the correlation between myocardial function and myocardial triglyceride content. Sixty-four subjects were initially assessed for inclusion into the study. All these 64 volunteers provided written informed consent before the first screening assessments. After the first screening assessments, 20 volunteers were excluded (19 volunteers showed abnormalities in their laboratory tests, one volunteer had an abnormal electrocardiogram). The remaining 44 subjects underwent MR scanning under the same fasting conditions (6–12 hours of fasting). One volunteer turned out to have cardiac abnormalities during MR scanning and was therefore excluded from further analysis. Therefore, data of 43 volunteers were analyzed.

Magnetic resonance spectroscopy

All MRI/ MRS studies were performed with the use of a 1.5T whole-body MR scanner (Gyrosan ACS/ NT1.5; Philips, Best, the Netherlands) with subjects resting in the supine position.

Cardiac ^1H -MR spectra (volume of interest = 8 ml) were obtained from the interventricular septum (Figure 7.1) as described previously.¹⁷ Spectroscopic data acquisition was double triggered using electrocardiographical triggering and respiratory navigator echoes to minimize breathing influences.^{17,25} Spectra were acquired at end-systole, with an echo time (TE) of 26 ms and a repetition time (TR) of at least 3000 ms. A total of 1024 data points were collected using a 1000 Hz spectral width and averaged over 128 acquisitions. Without changing any parameter, spectra without water suppression with a TR of 10 seconds and 4 averages were obtained, to be used as an internal standard.

^1H -MRS of the liver was performed with an 8 ml voxel positioned in the liver, avoiding gross vascular structures and adipose tissue depots. Spectra were obtained using the same parameters as described above. Sixty-four averages were collected with water suppression.

All ^1H -MRS data were fitted using Java-based MR user interface software (jMRUI version 2.2; developed by A. van den Boogaart, Katholieke Universiteit Leuven, Leuven, Belgium)²⁶ as described previously.¹⁷ Myocardial and hepatic triglyceride signals at 0.9 and 1.3 ppm from water-suppressed spectra and the water signal at 4.7 ppm from spectra without water suppression obtained from the same voxel were analyzed by using the Advanced Magnetic Resonance fitting algorithm within jMRUI.²⁷ Myocardial and hepatic triglyceride content relative to water were calculated as triglyceride/water $\times$ 100.

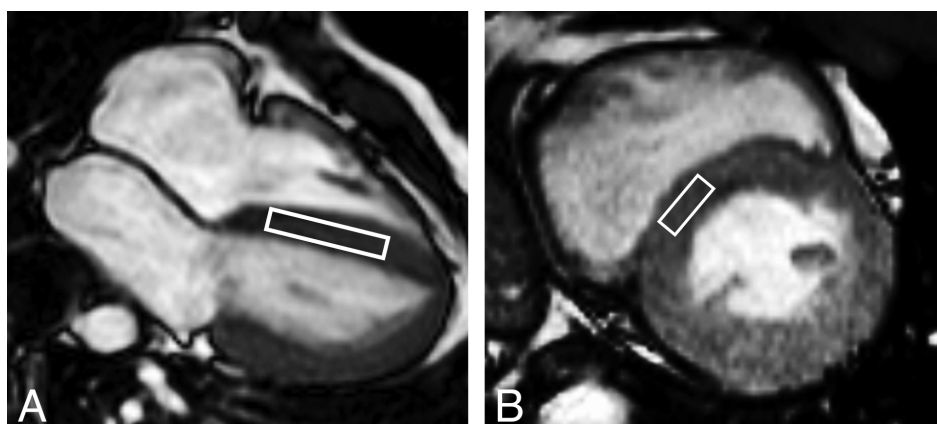


Figure 7.1

Myocardial voxel localization for ^1H -MRS. Voxel position in four-chamber (A) and short-axis (B) views.

Magnetic resonance imaging

The entire heart was imaged in the short-axis orientation ¹⁸ using electrocardiographically gated breath-hold balanced steady-state free precession imaging. Imaging parameters included the following: TE = 1.7 ms, TR = 3.4 ms. The temporal resolution was 25–39 ms depending on the heart rate, flip-angle = 35°, slice thickness = 10 mm, slice gap = 0.00 mm, field of view = 400 × 400 mm, reconstructed matrix size = 256 × 256. All images were analyzed quantitatively using dedicated software (MASS®, Medis, Leiden, the Netherlands). Left ventricular ejection fraction (EF) was assessed as measure for systolic function. Furthermore, in order to obtain aortic stiffness, aortic pulse wave velocity was assessed as described earlier.¹⁹

Moreover, an electrocardiographically-gated gradient-echo sequence with velocity encoding was performed to measure blood flow across the mitral valve for the determination of left ventricular diastolic function. Imaging parameters included the following: TE = 4.83 ms, TR = 14 ms, flip-angle = 20°, slice thickness = 8 mm, field of view = 350 × 350 mm, matrix size = 256 × 256, velocity-encoding gradient = 100 cm/s, scan percentage = 80%. Images were analyzed quantitatively using dedicated software (FLOW®, Medis). The resulting biphasic inflow pattern consists of two peaks, representing the early filling phase (E) and the atrial contraction (A). Analysis of E and A was performed by calculation of the early peak filling rate which is the maximal flow rate of E, the atrial peak filling rate, which is the maximal flow rate of A, and the ratio of E and A peak filling rates (E/A). Furthermore, the peak deceleration gradient ²⁸ of E was calculated automatically. During MRI, blood pressure and heart rate were measured.

Abdominal visceral fat depots were quantified by MRI.²⁹ A turbo spin echo imaging protocol was used and imaging parameters included the following: TE = 11 ms, TR = 168 ms, flip-angle = 90°, slice thickness = 10 mm. Three consecutive transverse images were obtained during one breath-hold with the middle image at a level just above the fifth lumbar vertebra. The volumes of the visceral fat depots of all slices were calculated by converting the number of pixels to square centimeters multiplied by the slice thickness. The total volume of the fat depots was calculated by summing the volumes of all three slices.

Statistical analysis

Statistical analysis was performed with SPSS for windows (version 12.0; SPSS, Chicago, IL). Data are expressed as mean ± standard deviation (SD). Correlations were calculated using Pearson's correlation coefficients. To detect determinants of myocardial triglyceride content univariate and multivariable linear regression analyses were performed. We studied univariate Pearson correlations between myocardial triglyceride content and its possible predictors (age, BMI, plasma glucose, NEFA, and triglycerides, HOMA index, hepatic triglyceride content, and visceral adipose tissue). To identify independent predic-

tors of myocardial triglyceride content in multivariable analysis, myocardial triglyceride content was entered as a dependent variable and age, BMI, visceral adipose tissue, and hepatic triglyceride content (which were all significantly correlated to myocardial triglyceride content in univariate analysis) were subsequently entered as independent variables into the model. Furthermore, possible confounders such as plasma NEFA, triglycerides, and glucose were separately entered into a model with age and BMI as independent variables and myocardial triglyceride content as dependent variable.

Secondly, to study the value of myocardial triglyceride content as predictor of diastolic function, an identical approach was used. First, univariate correlations between E/A and its possible predictors (age, BMI, blood pressure, heart rate, plasma glucose, NEFA, and triglycerides, HOMA index, myocardial and hepatic triglyceride content, pulse wave velocity, and visceral adipose tissue) were assessed. To identify independent predictors of E/A in multivariable analysis, E/A was entered as a dependent variable and myocardial triglyceride content, age, BMI, visceral adipose tissue, and hepatic triglyceride content (which were all negatively correlated to E/A in univariate analysis) were subsequently entered as independent variables into the model. Furthermore, all known possible confounders such as plasma NEFA, triglycerides, glucose, HOMA, diastolic blood pressure, heart rate, and pulse wave velocity were separately entered into a model with age, myocardial triglyceride content, and BMI as independent variables.

To test the satisfaction of the basic model assumptions, scatter plots of unstandardized predictive values and residuals were created and visually inspected for gross departures from model assumptions. $P < 0.05$ was considered statistically significant (two-sided). To correct for multiple testing in multivariable analysis we performed Bonferroni–Holm post hoc testing.

RESULTS

Characteristics of the subjects

Table 7.1 shows characteristics of the subjects. All subjects were Caucasian males with a mean age ($\pm$ SD; range) of 44 ± 15 ; 20–66 years and BMI was 25.9 ± 3.1 ; 19.4 – 34.9 kg/m². All subjects were normotensive (mean $\pm$ SD systolic blood pressure = 118 ± 12 mmHg, mean diastolic blood pressure = 70 ± 8 mmHg) and normoglycaemic (mean plasma glucose levels = 5.1 ± 0.4 mmol/l).

Regression analyses

Myocardial and hepatic ¹H-MRS were performed successfully in all participants. Figure 7.2 shows typical examples of myocardial ¹H-MR spectra of a younger and an older volunteer. Characteristics of the studied myocardial metabolic and functional parameters

Table 7.1 Mean characteristics of the volunteers and their univariate Pearson correlation coefficients to myocardial triglyceride content, E/A, and age

	Mean $\pm$ standard deviation	Myocardial triglyceride content (%)	E/A	Age
Age (years)	44 $\pm$ 15	0.57 (< 0.01)	-0.85 (< 0.01)	NA
Male gender / Caucasian (%)	100/100	NA	NA	NA
Body mass index (kg/m ²)	25.9 $\pm$ 3.1	0.44 (< 0.01)	-0.58 (< 0.01)	0.56 (< 0.01)
Systolic blood pressure (mmHg)	118 $\pm$ 12	-0.06 (0.69)	0.19 (0.23)	-0.25 (0.11)
Diastolic blood pressure (mmHg)	70 $\pm$ 8	0.25 (0.11)	-0.39 (0.01)	0.48 (< 0.01)
Heart rate (bpm)	59 $\pm$ 10	-0.01 (0.97)	-0.08 (0.61)	-0.09 (0.58)
Plasma glucose (mmol/l)	5.1 $\pm$ 0.4	0.14 (0.41)	-0.33 (0.05)	0.37 (0.02)
Plasma non-esterified fatty acids (mmol/l)	0.48 $\pm$ 0.22	-0.11 (0.51)	-0.02 (0.91)	-0.11 (0.52)
Plasma triglycerides (mmol/l)	1.11 $\pm$ 0.43	-0.20 (0.24)	0.25 (0.14)	-0.32 (0.06)
HOMA index	1.4 $\pm$ 0.8	-0.10 (0.58)	-0.19 (0.27)	0.08 (0.64)
Left ventricular ejection fraction (%)	59 $\pm$ 4	-0.14 (0.39)	0.20 (0.20)	-0.12 (0.47)
E/A	1.5 $\pm$ 0.5	-0.68 (< 0.01)	NA	-0.85 (< 0.01)
E deceleration (ml/s ² $\times$ 10 ⁻³)	4.5 $\pm$ 1.2	-0.46 (< 0.01)	0.55 (< 0.01)	-0.34 (0.03)
Pulse wave velocity (m/s)	5.1 $\pm$ 1.0	0.34 (0.03)	-0.70 (< 0.01)	0.77 (< 0.01)
Myocardial triglyceride content (%)	0.56 $\pm$ 0.29	NA	-0.68 (< 0.01)	0.57 (< 0.01)
Hepatic triglyceride content (%)	2.85 $\pm$ 2.81	0.32 (0.04)	-0.28 (0.08)	0.21 (0.19)
Visceral adipose tissue (ml)	229 $\pm$ 141	0.42 (< 0.01)	-0.64 (< 0.01)	0.66 (< 0.01)

Values are Pearson r (P -value).

Myocardial triglyceride content is expressed as a percentage relative to the unsuppressed water signal.

E/A = ratio of maximal left ventricular early peak filling rate and the maximal left ventricular atrial peak filling rate; HOMA = homeostasis model assessment; NA = not applicable.

are represented in Table 7.1. All subjects had a normal EF (mean $\pm$ SD: 59 $\pm$ 4%). Mean E/A and E deceleration peak were 1.5 $\pm$ 0.5 and 4.5 $\pm$ 1.2 ml/s² $\times$ 10⁻³, respectively. Myocardial and hepatic triglyceride content were 0.56 $\pm$ 0.29 and 2.85 $\pm$ 2.81%, respectively.

In addition, Table 7.1 lists the univariate Pearson's correlations between myocardial triglyceride content and age, biochemical parameters, and parameters of left ventricular function. Age was significantly correlated to myocardial triglyceride content ($r = 0.57$, $P < 0.05$; myocardial triglyceride content [%] = 0.088 + 0.011 $\times$ age [years]). Multivariable analysis was performed to study the association between age and myocardial triglyceride content. Therefore, myocardial triglyceride content was entered as a dependent variable and age, BMI, visceral adipose tissue, and hepatic triglyceride content (which were all significantly correlated to myocardial triglyceride content) were subsequently entered as independent variables into the model. Furthermore, possible confounders such as plasma NEFA, triglycerides, and glucose were separately entered into a model with age and BMI as independent variables. In all these models, only age showed a statistically significant association with myocardial triglyceride content also af-

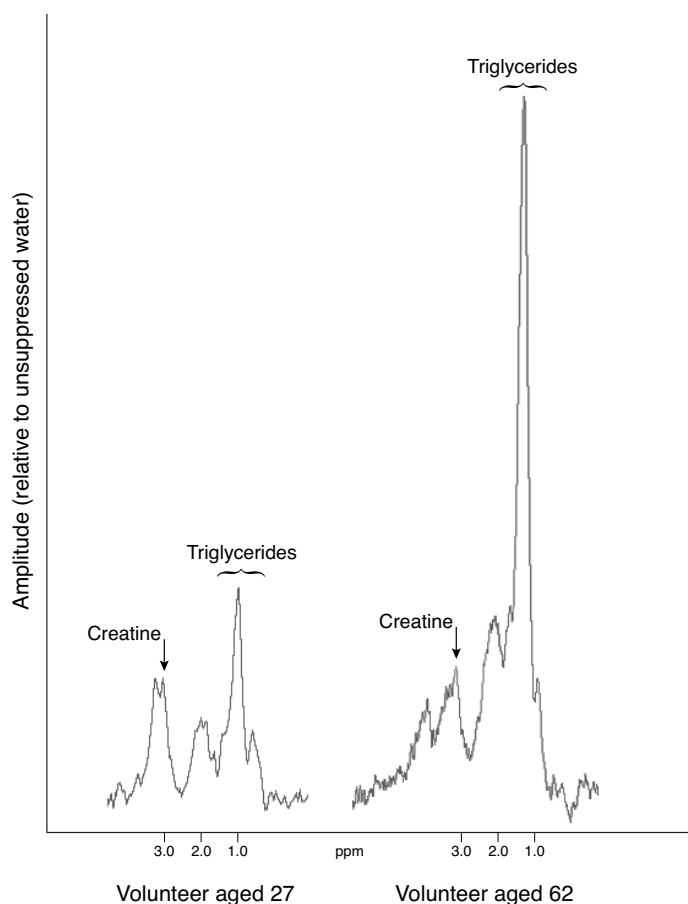


Figure 7.2

Typical examples of ^1H -MR spectra. Water-suppressed spectra are displayed. Myocardial triglyceride content of the younger volunteer was 0.24%, whereas myocardial triglyceride content in the 62-year old volunteer was 0.82%.

ter Bonferroni–Holm correction for multiple testing. Adjustment for BMI, visceral adipose tissue, hepatic triglyceride content, and biochemical parameters had no significant effect on the association between myocardial triglyceride content and age.

Several parameters of left ventricular function correlated significantly with myocardial triglyceride content (Table 7.1). E/A, as parameter of diastolic function, showed a significant inverse correlation with myocardial triglyceride content (Figure 7.3). To study the value of myocardial triglyceride content as predictor of diastolic function, a second multivariable analysis was performed to study the association between myocardial triglyceride content and E/A. Therefore, E/A was entered as a dependent variable and myocardial triglyceride content, age, BMI, visceral adipose tissue, and hepatic triglyceride

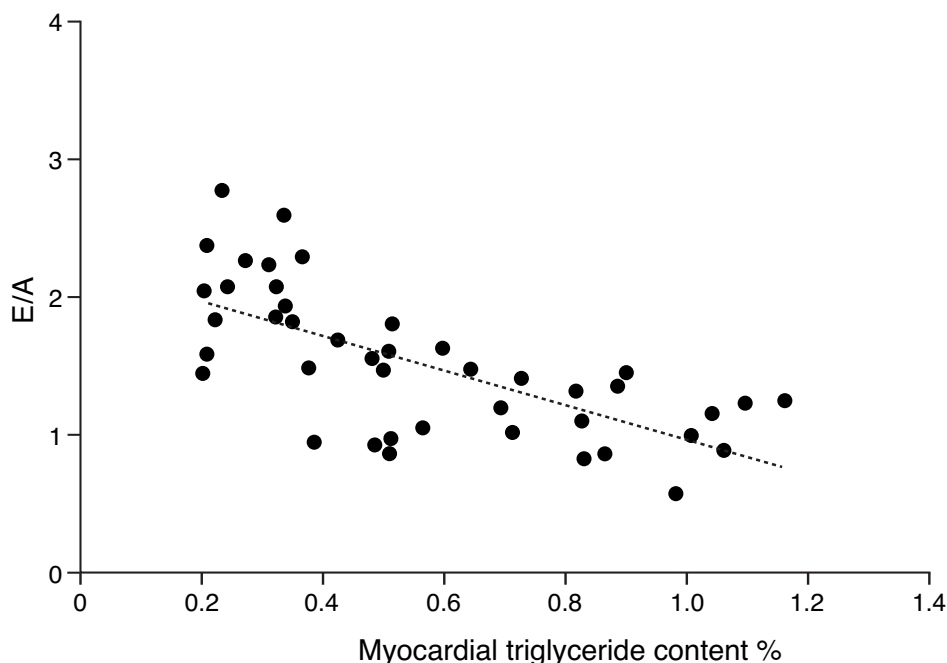


Figure 7.3

Correlation between myocardial triglyceride content and E/A. Myocardial triglyceride content is expressed as a percentage relative to the unsuppressed water signal.

$r = -0.68$, $P < 0.05$.

ide content (which were all negatively correlated to E/A) were subsequently entered as independent variables into the model (Table 7.2).

Furthermore, all known possible confounders such as plasma NEFA, triglyceride, glucose, HOMA, diastolic blood pressure, heart rate, and pulse wave velocity were separately entered into a model with age and BMI as independent variables. In all these models, both myocardial triglyceride content and age showed a statistically significant association with E/A, also after Bonferroni–Holm correction for multiple testing. Adjustment for BMI, visceral adipose tissue, hepatic triglyceride content, and biochemical parameters had no significant effect on the association of myocardial triglyceride content and age with E/A.

Myocardial triglyceride content also correlated inversely to E peak deceleration, and therefore a third multivariable analysis was performed. E peak deceleration was entered as dependent variable (instead of E/A) in the same models as shown in Table 7.2. Age, BMI, and visceral fat content had no influence on the association between e peak deceleration and myocardial triglyceride content. However, when biochemical parameters were entered as independent variables in the model, this significant association disappeared.

Table 7.2 Multivariable associations between E/A and myocardial triglyceride content

	E/A β -coefficient (95% CI)	P^a	r	r^2	P^b
Model 1			0.69	0.47	< 0.001
Myocardial triglyceride content (%)	-1.245 (-1.665; -0.826)	< 0.001			
Model 2 (model 1 + age)			0.88	0.78	< 0.001
Myocardial triglyceride content (%)	-0.541 (-0.872; -0.210)	0.002			
Age (years)	-0.023 (-0.029; -0.017)	< 0.001			
Model 3 (model 2 + body mass index)			0.89	0.79	< 0.001
Myocardial triglyceride content (%)	-0.507 (-0.843; -0.171)	0.004			
Age (years)	-0.022 (-0.028; -0.015)	< 0.001			
Body mass index (kg/m ²)	-0.017 (-0.047; 0.014)	0.275			
Model 4 (model 3 + visceral adipose tissue)			0.89	0.79	< 0.001
Myocardial triglyceride content (%)	-0.513 (-0.852; -0.174)	0.004			
Age (years)	-0.021 (-0.028; -0.013)	< 0.001			
Body mass index (kg/m ²)	-0.009 (-0.048; 0.029)	0.633			
Visceral adipose tissue (ml)	0.000 (-0.001; 0.001)	0.510			
Model 5 (model 4 + hepatic triglyceride content)			0.89	0.80	< 0.001
Myocardial triglyceride content (%)	-0.574 (-0.940; -0.208)	0.003			
Age (years)	-0.020 (-0.028; -0.012)	< 0.001			
Body mass index (kg/m ²)	-0.011 (-0.056; 0.034)	0.460			
Visceral adipose tissue (ml)	0.000 (-0.001; 0.001)	0.450			
Hepatic triglyceride content (%)	0.005 (-0.033; 0.044)	0.788			
Model 6 (model 3): Association between E/A and myocardial triglyceride content after adjusting for age, body mass index and each of the following variables					
a) Plasma non-esterified fatty acids (mmol/l)	-0.477 (-0.830; -0.124)	0.010	0.92	0.84	< 0.001
b) Plasma triglycerides (mmol/l)	-0.461 (-0.832; -0.089)	0.017	0.91	0.82	< 0.001
c) Plasma glucose (mmol/l)	-0.455 (-0.832; -0.078)	0.020	0.90	0.82	< 0.001
d) HOMA index	-0.492 (-0.869; -0.116)	0.012	0.92	0.84	< 0.001
e) Diastolic blood pressure (mmHg)	-0.487 (-0.811; -0.164)	0.004	0.89	0.79	< 0.001
f) Heart rate (bpm)	-0.490 (-0.814; -0.166)	0.004	0.90	0.81	< 0.001
g) Pulse wave velocity (m/s)	-0.574 (-0.915; -0.232)	0.002	0.90	0.80	< 0.001

CI = Confidence interval; HOMA = homeostasis model assessment; r and r^2 for the respective models, i.e. in model 1, with E/A as dependent and myocardial triglyceride content as independent variable; in model 2, myocardial triglyceride content and age are independent variables; in model 3, myocardial triglyceride content, age, and BMI are independent variables; in model 4, myocardial triglyceride content, age, BMI, and visceral adipose tissue are independent variables; in model 5, myocardial triglyceride content, age, BMI, visceral adipose tissue and hepatic triglyceride content are independent variables; in models 6 a-g the possible confounders are separately entered for adjustment and β coefficients and P -values for the association between myocardial triglyceride content and E/A are displayed.

^a Level of significance of the association between the separate components of the model and E/A. After Bonferroni-Holm correction for multiple testing, associations of myocardial triglyceride content and age with E/A remained statistically significant.

^b Level of significance of the model.

Finally, there was no significant correlation between myocardial triglyceride content and left ventricular EF ($r = -0.14$, $P > 0.05$).

DISCUSSION

In this study, we demonstrated that myocardial triglyceride content increases in the ageing male heart and is significantly correlated to the age-related decline in diastolic left ventricular function independently of BMI, blood pressure, and biochemical blood markers.

An age-associated accumulation of triglycerides in the myocardium of rodents has been shown earlier.^{30,31} Furthermore, it has been demonstrated in humans that pathological processes such as obesity and diabetes mellitus are associated with myocardial triglyceride accumulation.^{3,16}

In our study, multivariable analysis showed that myocardial triglyceride accumulation is physiological in the ageing human heart, independently of BMI. In addition, age was indicated as a better predictor of myocardial triglyceride accumulation than BMI, at least in healthy subjects.

A possible explanation for age-associated myocardial triglyceride accumulation is a discrepancy between myocardial uptake and myocardial oxidation of fatty acids. Impaired fatty acid oxidation has been shown in the ageing human heart.⁹ Although there is no evidence so far for a direct link between impaired myocardial fatty acid oxidation and myocardial triglyceride accumulation in humans, Cree et al.¹² demonstrated in skeletal muscle that an imbalance in the normal relationship between tissue uptake and disposal of fatty acids leads to an increase of triglyceride and intramyocellular fatty acyl intermediates in skeletal muscle of the elderly. The same mechanism may underlie myocardial triglyceride accumulation in the ageing male heart.

Myocardial triglyceride accumulation can be harmful to the heart. In a transgenic mouse model with increased myocardial fatty acid uptake, myocardial triglyceride content and fatty acid intermediates were increased resulting in lipotoxicity-induced heart failure and premature death.³² In the present study, we showed for the first time in healthy human subjects, that myocardial triglyceride accumulation is inversely correlated to E/A independently of age, BMI, blood pressure, aortic stiffness, or biochemical blood markers. In addition, E peak deceleration correlated negatively with myocardial triglyceride content, however, myocardial triglyceride content could not be indicated as an independent predictor of E peak deceleration when adjusted for biochemical markers. Finally, there was no significant correlation between myocardial triglyceride content and left ventricular EF. These findings indicate that myocardial triglyceride content is more closely correlated to diastolic function than to left ventricular systolic function.

Most likely, myocardial triglyceride stores themselves are inert, but rather are a reflection of increased intracellular concentrations of fatty acid intermediates that alter myocellular structure and function by complex molecular mechanisms.^{10,11} Increased fatty acid intermediates, reflected clinically by increased myocardial triglyceride content lead to myocardial lipotoxicity due to a continuous cycle of hydrolysis of the surplus triglyceride and re-esterification of fatty acids.^{10,11} During this process toxic intermediates (FA-acyl-CoA esters, ceramides) are formed which accumulate within the cardiomyocyte. Fatty acid intermediates are associated with increased apoptosis and can induce myocardial dysfunction.² Furthermore, altered membrane structure and increased expression of uncoupling proteins in a fatty acid-based metabolic system may lead to declined mitochondrial function³³ and consequently to lower levels of ATP at the sarcomeres. Lower ATP levels lead to impaired calcium (Ca^{2+}) sequestration by the sarcoplasmic reticulum.³⁴ Within ageing cardiomyocytes, an age-associated prolonged Ca^{2+} cytosolic calcium transience and contraction may impair myocardial relaxation during early diastole and, in part, may underlie the reduction in early diastolic filling rate that accompanies advancing age.⁷ Our findings support the evidence that the processes underlying myocardial triglyceride accumulation might be harmful to the human heart. Therefore, physiological triglyceride accumulation may become important in the ageing heart by increasing susceptibility to lipotoxicity in otherwise healthy individuals. However, excluding women from the study limits the generalizability of the present study. Besides effects of estrogens on lipid metabolism and gender differences in expression of certain cell surface receptors/ transporters of fatty acids,^{21,22} there is evidence of gender and estrogen levels-related effects on myocardial function.^{35,36} Further studies need to be initiated to extend the present findings to subjects from both genders and to clarify the possible confounding influences of fluctuation in lipid metabolism in women on hepatic and myocardial triglyceride accumulation and on gender-dependent myocardial functional changes.

In our study, we found an association between myocardial triglyceride content and myocardial function. However, using MRI and MRS, we cannot discriminate whether there is a causal relationship between increased myocardial triglyceride content and reduced left ventricular function. Neither can we discriminate if myocardial triglyceride accumulation per se hampers myocardial function due to a mechanical effect, the formation of cardiotoxic intermediates, or by intervening in other mechanisms such as calcium handling. Further studies need to be initiated to make these distinctions since, in animal models, therapeutic interventions aiming at reduction of myocardial triglyceride accumulation due to disturbed fatty acid metabolism have been shown to have beneficial effects on myocardial function.²

In conclusion, myocardial triglyceride content increases in the physiologically ageing male heart and is associated with the age-related decline in diastolic function, independent of BMI, blood pressure, and biochemical blood markers.

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Magnetic resonance of the diabetic heart

PART III

Short-term flexibility of myocardial triglycerides and diastolic function in patients with type 2 diabetes mellitus

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ABSTRACT

Background

Short-term caloric restriction increases plasma levels of non-esterified fatty acids (NEFA) and is associated with increased myocardial triglyceride content and decreased myocardial function in healthy subjects. Whether this flexibility of myocardial triglyceride stores and myocardial function is also present in patients with type 2 diabetes mellitus (T2DM) is yet unknown.

Methods

Myocardial triglyceride content and left ventricular (LV) ratio between the early (E) and atrial (A) diastolic filling phase (E/A) were determined using magnetic resonance (MR) spectroscopy and MR imaging respectively, before and after a 3-day very low calorie diet (VLCD) in 11 patients with T2DM. In addition, we studied these patients after a 3-day VLCD combined with the anti-lipolytic drug acipimox.

Results

The VLCD induced myocardial triglyceride accumulation (from 0.66 ± 0.09 (mean $\pm$ SE, baseline) to $0.98 \pm 0.16\%$ ($P = 0.028$), and a decrease in E/A ratio (from 1.00 ± 0.05 (baseline) to 0.90 ± 0.06 ($P = 0.002$). This was associated with increased plasma NEFA levels (from 0.57 ± 0.08 mmol/l at baseline to 0.92 ± 0.12 [$P = 0.019$]). After the VLCD with acipimox, myocardial triglyceride content, diastolic function and plasma NEFA levels were similar to baseline values.

Conclusion

In patients with T2DM a VLCD increases myocardial triglyceride content and is associated with a decrease in LV diastolic function. These effects were not observed when a VLCD was combined with acipimox, illustrating the physiologic flexibility of myocardial triglyceride stores and myocardial function in patients with T2DM.

Type 2 diabetes mellitus (T2DM) and obesity are associated with elevated plasma levels of non-esterified fatty acids (NEFA) ¹⁻³ and ectopic accumulation of triglycerides, reflected in hepatic ^{4,5} and cardiac steatosis.^{6,7} This accumulation of triglycerides in the heart is not merely an epiphenomenon, as it is associated with altered structure and function of the heart. For instance, increased plasma levels of NEFA are associated with increased myocardial triglyceride content and left ventricular (LV) mass.⁸ In rodents, cardiac triglyceride accumulation induces lipopoptosis and is associated with cardiac dysfunction.^{9,10} In humans parameters of myocardial fatty acid metabolism are predictors of LV mass in hypertension and diastolic dysfunction,² and increased myocardial triglyceride content may precede the onset of profound systolic dysfunction in patients with obesity and/or T2DM.⁶

Myocardial triglyceride content is not fixed as it is modulated by dietary interventions, at least in healthy subjects. We and others have previously shown that short-term caloric restriction is associated with myocardial triglyceride accumulation ¹²⁻¹⁴ and a decrease in LV diastolic function in healthy volunteers.^{13,14} As patients with uncomplicated T2DM show alterations in myocardial high-energy phosphate metabolism, illustrating the changes in normal myocardial substrate handling,¹⁵ we hypothesize this flexibility is diminished in patients with respect to myocardial triglyceride content and LV diastolic function. As short-term caloric restriction increases adipose tissue lipolysis, it is a suitable research tool to influence myocardial substrate selection and study the effects on myocardial triglyceride stores in relation to myocardial function.

The objective of the present study was therefore to assess the effects of short-term caloric restriction (3 days of a very low calorie diet, VLCD) on myocardial triglyceride content and function in patients with T2DM compared with control observations with no dietary restriction. Furthermore we assessed whether the effects of a VLCD could be prevented by co-administration of the anti-lipolytic drug acipimox.^{16,17} Acipimox has been extensively used to decrease plasma NEFA levels, and it therefore can be used to study the effects of changes in plasma NEFA levels during the interventions. To study the effects on tissue-specific distribution of ectopic triglyceride pools in patients with T2DM, hepatic triglyceride content was also measured.

METHODS

Patients

We included 11 well-controlled male patients with T2DM (mean $\pm$ SD age: 57.6 $\pm$ 4.7 years) in this prospective, cross-over intervention study. The sample size was based on our previous experiments in healthy subjects, in which we observed a statistical power

of 0.89 for detecting a mean increase in myocardial triglyceride content of 0.23% in 10 subjects.¹³

All patients used stable doses of metformin and glimepiride for at least 3 months. The use of other antidiabetic drugs was prohibited. In each patient a medical history was obtained and a physical examination was performed. In each patient an electrocardiogram was made and dobutamine-stress echocardiography was performed. Exclusion criteria were a history of or present cardiac disease (any abnormality on the electrocardiogram and/ or wall motion abnormalities at rest or during dobutamine-stress echocardiography) and any endocrine, hepatic or renal disease (standard laboratory and urinary tests). All patients signed informed consent prior to participation. The local ethics committee approved the study.

Study design

To obtain baseline measurements subjects followed their normal diet, only alcohol was restricted for a 3-day period. Four days prior to baseline measurements glimepiride was discontinued to avoid episodes of hypoglycemia during the second and third intervention period.

In addition, the subjects were studied after a 3-day VLCD alone (471 kcal/day, 50.2 g carbohydrates, 6.9 g fat of which 0.94 was saturated, Modifast Intensive, Nutrition & Santé Benelux, Breda, the Netherlands) and after a VLCD for 3 days plus acipimox (VLCD+acipimox). Acipimox (Nedios, ALTANA Pharma BV, Hoofddorp, the Netherlands) 250 mg was taken orally at 6-8 hour intervals during the last 24 hours of the 3-day period of VLCD (i.e. 24, 16, 10, and 4 hours prior to blood sampling). The sequence of the interventions was determined by balanced assignment. Both VLCD studies were separated by a wash-out phase of at least 14 days. For all study occasions patients used their last meal or last sachet of Modifast 4-hours prior to blood sampling. Blood samples were taken just before MR evaluation. The duration of the VLCD was chosen based on our previous experiments in healthy subjects.¹⁴

Determination of myocardial and hepatic triglyceride content

All magnetic resonance imaging (MRI) and MR spectroscopy (MRS) measurements were performed with the use of a 1.5T whole-body MR scanner (Gyrosan ACS/ NT15; Philips, Best, Netherlands) with subjects in supine position at rest in the afternoon. Single voxel (8 ml) spectra were obtained using a body coil for radiofrequency transmission and a circular surface coil (17 cm diameter) for signal receiving. The myocardial voxel was placed in the interventricular septum on four-chamber and short-axis images at end-systole, carefully avoiding contamination from epicardial fat. Data collection was double-triggered by using ECG-triggering and navigator echoes for compensation of respiratory motion.¹⁸ In short, an echo time (TE) of 26 ms and a repetition time (TR) of

at least 3000 ms were used. 1024 data points were collected using a spectral width of 1000 Hz, averaged over 128 acquisitions. To detect the resonances of the lipids, the water signal was suppressed. Furthermore, in the same voxel, the water signal (TE = 10 seconds) was measured to be used as an internal standard. For the liver we used the same parameters, except for 64 averaged acquisitions for the suppressed spectrum. Spectra were analyzed in the time-domain on the free-induction decays with Java-based MR user interface software and incorporated prior knowledge files (jMRUI version 2.2), as described earlier.^{18,19} Peak estimates of lipid resonances of myocardial and hepatic triglyceride at 0.9 parts per million (ppm) and 1.3 ppm were summed and calculated as a percentage of the unsuppressed water signal (triglyceride/water $\times$ 100).

Evaluation of myocardial systolic and diastolic function

During MR imaging, systolic and diastolic blood pressure and heart rate were measured with an automatic device (Dinamap DPC100X, Freiburg, Germany). To assess systolic function, the heart was imaged from apex to base with 12 to 14 imaging levels in short-axis view using an ECG-triggered sensitivity encoding balanced steady-state free precession sequence with breath-holds (1 for each slice). Imaging parameters included a field-of-view (FOV) of 400 $\times$ 320 mm, matrix size = 256 $\times$ 256, slice thickness = 10 mm, slice gap = 0 mm, flip angle = 35°, TE = 1.7 ms and TR = 3.4 ms. The temporal resolution was 25 to 39 ms depending on the heart rate. Left ventricular (LV) end-diastolic and end-systolic contours were drawn using dedicated software (MASS® post-processing software, Medis, Leiden, the Netherlands) as described earlier.¹⁷ LV ejection fraction (LVEF) and cardiac index (defined as cardiac output divided by body surface area) were calculated for assessment of systolic function. Furthermore, MRI is as accurate to assess diastolic function as compared to Doppler-derived results.²¹ Therefore, we measured blood flow across the mitral valve with an ECG-gated gradient-echo sequence with velocity encoding (Venc).^{21,22} Imaging parameters were: TE = 5 ms, TR = 14 ms, flip-angle = 20°, slice thickness = 8 mm, FOV = 350 $\times$ 350 mm, matrix size = 256 $\times$ 256, and Venc = 100 cm/s. Flow velocities in early diastole (E) and during the atrial contraction (A) were measured. Analyses were performed using dedicated analysis software (FLOW® analytical software package, Medis, Leiden, the Netherlands). The peak slope of the deceleration of the E (E deceleration) and the ratio between the peak filling rate of the E (EPFR) and A (APFR) were calculated (E/A ratio) as measures for diastolic function. The E/A ratio is load dependent and therefore an estimation of LV filling pressure was calculated (E/Ea).²³

Visceral fat quantification

Abdominal visceral fat depots were quantified by a turbo spin echo imaging protocol. Imaging parameters were: TE = 11 ms, TR = 168 ms, flip-angle = 90°, slice thickness =

10 mm. At the level of the fifth lumbar vertebra, three transverse images were acquired during a breath-hold. The volumes of the visceral fat depots of all slices were calculated by converting the number of pixels to square centimeters multiplied by the slice thickness. The total volume of the fat depots was calculated by summing the volumes of all three slices (using MASS® analytical software, Medis, Leiden, the Netherlands).

Assays

Plasma concentrations of glucose, total cholesterol (TC) and triglycerides were measured on a fully automated P800 analyzer (Roche, Almere, the Netherlands). Insulin concentrations were measured on an Immulite 2500 random access analyzer with a chemoluminescence immunoassay (DPC, Los Angeles, CA, USA). Coefficients of variation for glucose, TC and triglycerides were < 2%, and for insulin < 5 %. Plasma NEFA concentrations were measured by a commercial kit (NEFA-C; Wako Chemicals, Neuss, Germany).

Statistical analysis

Statistical analyses were performed using SPSS for windows (version 14; SPSS, Chicago, IL). Statistical comparisons between the conditions were made by paired T-tests. *P*-values reflect data compared to baseline unless indicated otherwise. Data are shown as mean ± standard error (SE). *P* < 0.05 (two-tailed) was considered significant.

RESULTS

Metabolic changes

Patient characteristics and metabolic changes are listed in Table 8.1. Plasma NEFA levels increased after the VLCD compared to baseline (from 0.57 ± 0.08 to 0.92 ± 0.12 mmol/l, *P* = 0.019). In contrast, plasma NEFA levels after the VLCD+acipimox were not significantly different compared to baseline (*P* = 0.142), but decreased significantly compared to VLCD alone (0.35 ± 0.12 mmol/l, *P* = 0.006).

Myocardial and hepatic triglyceride content

Myocardial triglyceride content at baseline was $0.66 \pm 0.09\%$. After the VLCD myocardial triglyceride content increased to $0.98 \pm 0.16\%$ (*P* = 0.028), whereas it returned to baseline values after the VLCD+acipimox ($0.73 \pm 0.15\%$, *P* = 0.485 [Figures 8.1 and 8.2A]). Moreover, myocardial triglyceride content was decreased after the VLCD+acipimox compared to the VLCD alone (*P* = 0.044). Myocardial ¹H-MR spectra could not be obtained in 1 patient due to technical problems. Hepatic triglyceride content did not change significantly with either intervention (Table 8.1).

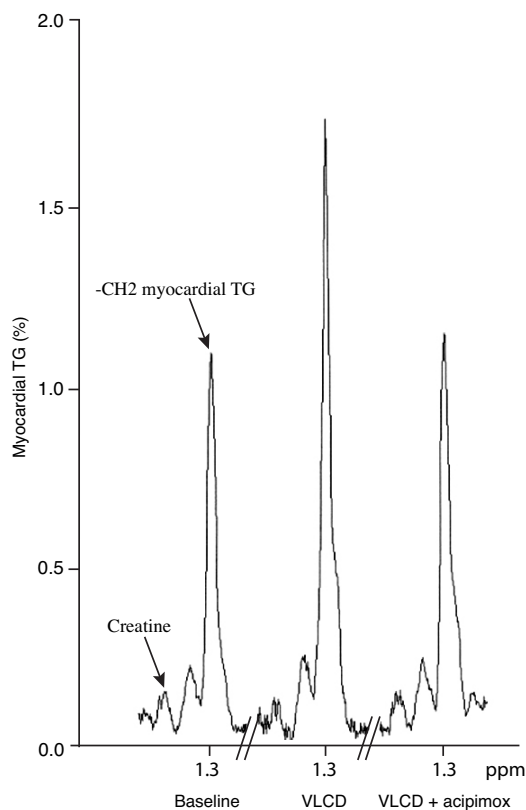
Table 8.1 Metabolic parameters at baseline, after the very low calorie diet and after the very low calorie diet plus acipimox

	Baseline	VLCD	VLCD+ acipimox
Glycated hemoglobin (%)	6.0 ± 0.2		
Body mass index (kg/m ²)	26.6 ± 0.9	25.8 ± 0.8*	25.9 ± 0.9*
Glucose (mmol/l)	6.0 ± 0.4	5.2 ± 0.3 [‡]	4.9 ± 0.2 [†]
Insulin (mU/l)	6.6 ± 1.3	3.3 ± 0.6 [‡]	2.3 ± 0.2 [†]
Triglycerides (mmol/l)	2.2 ± 0.4	1.3 ± 0.2 [†]	1.0 ± 0.1 [†]
Non-esterified fatty acids (mmol/l)	0.57 ± 0.08	0.92 ± 0.12 [‡]	0.35 ± 0.12
Total cholesterol (mmol/l)	4.5 ± 0.4	4.7 ± 0.2	4.5 ± 0.3
Visceral adipose tissue (ml)	375 ± 55	295 ± 35 [†]	303 ± 39
Hepatic triglyceride content (%)	16.4 ± 1.4	14.2 ± 1.0	14.2 ± 1.2

Values are mean ± standard error.

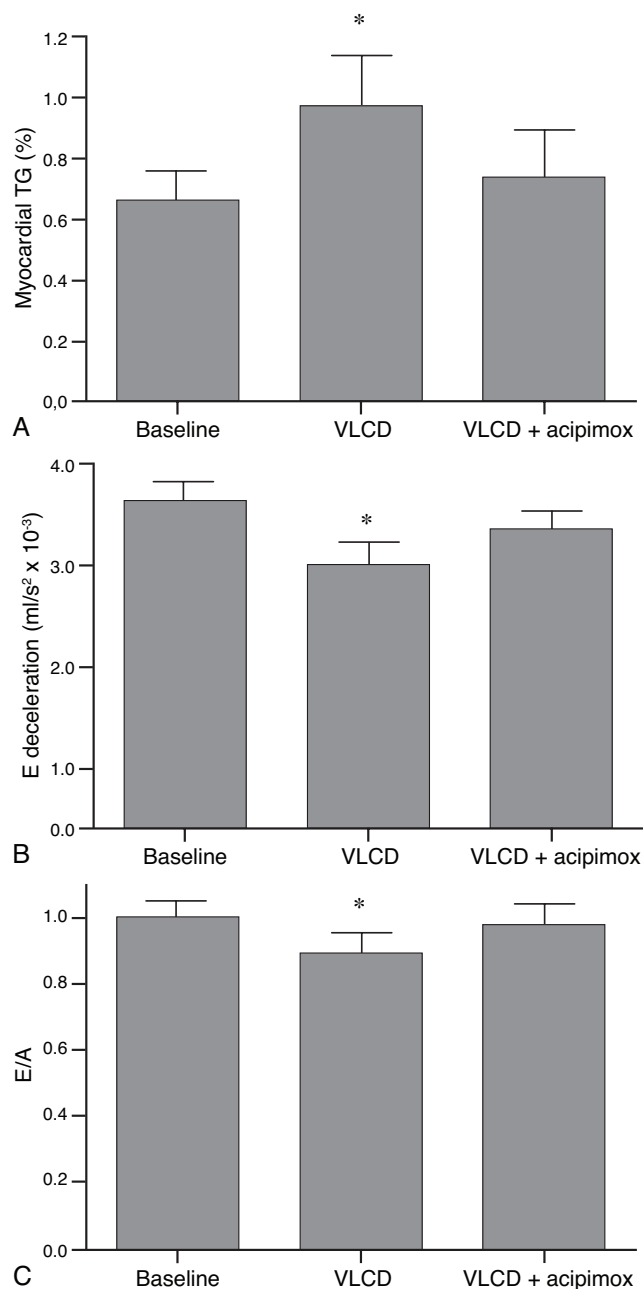
* $P < 0.001$, [†] $P < 0.01$, [‡] $P < 0.05$ vs. baseline.

VLCD = very low calorie diet.

**Figure 8.1**

Proton spectra of 1 patient at baseline, after the VLCD and after the VLCD plus acipimox (relative to the unsuppressed water).

VLCD = very low calorie diet; TG = triglyceride; CH2 = methyl groups of myocardial lipid content.

**Figure 8.2**

Myocardial triglyceride content is significantly increased after the VLCD and unchanged after administration of acipimox during the VLCD (A) and associated with changes in diastolic E deceleration (B) and E/A ratio (C).

VLCD = very low calorie diet; TG = triglyceride.

Values are mean $\pm$ standard error. * $P < 0.05$.

Myocardial function

Systolic function was unaffected by the dietary interventions (Table 8.2). Diastolic blood pressure was equally decreased after the VLCD and after the VLCD+acipimox. E deceleration decreased significantly from 3.6 ± 0.2 to 2.9 ± 0.2 $\text{ml/s}^2 \times 10^{-3}$ after the VLCD compared to baseline ($P = 0.004$, Figure 8.2B). E/A peak ratio decreased from 1.00 ± 0.05 to 0.90 ± 0.06 after the VLCD compared to baseline ($P = 0.002$, Figure 8.2C). In contrast, after the VLCD+acipimox the E deceleration (3.3 ± 0.2 $\text{ml/s}^2 \times 10^{-3}$) and the E/A peak ratio (0.98 ± 0.06) were unchanged compared to baseline ($P = 0.270$ and $P = 0.590$ respectively, Figures 8.2B and 8.2C).

Table 8.2 Magnetic resonance parameters at baseline, after the very low calorie diet and after the very low calorie diet plus acipimox

	Baseline	VLCD	VLCD+ acipimox
Systolic blood pressure (mmHg)	115 ± 5	114 ± 6	110 ± 5
Diastolic blood pressure (mmHg)	73 ± 2	$69 \pm 3^\dagger$	$68 \pm 2^\dagger$
Heart rate (bpm)	64 ± 3	63 ± 2	64 ± 3
LVEF (%)	55 ± 1	58 ± 2	55 ± 2
Cardiac index (l/min/m^2)	2.8 ± 0.1	2.7 ± 0.1	2.8 ± 0.2
E peak filling rate (ml/s)	415 ± 27	$342 \pm 30^\dagger$	380 ± 19
A peak filling rate (ml/s)	415 ± 16	394 ± 33	395 ± 17
E/Ea	8.5 ± 0.8	9.1 ± 1.0	9.9 ± 0.9

Values are mean $\pm$ standard error.

$^\dagger P < 0.01$, $^\ddagger P < 0.05$ vs. baseline.

VLCD = very low calorie diet; E = early diastolic wave; A = atrial diastolic wave; LVEF = left ventricular ejection fraction; E/Ea = estimated left ventricular filling pressure.

DISCUSSION

This study shows that in well-controlled patients with T2DM short-term caloric restriction increases myocardial triglyceride content by approximately 50%. This increase in myocardial triglyceride content is accompanied by a decrease in myocardial diastolic function. A VLCD combined with acipimox has no effects on myocardial triglyceride content and myocardial function. These data demonstrate the flexibility of the diabetic myocardium during short-term caloric restriction.

In the present study we show that a physiological increase in circulating NEFA levels is accompanied by increased myocardial uptake and re-esterification of fatty acids in T2DM patients. As patients with T2DM have altered myocardial metabolism,¹⁵ the short-term flexibility of myocardial triglyceride stores is remarkable during caloric restriction. The T2DM patients in our cohort were under good glycemic control and only moderately obese. Therefore, in more severe obesity and/or poor glycemic control the effects of a

short-term VLCD may be different. Moreover, future studies should address the differences in the response to a VLCD between patients with T2DM and healthy subjects matched for BMI and age, as these parameters influence myocardial triglyceride content and diastolic function.²⁴

During caloric restriction, elevated plasma levels of NEFA increase hepatic VLDL- triglyceride production,²⁵ which is an important supplier of fatty acids to the myocardium.^{26,27} During the VLCD with acipimox, no changes were observed in myocardial triglyceride content. This supports the notion that there is a relationship between increased fatty acid fluxes from the adipose tissue and myocardial triglyceride stores, although we can not exclude the possibility of a direct effect of acipimox. This appears however unlikely, as the anti-lipolytic effects would lead to an increase, rather than a decrease in myocardial triglyceride content. Furthermore, as acipimox was added in a hypocaloric situation, its effects underline the potential of the heart to switch substrate metabolism, even in a situation of increased fatty acid dependence.

We hypothesize that the decrease in visceral adipose tissue contributes to the increased levels of circulating fatty acids and possibly to the myocardial triglyceride accumulation after the VLCD.

Although our results can not be extrapolated to the long-term implications of chronic (hyper- or eucaloric) exposure to elevated NEFA levels in obesity and T2DM, our data suggest that in general, interventions aiming to decrease plasma lipids or pathological elevated myocardial triglyceride content seem promising. Accordingly, it was recently shown in insulin treated T2DM patients that adding pioglitazone to insulin therapy decreased myocardial triglyceride stores.²⁸

We used MR velocity mapping to assess blood flow across the mitral valve. Values of EPFR, APFR and their ratio (E/A) obtained with MR velocity mapping are highly correlated to the values of the same parameters when obtained with echocardiography.⁸ Early deceleration is an MR reflection of the early deceleration time which is used in echocardiography. Therefore, the observed changes in parameters of diastolic function as observed in the present study would be observed likewise when the study was performed with ultrasound. The flow measurements can be affected by changes in preload. Furthermore, systemic effects of acipimox include vasodilatation.²⁹ However, MR estimated LV filling pressures were unaffected after the interventions and therefore, the preload was unchanged. Accordingly, the observed changes in diastolic function are likely to be caused by changes in elastic recoil of the LV. This extends the previously documented relation between plasma NEFA levels and diastolic function in obesity.³⁰ Furthermore, the results are in accordance with results obtained in animal models of obesity, documenting the association between myocardial triglyceride accumulation and myocardial function.^{9,10,31} Alternatively, caloric restriction and increased plasma NEFA levels may change myocardial calcium handling and thereby influence diastolic

function.³²⁻³⁴ A causal relationship between myocardial triglyceride stores and diastolic function can therefore not be derived from the present data. Acipimox is not suitable for long-term administration because of the rebound effects on plasma levels of NEFA,³⁵ but the present data, however, warrant future studies in a clinical setting to study the effects of therapeutic interventions on myocardial triglyceride content and myocardial function. We believe that the differences observed in diastolic function are too small to reflect clinically relevant diastolic dysfunction but merely reflect the interaction between short-term metabolic fluctuations and diastolic function. These mechanisms may be relevant for the pathogenesis of cardiac dysfunction in patients with T2DM,⁶ although this can not be concluded from the present data.

We also studied the effects of a VLCD on hepatic triglyceride content in patients with T2DM. Hepatic triglyceride content was almost 7-fold increased at baseline compared to our previous observations in healthy subjects.^{13,14} In contrast to myocardial triglyceride content, hepatic triglyceride content was not significantly affected by the short-term VLCD, either with or without acipimox. We postulate that the duration of the VLCD is too short to induce reductions in hepatic triglyceride content in T2DM subjects with hepatic steatosis, since a prolonged VLCD in obese T2DM subjects induces major reductions in hepatic triglyceride content.³⁶ Nonetheless, the present study documents that the heart and the liver have a differential response to short-term caloric restriction in patients with T2DM. Our study has some limitations. Although the study was powered to detect relevant differences in the patient and patients are their own controls, the number of patients in the study is still limited. Second, we evaluated the effects of a VLCD only with MRI and MRS. It would however be interesting to combine data on myocardial triglyceride content with data on fatty acid and glucose uptake obtained using positron emission tomography (PET) because the balance between the use of glucose and plasma fatty acids determines myocardial energy supply and cardiac function. Unfortunately, these data could not be obtained in the present study, as a PET scanner is unavailable at our institution.

In conclusion, in patients with well-controlled T2DM a VLCD increases myocardial triglyceride content and is associated with a decrease in LV diastolic function. These effects were not observed when a VLCD was combined with acipimox. These data illustrate physiologic flexibility of myocardial triglyceride stores and myocardial function in patients with T2DM.

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Magnetic resonance assessment of aortic pulse wave velocity, aortic distensibility, and cardiac function in uncomplicated type 2 diabetes mellitus

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ABSTRACT

Background

Type 2 diabetes mellitus (T2DM) may augment arterial stiffening and thereby modulates left ventricular (LV) function. Cardiovascular magnetic resonance (CMR) is well suited to assess aortic pulse wave velocity (PWV) and aortic distensibility, both markers of arterial stiffness, without the use of geometric assumptions. Furthermore, CMR is a reliable method for assessing left ventricular (LV) function. The purpose of this study was to assess LV function, PWV, and aortic distensibility in patients with T2DM using CMR.

Methods

Fourteen patients with well-controlled, uncomplicated T2DM, and 16 age and gender matched healthy subjects were included. PWV was calculated based on MR velocity mapping at two predefined aortic locations. Aortic distensibility was measured in the mid ascending aorta. LV volumes were measured by fast gradient-echo imaging to assess systolic function. Furthermore, mitral inflow was measured by MR velocity mapping to assess diastolic LV function.

Results

Mean PWV was higher in patients as compared to healthy subjects (6.83 ± 1.60 vs. 5.65 ± 0.75 m/s, $P < 0.05$). This difference was independent of blood pressure. PWV correlated significantly ($P < 0.05$) with fasting plasma glucose and insulin levels. Aortic distensibility was lower in patients as compared to healthy subjects ($4.50 \times 10^{-3} \pm 2.24 \times 10^{-3}$ vs. $7.42 \times 10^{-3} \pm 3.34 \times 10^{-3}$ mmHg⁻¹, $P < 0.05$). Distensibility correlated negatively with PWV and positively with LV diastolic function ($P < 0.05$).

Conclusion

A combined CMR assessment of aortic PWV, aortic distensibility, and heart function reveals abnormal PWV and distensibility in patients with T2DM, independent of blood pressure. Furthermore, aortic distensibility correlates with diastolic left ventricular function.

Type 2 diabetes mellitus (T2DM) is rapidly becoming a worldwide epidemic¹ and is associated with high morbidity and mortality due to cardiovascular complications.² Many mechanisms have been proposed to be the underlying cause of the deleterious effects of diabetes on the cardiovascular system. It has been shown that T2DM may augment arterial stiffening and thereby modulates left ventricular (LV) function, coronary blood flow and arterial function throughout the cardiovascular system.³⁻⁵ A strong and independent association between increased arterial stiffness and the presence of coronary artery disease has been demonstrated.⁶

As arterial stiffness has become established as a cardiovascular risk factor in its own right,^{3,7-9} there is a need for a simple, reliable, non-invasive method of detecting early disturbances in arterial stiffness at a time when therapeutic intervention can be most beneficial.¹⁰ Arterial stiffness can be estimated from area or diameter changes of the artery related to the distending pressure (distensibility) or by measuring the pulse wave velocity (PWV).

Currently, ultrasound and tonometry are the most often used methods to assess pulse wave velocity by measuring the pulse wave at 2 points in the vasculature and by estimating the path length of the pulse wave ($PWV = \text{distance}/\text{time}$ in m/s). However, body habitus and age-related vessel tortuosity may affect estimation of the path length when the distance of the pulse wave is estimated over the body surface.¹¹

Cardiovascular magnetic resonance (CMR) is well suited to assess aortic PWV.^{12,13} CMR is a non-invasive technique that allows direct imaging of the thoracic and abdominal aorta without the use of geometric assumptions. In contrast to ultrasound and tonometry, CMR allows the accurate and direct measurement of the path length of pulse waves in the proximal and distal aorta, even in the presence of a tortuous vessel, which is a major advantage over other techniques.¹⁴ Furthermore, CMR has proven to be a reliable and accurate method for assessing LV function.^{15,16} Although previous studies with non-CMR techniques have demonstrated the pathophysiological changes in aortic distensibility, PWV, and cardiac function in patients with T2DM, there are no previous reports on the combined evaluation of aortic and cardiac function using a comprehensive CMR protocol.

Accordingly, the purpose of the present study was to combine the assessment of aortic PWV, aortic distensibility, and LV function using CMR in patients with T2DM in one comprehensive evaluation.

METHODS

Study subjects

Fourteen patients (11 male and 3 female) with well-controlled, uncomplicated T2DM and 16 age and gender matched healthy subjects were studied (Table 9.1). Consecutive patients were selected from general practices after approval of their physicians. Selection criteria were T2DM of short duration (< 5 years, diagnosed according to WHO criteria),¹⁷ no signs or symptoms or history of cardiovascular disease, a normal electrocardiogram (ECG), blood pressure (BP) < 150/90 mmHg, good metabolic control (glycated hemoglobin [HbA1c] < 7.0%), no use of drugs other than sulfonylureas and/ or metformin and no diabetic complications including albuminuria, retinopathy, and neuropathy. Healthy subjects had no history or clinical evidence of cardiovascular disease and diabetes (screening visits consisted of a medical history, physical examination, an ECG and screening laboratory tests such as fasting plasma glucose, lipids and HbA1c). HbA1c was determined by HPLC after hemolysis (reference range 4.3-6.3% in non-diabetic subjects; Bio Rad, Richmond, California, USA). C-reactive protein (CRP) levels were measured using an enzyme linked immunosorbent assay (CRP EIA HS assay, [Kordia, Leiden, the Netherlands]; normal range 0.2-6.0 mg/l).

The local ethics committee approved the protocol and all subjects gave informed consent.

Table 9.1 Patient characteristics

	Healthy subjects (n = 16)	Patients (n = 14)
Male/Female (n)	12 / 4	11 / 3
Age (years)	55 ± 7	55 ± 8
Duration of type 2 diabetes mellitus (months)	NA	26 ± 22
Body Mass Index (kg/m ²)	24.6 ± 1.3	26.0 ± 1.8*
Systolic blood pressure (mmHg)	127 ± 12	133 ± 15
Diastolic blood pressure (mmHg)	75 ± 8	79 ± 8
Pulse Pressure (mmHg)	52 ± 8	54 ± 10
Mean arterial pressure (mmHg)	92 ± 9	97 ± 10
Heart rate (bpm)	63 ± 11	70 ± 10
Fasting plasma glucose (mmol/l)	5.3 ± 0.4	7.5 ± 1.9*
Fasting plasma insulin (pmol/l)	9.1 ± 3	18.9 ± 10*
Glycated hemoglobin (%)	4.9 ± 0.6	5.8 ± 1.2*
High-sensitivity C-reactive protein (mg/l) [†]	0.72 ± 1.21	0.93 ± 2.62

Values are mean ± standard deviation.

* P < 0.05.

[†] Values are medians (interquartile range).

NA = not applicable.

Cardiovascular magnetic resonance acquisition technique and image analysis

CMR studies were performed with the use of a 1.5T whole-body MR scanner (Gyroscan ACS/ NT15, Philips, Best, the Netherlands) in the supine position at rest. Images were analyzed quantitatively using dedicated software (FLOW or MASS, Medis, Leiden, the Netherlands).

Aortic pulse wave velocity

A retrospectively ECG-gated gradient-echo pulse sequence with velocity encoding (Venc) was applied to measure through-plane flow at 2 predefined positions in the ascending and abdominal aorta. Imaging parameters included the following: echo time = 4.83 ms, repetition time = 14 ms, flip-angle = 20°, slice thickness = 8 mm, field of view = 350 × 350 mm, matrix size = 256 × 256, Venc = 150 cm/s, scan percentage = 80%. The predefined imaging planes were perpendicular to the aorta at 2 levels: 1) at the mid ascending aorta and 2) just above the bifurcation of the abdominal aorta (Figure 9.1). The temporal resolution was approximately 25 ms depending on the heart rate. The in-plane spatial resolution was 1.37 × 1.76 mm after reconstruction.

A single observer, blinded to the clinical status of the subjects, analyzed these flow measurements to calculate aortic PWV. Aortic PWV was calculated as $\Delta x / \Delta t$ (expressed in m/s), where Δx is the aortic path length between the 2 imaging levels and Δt is the time delay between the arrival of the foot of the pulse wave^{13,18} at these levels (Figure 9.1). Aortic PWV was calculated twice within a month to examine intra-observer variability.

Aortic distensibility

Distensibility of the aorta derived from flow measurements at the mid ascending aorta was calculated using the following formula:

$$D = (A_{\max} - A_{\min}) / (A_{\min} \times \text{pulse pressure}),^{19}$$

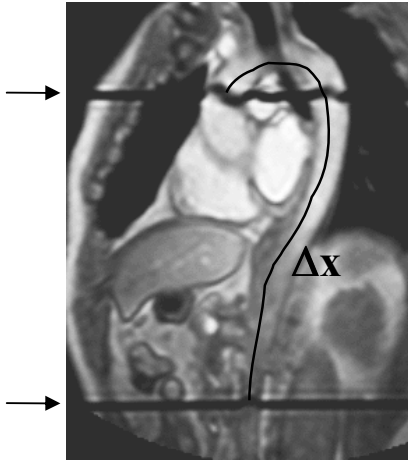
where D = distensibility (mmHg^{-1}), $A_{\max}$ = maximal aortic area (mm^2), $A_{\min}$ = minimal aortic area (mm^2), pulse pressure = systolic BP – diastolic BP (mmHg). The blood pressure was recorded using a semi-automated sphygmomanometer (Dinamap, Critikon, Tampa, Florida, USA) during the CMR examination. Mean arterial pressure (MAP) was calculated as $[\text{systolic BP} - (2 \times \text{diastolic BP})] / 3$.

Aortic contours were drawn twice within a month interval to assess intra-observer variability in distensibility.

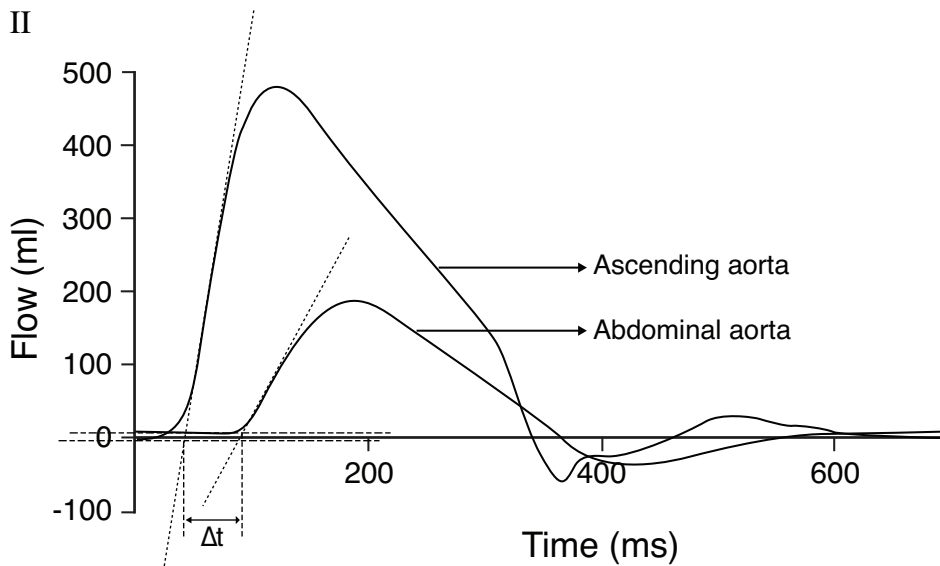
Left ventricular function

Systolic and diastolic LV functions were measured. The entire heart was imaged in the short-axis orientation using ECG-gated breath-hold multishot echo-planar imaging as

I



II

**Figure 9.1**

Pulse wave velocity determination. In Panel I, a spin echo image in plane with the long axis of the aorta is shown. A pre-saturation slab is noted proximal in the ascending aorta and at the abdominal level (arrows). Velocity maps were obtained at both levels. A standardized approach was used to provide consistent data to measure the path length between the middle of both levels (Δx) indicated by the curved line following the midline course of the aorta.

In panel II the flow curves in the ascending and abdominal aorta are shown. The intersection of the tangent line to the upstroke and the baseline was considered as the arrival time of the pulse wave. Δt denotes the time delay of the arrival of the proximal and abdominal flow curve. Aortic pulse wave velocity is defined by $\Delta x / \Delta t$ (m/s).

described before.²⁰ Imaging parameters included the following: echo time = 6 ms, repetition time = 11 ms, temporal resolution = 35–39 ms per cardiac phase, depending on the heart rate, flip angle = 30°, slice thickness = 10 mm, field of view = 400 × 400 mm, reconstructed matrix size = 256 × 256. End-diastolic chamber volume (EDV), end-systolic chamber volume (ESV), stroke volume (SV), ejection fraction (EF), and left ventricular mass (LVM) were assessed. Furthermore, an ECG-gated gradient-echo sequence with velocity encoding was performed to measure blood flow across the mitral valve for the determination of LV diastolic function. Imaging parameters included the following: echo time = 4.83 ms, repetition time = 14 ms, flip-angle = 20°, slice thickness = 8 mm, field of view = 350 × 350 mm, matrix size = 256 × 256, Venc = 100 cm/s, scan percentage = 80%. In each cardiac phase, the area of the mitral valve was manually traced, and the corresponding flow-versus time curve was derived automatically. Flow velocities in early diastole (E) and at atrial contraction (A) were assessed and the early peak filling rate, which is the maximal flow rate of E, the atrial peak filling rate, which is the maximal flow rate of A, and the ratio of E and A peak filling rates (E/A) were used for analysis. Furthermore, the peak acceleration and peak deceleration gradients of E were calculated automatically (Figure 9.2).^{21,22}

Statistical analysis

Statistical analysis was performed with SPSS for windows (version 11.5; SPSS, Chicago, IL). Data are expressed as mean (standard deviation [SD]). Only CRP, due to abnormal distribution, was expressed as median (interquartile range) and logarithmically transformed when used in the analyses. Between group differences were calculated using a two-tailed independent sample T-test. Multivariable testing was performed by using the general linear model. Data of patients and healthy subjects were pooled to calculate Pearson r-values for correlations. Significance was assumed when $P < 0.05$ (two-tailed).

RESULTS

Patient characteristics

Table 9.1 lists the characteristics of the participants. Patients, as compared to healthy subjects, showed a higher body mass index (BMI) as well as higher fasting plasma glucose and insulin levels. There were no differences in age, blood pressure, and heart rate between patients and healthy subjects.

Pulse wave velocity

Aortic PWV was significantly higher in patients than in healthy subjects (Table 9.2). CMR measured aortic PWV showed an inverse correlation with aortic distensibility (Table 9.2)

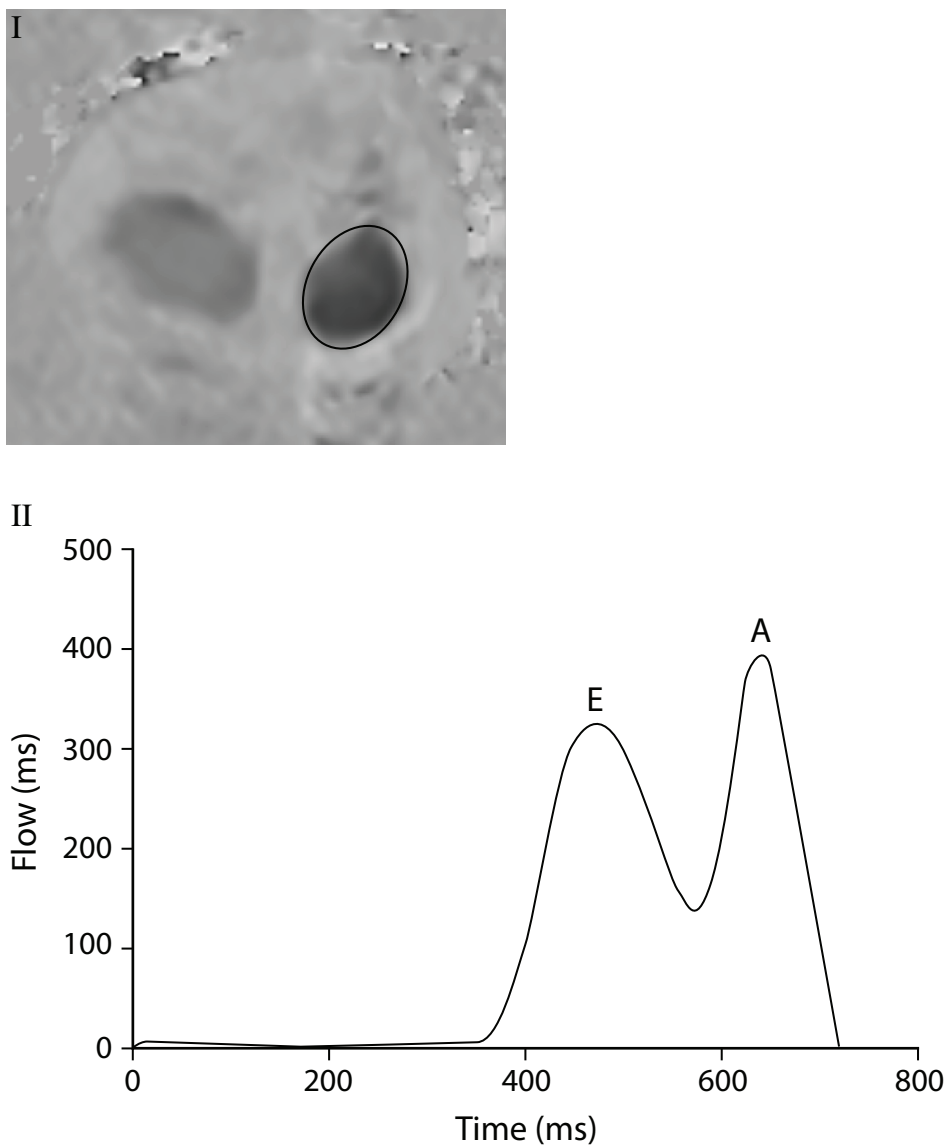


Figure 9.2
Left ventricular diastolic function. Panel I shows a phase contrast velocity map across the mitral valve in one cardiac phase. The mitral valve contour is traced. Panel II shows a flow-versus-time curve of a patient with type 2 diabetes mellitus across the mitral valve. The biphasic inflow pattern consists of two peaks, representing the early filling phase (E) and the atrial contraction (A). Analysis of E and A was performed by calculation of the early peak filling rate, which is the maximal flow rate of E, the atrial peak filling rate, which is the maximal flow rate of A, and the ratio of E and A peak filling rates. Furthermore, the peak acceleration and peak deceleration gradients of E were automatically calculated by assessing the steepest tangent to the upstroke and downstroke of E.

Table 9.2 Aortic vascular and left ventricular dynamics

	Healthy subjects (n = 16)	Patients (n = 14)	Correlation with aortic distensibility (n = 28)
Aortic pulse wave velocity (m/s)	5.65 ± 0.75	6.83 ± 1.60*	-0.517*
Distensibility ($\times 10^{-3}$ mmHg ⁻¹)	7.42 ± 3.34	4.50 ± 2.24*	NA
Left ventricular end-diastolic volume (ml)	141 ± 26	146 ± 35	0.288
Left ventricular end-systolic volume (ml)	54 ± 9	59 ± 18	0.201
Left ventricular stroke volume (ml)	87 ± 20	87 ± 22	0.278
Left ventricular ejection fraction (%)	60.9 ± 6.6	59.9 ± 7.0	0.079
Left ventricular end-systolic mass (g)	113.9 ± 30.6	119.5 ± 33.5	0.088
E peak filling rate (ml/s)	454.8 ± 108.6	379.5 ± 70.1*	0.517*
E acceleration peak (ml/s ² × 10 ³)	7.66 ± 1.85	6.38 ± 1.42*	0.285
E deceleration peak (ml/s ² × 10 ³)†	3.72 ± 1.32	2.73 ± 0.74*	0.542*
E/A peak flow ratio	1.32 ± 0.36	1.08 ± 0.28	0.413*

* Mean values ± standard deviation are significantly different (independent samples T-tests) or correlation is significant (Pearson correlation, $P < 0.05$).

† One patient was considered as an outlier and censored for this parameter.

A = atrial contraction; E = early diastole; NA = not applicable.

whereas positive correlations were found with systolic and diastolic blood pressure, MAP, and fasting plasma glucose and insulin levels (Table 9.3). Multivariable analysis showed that the difference in PWV between patients and controls was statistically significant after correction for SBP and PP separately ($P < 0.05$). The difference in PWV between patients and controls was borderline significant after correction for DBP and MAP separately ($P = 0.06$). In healthy subjects, but not in T2DM patients, aortic PWV correlated with age ($r = 0.53$, $P < 0.05$ and 0.12 , $P > 0.05$, respectively).

Intra-observer reproducibility for CMR measured aortic PWV was excellent. The average difference was -0.08 ± 0.25 ($P > 0.05$). Limits of agreement were -0.59 to 0.42 , and

Table 9.3 Diabetic and anthropometric parameters correlated with aortic pulse wave velocity and distensibility

	Correlation with pulse wave velocity (n = 30)	Correlation with aortic distensibility (n = 28)
Systolic blood pressure (mmHg)	0.387*	-0.490*
Diastolic blood pressure (mmHg)	0.423*	-0.467*
Mean arterial pressure (mmHg)	0.431*	-0.517*
Fasting plasma insulin (mU/l)	0.523*	-0.422*
Fasting plasma glucose (mmol/l)	0.599*	-0.388*
Log C-reactive protein (mg/l)	0.358	-0.429*
Heart rate (bpm)	0.245	-0.441*
Patient length (cm)	0.084	-0.071

* $P < 0.05$ (Pearson correlation).

no trend was observed. The two calculations were significantly correlated ($r = 0.98$, $P < 0.001$).

Distensibility

Distensibility could not be calculated in 1 out of 14 patients and 1 out of 16 healthy subjects due to technical problems with BP measurements during MR data acquisition. Distensibility of the aorta was significantly lower in patients as compared to healthy subjects (Table 9.2).

Furthermore, fasting plasma insulin, glucose, CRP levels and heart rate showed statistically significant correlations with aortic distensibility (Table 9.3).

Intra-observer reproducibility for CMR measured aortic distensibility was excellent. The average difference was 0.02 ± 0.90 ($P > 0.05$). Limits of agreement were -1.79 to 1.82 . There was no trend observed. The two calculations were significantly correlated ($r = 0.96$, $P < 0.001$).

Left ventricular function

Parameters of systolic function were similar in patients as compared to controls (Table 9.2). There was no correlation between aortic stiffness and left ventricular systolic function. Patients and healthy subjects showed similar LV masses ($P > 0.05$), and there was no correlation between PWV or distensibility of the aorta and LV mass (Table 9.2). A positive correlation was observed between aortic distensibility and diastolic functional parameters (Table 9.2). Furthermore, E peak filling rate, E acceleration peak of mitral flow velocity, and E deceleration peak of mitral flow velocity were significantly lower in patients as compared to healthy subjects. In addition, the E/A peak flow ratio showed a borderline significant difference ($P = 0.055$) between patients and healthy subjects.

DISCUSSION

This study demonstrates the potential value of an integrated CMR protocol for comprehensive evaluation of aortic and cardiac function in patients with type 2 diabetes mellitus. Although the pathophysiology of aortic and cardiac function in diabetic patients has been extensively studied with other techniques than CMR, there are only a few studies that have studied the possible relationship between aortic and cardiac function using CMR as a non-invasive tool. The main findings in our study are that aortic stiffening as characterized by pulse wave velocity and aortic distensibility was significantly increased in patients as compared to healthy subjects. In addition, aortic distensibility correlated with left ventricular diastolic function. The sample size was adequate to identify significant differences in aortic pulse wave velocity and aortic distensibility between patients and

healthy controls. The observations are similar to that found using less-precise modalities in previous studies that required larger sample sizes, owing to the high reproducibility of MR measurements.

The present magnetic resonance findings of an increased pulse wave velocity and a decreased aortic distensibility in diabetic subjects are in agreement with previous studies that used techniques other than MRI.^{3-5,23,24} Cruickshank et al. used ultrasound to demonstrate that pulse wave velocity is an independent predictor of mortality in the diabetic population.³ Whereas previous studies mainly used ultrasound or applanation tonometry to assess pulse wave velocity or vascular distensibility, our study showed the usefulness of CMR in assessing both parameters of aortic stiffness in one session. Another important advantage of CMR is that aortic path length can be measured directly, thereby providing a potentially more accurate pulse wave velocity than ultrasound or applanation tonometry, where path length has to be estimated. Furthermore, CMR is capable of assessing the pulse waves locally in the aorta, thus minimizing influences of peripheral arteries on aortic pulse wave velocity determination as may be the case with peripheral applanation tonometry.²⁵ This is of importance since it is known that diabetes has greater impact on pulse wave velocity of the elastic central arteries as compared to the muscular peripheral arteries.²⁶

We were able to show correlations between both parameters of aortic stiffness and fasting plasma glucose and insulin levels. Raised blood glucose and insulin levels in diabetic subjects can partially explain the stiffening of the aorta. Hyperglycemia causes vascular damage through various mechanisms, including the formation of advanced glycation end products (AGEs), oxidative stress and vascular inflammation. Collectively, all these mechanisms can contribute to aortic stiffening. We observed that CMR measured aortic pulse wave velocity and distensibility correlate to systolic and diastolic blood pressure as well as to mean arterial pressure. The difference in pulse wave velocity between patients and controls was still statistically significant after correction for systolic blood pressure and pulse pressure. The difference in pulse wave velocity between patients and controls was borderline significant after correction for diastolic blood pressure and mean arterial pressure separately ($P = 0.06$). Therefore, we conclude that diabetes mellitus type 2 may induce an aortopathy independent of blood pressure. Furthermore, aortic distensibility correlated to heart rate. There was no correlation between pulse wave velocity and heart rate as suggested previously.²⁷ The younger age group and smaller range of heart rate in the present study might explain this observation. The correlation of aortic pulse wave velocity with age is already well known.²⁸ We found a correlation of pulse wave velocity with age in the healthy population only. No such correlation was found in diabetic subjects, probably because pulse wave velocity in these patients is affected by multiple factors.

In the current study, no difference in left ventricular systolic function between patients and healthy controls was observed. However, markers of left ventricular diastolic function (early peak filling rate, early acceleration peak, and early deceleration peak, calculated from the transmitral filling patterns) were decreased in diabetic subjects. Furthermore, the difference in the E/A peak flow ratio showed borderline significance between patients and healthy subjects. These findings are in accordance with previous ultrasound studies that indicate that diastolic dysfunction may occur in approximately 40% of diabetic patients.^{24,29,30} Interestingly, the present CMR data show that when aortic distensibility decreased, the early filling rate, the early deceleration peak, and the E/A peak flow ratio decreased as well. This observation suggests that aortic distensibility is related to changes in left ventricular diastolic function. This pattern of changes in early diastolic flow velocities corresponds to an increase in myocardial compliance and a decrease in active relaxation of the left ventricle.³¹ Although our study does not allow establishing a causal relation between aortic distensibility and left ventricular diastolic function, it is known that left ventricular diastolic function may become impaired by aortic stiffness through various mechanisms. Stiffening of the aorta increases end-systolic wall stress and reduces aortic pressure throughout diastole.³² This leads to an increased myocardial oxygen demand, hypertrophy and compromised coronary perfusion. An increased systolic left ventricular pressure, left ventricular hypertrophy, and compromised coronary perfusion can delay myocardial relaxation and cause diastolic heart failure. There is some evidence in patients with syndrome X, indicating a link between increased arterial stiffening and myocardial perfusion impairment.³³ Panting et al. were able to show sub-endocardial hypoperfusion during the intravenous administration of adenosine in patients with syndrome X but did not report any relation with subendocardial hypoperfusion and LV systolic function, diastolic function, or aortic physiology.³⁴ Nevertheless, adenosine stress myocardial perfusion might be a useful tool to test the relationship between aortic pulse wave velocity, aortic distensibility, myocardial function and myocardial perfusion impairment, especially in patients with type 2 diabetes mellitus of whom a considerable part is suffering from silent myocardial ischemia.^{35,36} Furthermore, stiffening of the aorta may cause myocardial hypertrophy,³⁷ which may lead to an increase in systolic and diastolic myocardial stiffness.³⁸ In our study, no significant left ventricular hypertrophy was noted in diabetic patients. However, LV diastolic dysfunction may occur even in the absence of left ventricular hypertrophy.^{21,39}

A limitation of this study is that local assessment of aortic pulse pressure was not performed, which would have defined aortic distensibility more accurately than the use of brachial pulse pressure.⁴⁰

In conclusion, a comprehensive CMR protocol is well suited to assess aortic and cardiac function in diabetic patients. A combined CMR assessment of aortic PWV, aortic distensibility, and heart function reveals abnormal PWV and distensibility in patients with T2DM,

independent of blood pressure. Furthermore, aortic distensibility correlates with diastolic left ventricular function.

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

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ABSTRACT

Background

Type 2 diabetes mellitus (T2DM) is a major risk factor for cardiovascular disease. Increasing evidence is emerging indicating that lipid oversupply to cardiomyocytes plays a role in the development of diabetic cardiomyopathy by causing lipotoxic injury and myocardial steatosis. Therefore, the purpose of this study was to compare myocardial triglyceride content and function between patients with uncomplicated T2DM and healthy subjects within the same range of age and body mass index (BMI), and to study the associations between myocardial triglyceride content and function.

Methods

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

Results

Myocardial triglyceride content was significantly higher in T2DM patients compared to healthy volunteers (0.96 ± 0.07 vs. $0.65 \pm 0.05\%$, $P < 0.05$). Systolic function did not significantly differ between both groups. Indices of diastolic function, including left ventricular E/A ratio and E peak deceleration, were significantly impaired in T2DM compared to healthy subjects (1.08 ± 0.04 vs. 1.24 ± 0.06 and 3.6 ± 0.2 vs. 4.4 ± 0.3 $\text{ml/s}^2 \times 10^{-3}$ respectively, $P < 0.05$). Multivariable analysis indicated that myocardial triglyceride content was associated with E/A and E peak deceleration, independently of diabetic state, age, BMI, heart rate, visceral fat and diastolic blood pressure.

Conclusion

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

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

Left ventricular (LV) diastolic function can be assessed using flow velocity encoded magnetic resonance imaging (MRI). Using this technique, myocardial diastolic functional parameters have been shown to be associated with myocardial triglyceride accumulation in healthy volunteers.¹¹ Furthermore, in explanted hearts of obese and T2DM patients with end-stage heart failure, lipid staining was a common finding.⁴

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

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

METHODS

Subjects

Forty-one male T2DM patients were included in this study, which was approved by the local ethics committee. The hormonal status or use of contraceptives may affect lipid metabolism in women. Plasma estrogen levels influence lipid metabolism (including plasma lipid levels, adipose tissue) and gender differences in expression of certain cell surface receptors/ transporters of fatty acids have been reported.^{12,13} Therefore, we decided to exclude women to avoid possible confounding influences of fluctuation in lipid metabolism in women on hepatic and myocardial triglyceride accumulation. All participants signed informed consent. Patients were recruited by advertisements in the local newspapers according to the following inclusion criteria: 1) age 45-65 years, 2) T2DM diagnosed according to WHO criteria¹⁴ and treated by sulfonylurea derivatives in stable doses, 3) glycated hemoglobin below 8.5%, and 4) sitting blood pressure <150/85 mmHg, with or without antihypertensive drugs. Exclusion criteria included: impaired hepatic function or a history of liver disease, substance abuse, known cardiovascular disease or diabetes-related complications including proliferative retinopathy, autonomic neuropathy as defined by Ewing's tests,¹⁵ microalbuminuria as defined as measurements of albumin-creatinine ratio in a urine sample, and all contra-indications for MRI. Furthermore, to avoid interference with the main study parameters, patients on lipid lowering therapy (such as statins, fibrates) were excluded. Myocardial ischemia was excluded by means of a high-dose dobutamine stress echocardiography.

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

Exclusion criteria included chronic use of any drug, substance abuse, hypertension and impaired glucose tolerance (as determined by a 75-g oral glucose tolerance test).¹⁶

Magnetic resonance spectroscopy

All subjects underwent MR scanning in the morning for the assessment of ectopic triglyceride content and heart function after an overnight fast and after blood sampling.

All MR studies were performed with the use of a 1.5T whole-body MR scanner (Gyrosan ACS/ NT15; Philips, Best, the Netherlands) with subjects in supine position at rest. Myocardial ¹H-MR spectra were obtained as described before.¹⁷ Briefly, the body coil was used for radiofrequency transmission and a 17 cm diameter circular surface coil was used for signal reception. An 8 ml voxel was positioned in the interventricular sep-

tum. Spectroscopic data acquisition was double triggered using electrocardiographical triggering and respiratory navigator echoes to minimize breathing influences.¹⁷ Water-suppressed spectra were acquired for the detection of the triglyceride signals at end systole, with an echo time of 26 ms and a repetition time of at least 3000 ms. 1024 data points were collected using a 1000 Hz spectral width and averaged over 128 acquisitions. Without changing any parameter, spectra without water suppression with a repetition time of 10 seconds and 4 averages were obtained from the same voxel, to be used as an internal standard.

¹H-MR spectroscopy of the liver was performed with an 8 ml voxel positioned in the liver, avoiding gross vascular structures and adipose tissue depots. Spectra were obtained using the same parameters as described for myocardial ¹H-MR spectroscopy. Only 64 averaged acquisitions were collected with water suppression.

All ¹H-MR spectroscopic data were fitted using Java-based MR user interface software (jMRUI version 2.2; developed by A. van den Boogaart, Katholieke Universiteit Leuven, Leuven, Belgium) as described before.^{17,18} Myocardial and hepatic triglyceride content were calculated as a percentage relative to water as (signal amplitude of triglyceride)/(signal amplitude of water) × 100.

Magnetic resonance imaging

All images were analyzed quantitatively using dedicated software (FLOW® or MASS®, Medis, Leiden, the Netherlands). The entire heart was imaged in short-axis orientation using electrocardiographically gated breath-holds with a sensitivity encoding balanced turbo-field echo sequence. Imaging parameters included the following: echo time = 1.7 ms, repetition time = 3.4 ms, flip-angle = 35°, slice thickness = 10 mm with a gap of 0 mm, field of view = 400 × 400 mm, reconstructed matrix size = 256 × 256. LV ejection fraction was assessed for the determination of LV systolic function. In addition, LV mass/(end diastolic) volume ratio was calculated.

An electrocardiographically gated gradient-echo sequence with velocity encoding (Venc) was performed to measure blood flow across the mitral valve for the determination of LV diastolic function. Imaging parameters included the following: echo time = 5 ms, repetition time = 14 ms, flip-angle = 20°, slice thickness = 8 mm, field of view = 350 × 350 mm, matrix size = 256 × 256, Venc = 100 cm/s, scan percentage = 80%. The resulting biphasic diastolic inflow pattern consists of two peaks, representing the early filling phase and the atrial contraction. Analysis of the early filling phase and the atrial contraction was performed by calculation of their peak filling rates and ratio of the peak filling rates (E/A). Furthermore, the peak deceleration gradient of the early filling phase (E deceleration peak) was calculated automatically. In addition, an estimation of LV filling pressures (E/Ea) was assessed as described before.¹⁹ During MR imaging, blood pressure and heart-rate were measured.

Abdominal visceral fat depots were quantified by magnetic resonance imaging.²⁰ A turbo spin echo imaging protocol was used and imaging parameters included the following: echo time = 11 ms, repetition time = 168 ms, flip-angle = 90°, slice thickness = 10 mm. Three consecutive transverse images were obtained during 1 breath-hold with the middle image at a level just above the fifth lumbar vertebra. The volumes of the visceral fat depots of all slices were calculated by converting the number of pixels to square centimeters multiplied by the slice thickness. The total volume of the fat depots was calculated by summing the volumes of all three slices.

Assays

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

Statistical analysis

Statistical analysis was performed using SPSS for windows (version 12.0; SPSS, Chicago, IL). Data are expressed as mean $\pm$ standard error when normally distributed; nonnormally distributed data are expressed as the median (interquartile range). Nonnormally distributed data were log-transformed, and unpaired T-tests (or, when appropriate, non-parametric tests) were used for comparisons. To detect determinants of myocardial triglyceride content and LV function, univariate and multivariable linear regression analyses were performed. $P < 0.05$ was considered statistically significant.

RESULTS

Baseline characteristics of patients and healthy subjects are displayed in Table 10.1. The MR scan protocol could not successfully be completed due to technical constraints in 3 patients and in 2 healthy subjects. Therefore, data obtained from 38 patients and 28 healthy subjects was used for analysis. Mean systolic blood pressure in both groups was well within the normal range, although it was higher in patients than in controls (127 ± 2 vs. 116 ± 2 mmHg, $P < 0.05$). Although no patient met official criteria for

Table 10.1 Clinical and biochemical characteristics

	Healthy subjects (n = 28)	Patients with T2DM (n = 38)
Age (years)	54 ± 1	57 ± 1
Body mass index (kg/m ²)	26.9 ± 0.5	28.1 ± 0.6
Systolic blood pressure (mmHg)	116 ± 2	127 ± 2*
Diastolic blood pressure (mmHg)	73 ± 2	76 ± 1
Heart rate (bpm)	59 (52 – 63)	65 (62 – 72)*
Plasma glucose (mmol/l)	5.3 ± 0.1	9.1 ± 0.3*
Plasma insulin (pmol/l)	35 (24 – 51)	55 (35 – 100)*
Glycated hemoglobin (%)	5.33 ± 0.05	7.21 ± 0.17*
Plasma triglycerides (mmol/l)	1.0 (0.8 – 1.3)	1.6 (1.1 – 2.8) *
Non-esterified fatty acids (mmol/l)	0.45 ± 0.04	0.49 ± 0.03
High density lipoprotein cholesterol (mmol/l)	1.39 ± 0.06	1.09 ± 0.04*
Low density lipoprotein cholesterol (mmol/l)	3.3 (3.0 – 3.7)	2.9 (2.6 – 3.4)*
Total cholesterol (mmol/l)	5.29 ± 0.14	5.00 ± 0.13

Values are mean ± standard error or median (interquartile range).

* $P < 0.05$ compared to healthy subjects.

T2DM = Type 2 diabetes Mellitus.

autonomic neuropathy, their resting heart-rate was increased relative to controls (median [interquartile range]: 65 [62–72] vs. 59 [52–63] bpm, $P < 0.05$).

Myocardial and hepatic triglyceride content

Myocardial triglyceride content was significantly higher in patients with T2DM compared to healthy volunteers (0.96 ± 0.07 vs. $0.65 \pm 0.05\%$) as was hepatic triglyceride content (8.6 ($2.7 - 24.3$) vs. 2.2 ($1.2 - 3.8$)%, $P < 0.05$, Table 10.2 and Figure 10.1). Myocardial triglyceride content showed significant univariate correlations with age, visceral adipose tissue volume, plasma triglycerides, plasma high density lipoprotein cholesterol, plasma glucose, plasma insulin concentrations and hepatic triglyceride content (Pearson $r = 0.28, 0.36, 0.37, -0.39, 0.45, 0.30$, and 0.37 respectively, $P < 0.05$), but not with BMI, in all study subjects pooled. Visceral adipose tissue volume and plasma high density lipoprotein cholesterol and glucose concentrations as well as hepatic triglyceride content levels remained significantly correlated to myocardial triglyceride content after adjustment for diabetic state, whereas the association between plasma triglyceride levels and myocardial triglyceride content showed borderline significance when adjusted for diabetic state ($P = 0.051$).

Association between myocardial steatosis and myocardial function

Differences in cardiovascular function between T2DM patients and healthy volunteers are displayed in Table 10.2. LV diastolic parameters were significantly impaired in T2DM

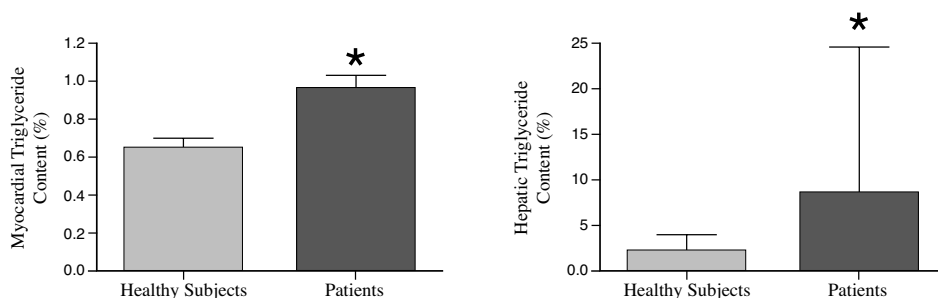


Figure 10.1

Myocardial and hepatic triglyceride content in patients and controls. Bar graphs show increased myocardial and hepatic triglyceride content in diabetic patients as compared to healthy control subjects. Bars represent mean + standard error for myocardial triglycerides and median + interquartile range for hepatic triglycerides. * $P < 0.05$.

patients. LV systolic function was not significantly different between healthy subjects and T2DM patients (ejection fraction = 58 ± 1 vs. $60 \pm 1\%$, $P > 0.05$).

E/A and E peak deceleration were used as parameters of LV diastolic function for further analysis. Table 10.3 lists Pearson correlations of E/A and the E deceleration peak with several parameters in all study subjects and shows that both parameters were significantly inversely correlated to age, heart rate, blood pressure, plasma glucose levels and myocardial triglyceride content ($P < 0.05$, Figure 10.2).

Table 10.2 Magnetic resonance study parameters

	Healthy subjects (n = 28)	Patients with T2DM (n = 38)
Myocardial triglyceride content (%)	0.65 ± 0.05	$0.96 \pm 0.07^*$
Hepatic triglyceride content (%)	2.2 (1.2 – 3.8)	8.6 (2.7 – 24.3)*
Visceral fat (ml)	284 ± 24	$420 \pm 31^*$
Left ventricular mass (g)	108 ± 5	105 ± 3
LV mass/volume ratio (g/ml)	0.09 ± 0.02	$0.11 \pm 0.02^*$
Left ventricular ejection fraction (%)	58 ± 1	60 ± 1
E peak filling rate (ml/s)	490 ± 21	$426 \pm 14^*$
E deceleration peak ($\text{ml/s}^2 \times 10^{-3}$)	4.4 ± 0.3	$3.6 \pm 0.2^*$
A peak filling rate (ml/s)	402 ± 9	401 ± 10
E/A	1.24 ± 0.06	$1.08 \pm 0.04^*$
E/Ea	9.1 ± 0.6	10.0 ± 0.7

Values are mean $\pm$ standard error or median (interquartile range).

* $P < 0.05$ compared to healthy subjects.

T2DM = Type 2 diabetes mellitus; LV mass/volume ratio = left ventricular mass/left ventricular end-diastolic volume; E = early diastolic filling phase; A = atrial contraction; E/A = ratio of maximal left ventricular early peak filling rate and the maximal left ventricular atrial peak filling rate; E/Ea = estimation of LV filling pressures.

Table 10.3 Univariate correlations between diastolic function and anthropometric and biochemical markers and fat compartments.

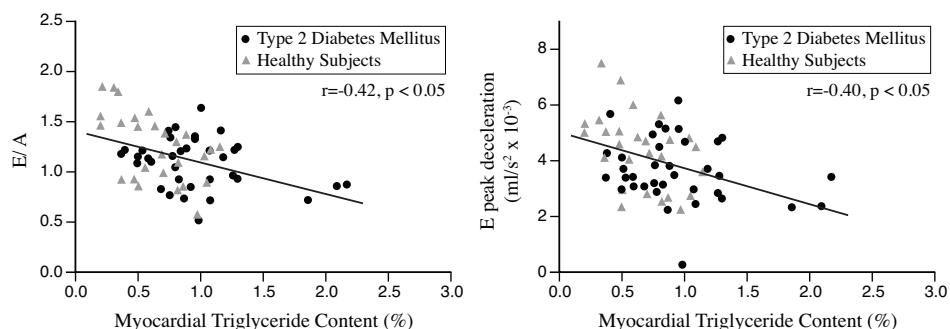
	E/A	E peak deceleration (ml/s ² × 10 ³)
Age (years)	-0.58*	-0.44*
Body mass index (kg/m ²)	-0.25*	-0.18
Log heart rate (bpm)	-0.36*	-0.36*
Systolic blood pressure (mmHg)	-0.26*	-0.24
Diastolic blood pressure (mmHg)	-0.41*	-0.45*
LV mass/volume ratio (g/ml)	-0.35*	-0.46*
Plasma glucose (mmol/l)	-0.32*	-0.34*
Log plasma triglyceride (mmol/l)	0.01	-0.05
Non-esterified fatty acids (mmol/l)	0.01	0.09
High density lipoprotein cholesterol (mmol/l)	0.21	0.28*
Visceral fat (ml)	-0.31*	-0.20
Hepatic triglyceride content (%)	-0.30*	-0.21
Myocardial triglyceride content (%)	-0.42*	-0.40*

Values are Pearson *r*.

* *P* < 0.05.

LV mass/volume ratio = left ventricular mass/left ventricular end-diastolic volume; E = early diastolic filling phase; E/A = ratio of maximal left ventricular early peak filling rate and the maximal left ventricular atrial peak filling rate.

Multivariable analysis was performed in all subjects to study the association between diastolic function and myocardial triglyceride content. To this purpose, E/A was entered as a dependent variable, and subsequently, myocardial triglyceride content, the presence of type 2 diabetes mellitus, age, heart rate, and diastolic blood pressure were entered as independent variables into the model (Table 10.4). The diabetic state had no

**Figure 10.2**

Correlations between myocardial triglyceride content and left ventricular diastolic function.

Increased myocardial triglyceride content is significantly associated with decreased myocardial function.

E = early diastolic filling phase; E/A = ratio of maximal left ventricular early peak filling rate and the maximal left ventricular atrial peak filling rate.

Table 10.4 Multivariable associations between E/A and myocardial triglyceride content

	E/A β -coefficient (95% CI)	P^a	r^2	P^b
Model 1			0.18	< 0.001
Myocardial triglyceride content %	-0.31 (-0.48; -0.15)	< 0.001		
Model 2 (model 1 + diabetic state)			0.19	0.001
Myocardial triglyceride content %	-0.28 (-0.46; -0.10)	0.003		
T2DM/Healthy subject	0.07 (-0.07; 0.22)	0.320		
Model 3 (model 2 + age)			0.42	< 0.001
Myocardial triglyceride content %	-0.20 (-0.36; -0.04)	0.015		
T2DM /Healthy subject	0.02 (-0.10; 0.15)	0.725		
Age (years)	-0.02 (-0.03; -0.01)	< 0.001		
Model 4 (model 3 + heart rate)			0.46	< 0.001
Myocardial triglyceride content %	-0.20 (-0.35; -0.04)	0.014		
T2DM /Healthy subject	-0.03 (-0.16; 0.10)	0.671		
Age (years)	-0.02 (-0.03; -0.01)	< 0.001		
10% increase in Heart rate (bpm)	-0.97 (-1.81; -0.14)	0.023		
Model 5 (model 4 + diastolic blood pressure):			0.50	< 0.001
Myocardial triglyceride content %	-0.20 (-0.35; -0.05)	0.009		
T2DM /Healthy subject	-0.05 (-0.17; 0.08)	0.487		
Age (years)	-0.02 (-0.03; -0.01)	< 0.001		
10% increase in Heart rate (bpm)	-0.89 (-1.71; -0.08)	0.032		
Diastolic blood pressure (mmHg)	-0.01 (-0.02; -0.001)	0.031		
Model 6 (model 3):				
Association between E/A and myocardial triglyceride content after adjusting for diabetic state, age, and each of the following variables				
a) LV mass/volume ratio	-0.19 (-0.34; -0.03)	0.017	0.47	< 0.001
b) Visceral fat (ml)	-0.19 (-0.35; -0.02)	0.026	0.42	< 0.001
c) 10% increase in hepatic triglyceride content	-0.20 (-0.38; -0.01)	0.036	0.42	< 0.001
d) Fasting plasma glucose (mmol/l)	-0.19 (-0.36; -0.02)	0.025	0.43	< 0.001
e) Body mass index (kg/m ²)	-0.23 (-0.33; -0.01)	0.033	0.44	< 0.001

T2DM = Type 2 diabetes mellitus; CI = Confidence interval; r^2 for the respective models, i.e. in model 1, with E/A as dependent and myocardial triglyceride content as independent variable; in model 2, myocardial triglyceride content and diabetic state are independent variables; in model 3, myocardial triglyceride content, diabetic state, and age are independent variables; in model 4, myocardial triglyceride content, diabetic state, age, and heart rate are independent variables; in model 5, myocardial triglyceride content, age, diabetic state, heart rate, and diastolic blood pressure are independent variables; in models 6 a-e the possible confounders are separately entered for adjustment and β -coefficients and P -values for the association between myocardial triglyceride content and E/A are displayed.

^a Level of significance of the association between E/A and the separate components of the model.

^b Level of significance of the model.

effect on the association between E/A and myocardial triglyceride content. Furthermore, adjustment for age, heart rate, and diastolic blood pressure, which were all significantly correlated with E/A, had no effect on the association between E/A and myocardial triglyceride content. Identical analyses were performed with E peak deceleration as independent variable. This analysis indicated myocardial triglyceride content was associated with E peak deceleration, independently of diabetic state, age, heart rate, and diastolic blood pressure.

DISCUSSION

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

We were able to extend the findings in previous studies showing myocardial steatosis in T2DM patients.^{4,10} McGavock et al. demonstrated that excessive triglyceride accumulation in human cardiomyocytes occurs early in the natural history of T2DM. In their study, a heterogeneous group of T2DM patients showed increased myocardial triglyceride content compared to healthy subjects. The use of insulin (a well-known lipogenic agent, which might have increased myocardial triglyceride levels) by the T2DM patients and differences in age and BMI between the two groups could have influenced their observations. In addition, the occurrence of silent ischemia, which can be present in up to 22% of asymptomatic T2DM patients with a normal electrocardiogram,²¹ could influence accumulation of triglycerides in the reversibly injured myocardium during reperfusion.^{22,23} Therefore, in the present study, we used controls of the same gender and within the same range of age and BMI as patients, and included only T2DM patients with normal dobutamine stress echocardiography to control for reversible ischemia. By using these well-defined groups we could confirm that myocardial triglyceride content in T2DM patients is significantly elevated in comparison to healthy volunteers, independently of age or BMI.

In our study, diabetic patients had higher plasma triglyceride levels and showed significantly lower levels of high density lipoprotein cholesterol compared to control subjects. Interestingly, plasma triglyceride concentrations were positively associated with myocardial triglyceride content. We also found an inverse correlation between myocardial triglyceride content and high density lipoprotein cholesterol levels, also after adjustment for diabetic state. These data are in line with prior observations demonstrating that increased serum triglyceride and decreased serum high density lipoprotein cholesterol concentrations correlate with lipid content in skeletal muscle in patients with HIV-lipodystrophy.²⁴ High density lipoprotein cholesterol is considered an important mediator of reverse cho-

lesterol transport, a process that involves the transfer and uptake of free cholesterol from the peripheral tissues, with subsequent delivery to the liver where it can be eliminated. Artificial elevation of high density lipoprotein in cholesterol-fed rabbits induced the regression of early aortic fatty streaks.²⁵ Based on our results, we hypothesize that reverse cholesterol transport might also play a role in protecting the heart from accumulating lipids.

In the present study, we are the first to demonstrate that myocardial triglyceride accumulation in patients with uncomplicated T2DM is associated with left ventricular diastolic dysfunction, independently of age, BMI, blood pressure and heart-rate. Neutral triglycerides are probably inert and harmless to cells and may initially even provide a protective buffer by diverting NEFA from deleterious pathways.²⁶ Eventually, however, excessive triglyceride stores enter a continuous cycle between hydrolysis and fatty acid re-esterification, yielding cardiotoxic intermediates, such as ceramide and diacylglycerol, which seem to be an important route leading to myocardial dysfunction, at least in animal models.^{7,27} In the present study, left ventricular ejection fraction was not different between patients and healthy control subjects and was not associated with myocardial triglyceride content. We excluded patients with myocardial ischemia and contractile abnormalities, using dobutamine stress echocardiography, however, microvascular disease cannot be ruled out. Prior experimental studies have shown decreased contractility and diastolic function in combination with hypertrophy and concentric remodeling.²⁸ In our patient population with uncomplicated diabetes mellitus, cardiac mass was not different than in healthy volunteers. LV mass/ volume ratio was increased in T2DM patients, indicating mild concentric remodeling.²⁹ Therefore, LV mass/ volume ratio was included in the multivariable analysis, but had no influence on the independent relationship between myocardial triglyceride content and diastolic function.

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

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

In the present study design, we can not establish a causal relationship between increased myocardial triglyceride content and reduced left ventricular diastolic function. We cannot discriminate if myocardial triglyceride accumulation per se hampers myocardial function due to a mechanical effect, the formation of cardiotoxic intermediates, or by intervening in other mechanisms such as calcium handling. In addition, excluding women from the

study limits the generalizability of the present study. Further studies need to be initiated to make these distinctions and to extend the present findings to female subjects to identify the role of myocardial triglyceride accumulation in the human diabetic heart, because in animal models, therapeutic interventions aiming at reduction of myocardial triglyceride accumulation due to disturbed fatty acid metabolism have been shown to have beneficial effects on myocardial function.⁷

Furthermore, pioglitazone, a peroxisome proliferator-activated receptor γ agonist, which has the capacity to divert fat from non-adipose tissue to subcutaneous tissue, has been shown to lower myocardial triglyceride content in T2DM patients on a insulin-based treatment regimen.³³ Therefore, myocardial steatosis might be a useful indicator for predicting the severity of diabetic cardiomyopathy and for evaluating the effects of antisteatotic therapy.

In conclusion, myocardial triglyceride content is increased in uncomplicated T2DM and associated with impaired left ventricular diastolic function, independently of age, BMI, heart rate, visceral fat, and diastolic blood pressure.

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

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ABSTRACT

Background

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

Methods

Seventy-eight males with T2DM without cardiac ischemia as verified by dobutamine-stress echocardiography were randomly assigned to pioglitazone (30mg/day) or metformin (2000mg/day) and matching placebo during 24 weeks. The primary endpoint was change from baseline in cardiac diastolic function relative to myocardial metabolic changes, measured by magnetic resonance (MR) imaging, proton and phosphorus MR-spectroscopy and positron-emission tomography using ^{18}F -2-fluoro-2-deoxy-D-glucose and ^{11}C -palmitate. Seventy-one patients completed the study.

Results

No patient developed congestive heart failure. Both therapies similarly improved glyce-mic control, whole-body insulin sensitivity and systolic blood pressure. Pioglitazone, but not metformin, improved left ventricular (LV) diastolic function ($P = 0.034$) and ameliorated LV compliance. Pioglitazone increased myocardial glucose uptake ($P = 0.011$), but pioglitazone-related diastolic improvement was not associated with changes in myocardial substrate metabolism. Metformin did not affect myocardial function, but reduced myocardial glucose uptake ($P = 0.005$) and fatty-acid oxidation ($P = 0.024$). Neither treatment affected high-energy phosphate metabolism or myocardial triglyceride content. Only pioglitazone reduced liver fat content ($P < 0.001$).

Conclusion

In the absence of myocardial ischemia, pioglitazone may have beneficial cardiac effects in T2DM patients, as demonstrated by the improved diastolic function and LV compliance.

Cardiac disease is the leading cause of mortality in type 2 diabetes mellitus (T2DM).¹ In asymptomatic patients, cardiac abnormalities exist, even in the absence of coronary artery disease (CAD) or hypertension, due to diabetic cardiomyopathy.^{2,3} Increased left ventricular (LV) diastolic stiffness is a common and early finding.³ Although diabetic cardiomyopathy is a multi-causal condition, evidence obtained from animal studies indicates that diabetes-related metabolic abnormalities are the major contributors to the observed cardiac defects.³ Thus, increased non-esterified fatty acid (NEFA) fluxes resulting in myocardial triglyceride accumulation, the formation of toxic intermediates, mitochondrial dysfunction and oxidative stress have been implicated.³ Although NEFA are the preferred cardiac substrate under physiological conditions, the heart should be able to readily switch to glucose oxidation during stress or ischemia. Due to prolonged exposure to the abnormal metabolic environment the diabetic heart loses its metabolic flexibility. Consequently, the initially adaptive mechanism will transform into a maladaptive vicious circle leading to altered energy metabolism and contractile dysfunction.^{3,4} Mechanistic *in vivo* studies in humans are limited, but similar mechanisms have been proposed to underlie human diabetic cardiomyopathy.^{2,5-8}

By targeting lipotoxicity and insulin resistance, the blood-glucose lowering agent pioglitazone may favorably influence cardiac risk in T2DM.^{9,10} In the PROactive study, pioglitazone reduced cardiovascular disease in high-risk patients with T2DM.¹¹ However, while pioglitazone improved cardiac function in experimental diabetic cardiomyopathy,^{12,13} its use in patients may result in heart failure due to fluid retention.¹⁴ Inasmuch as the majority of patients in the PROactive study had CAD and longstanding diabetes, it is feasible that cardiac metabolic inflexibility may have contributed to the pioglitazone-related heart failures. Indeed, substrate manipulation in metabolic inflexible states like heart failure due to CAD decreased myocardial efficiency and cardiac function,¹⁵ revealing the close connection of metabolism and function in the compromised heart. At present, however, it is unknown whether interventions aimed at altering cardiac metabolism will lead to changes in function in the non-ischemic diabetes heart. We studied the effect of pioglitazone, versus metformin, on myocardial function, dimensions and perfusion, in association with cardiac glucose and fatty acid metabolism, as well as triglyceride content and high-energy phosphate metabolism, using magnetic resonance (MR) imaging and spectroscopy and positron emission tomography (PET). In order to avoid confounding by cardiac ischemia we performed the studies in patients with well-controlled T2DM of short duration and with verified absence of cardiac ischemia.

METHODS

Study design and patients

The PIRAMID study (Pioglitazone Influence on tRiglyceride Accumulation in the Myocardium In Diabetes) was a 24-week prospective randomized double-blind double-dummy with active comparator, 2-center parallel-group intervention. Males with uncomplicated T2DM, aged 45-65 years, were eligible. Inclusion criteria were a glycated hemoglobin level of 6.5-8.5% at screening, body-mass index (BMI; weight/[length]²) of 25-32 kg/m², blood pressure not exceeding 150/85 mmHg (with or without the use of anti-hypertensive drugs). Exclusion criteria were any clinically significant disorder, particularly any history or complaints of cardiovascular and liver disease or diabetes-related complications, prior use of thiazolidinediones or insulin. Written informed consent was obtained from all participants. The protocol was approved by the Medical Ethics Committee of both centers, and performed in full compliance with the Declaration of Helsinki.

Study procedures

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

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

weeks and underwent outcome measurements at baseline and at study termination as outlined below. They were requested to adhere to pre-study lifestyle and dietary habits throughout the study.

Cardiac magnetic resonance imaging protocol

MR assessments were performed after an overnight fast at one single site (Leiden), using a 1.5T whole-body MR scanner (Gyroscan ACS/ NT15; Philips, Best, the Netherlands). Before MR examinations, blood samples were collected to determine substrates and during MR imaging, blood pressure and heart rate were monitored. Rate pressure product (RPP) was calculated as the product of systolic blood pressure and heart rate. The entire heart was imaged in the short-axis orientation using ECG-gated breath-hold balanced steady-state free precession imaging.¹⁶ Measures of systolic function were left ventricular (LV) ejection fraction (EF) and cardiac index (CI = cardiac output/body surface area). As measures of cardiac dimensions LV end-diastolic volume (LVEDV), LV end-systolic volume (LVESV) and LV stroke volume (LVSV) were used. An ECG-gated gradient-echo sequence with velocity encoding was performed to measure blood flow across the mitral valve for the determination of LV diastolic function parameters, including peak filling rates of the early filling phase (E) and the atrial contraction (A) and their ratio (E/A). Also, the peak ($E\text{-dec}_{\text{peak}}$) and mean deceleration gradient of E ($E\text{-dec}_{\text{mean}}$) and E peak flow rate (EPFR) were calculated.¹⁶ LV filling pressures (E/E_a) were estimated.¹⁷ Images were analyzed quantitatively using dedicated software (MASS® and FLOW®, Medis, Leiden, the Netherlands).

Myocardial and hepatic proton magnetic resonance spectroscopy

Myocardial and hepatic proton (¹H)-MR spectroscopy were performed as described previously.^{16,18} Briefly, Myocardial ¹H-MR spectra were obtained from the interventricular septum carefully avoiding contamination from epicardial fat. Spectroscopic data acquisition was double triggered using electrocardiographical triggering and respiratory navigator echoes to minimize motion artifacts. Water-suppressed spectra were acquired to detect weak triglyceride signals and spectra without water suppression were acquired and used as an internal standard.¹⁶ ¹H-MR spectroscopic data were fitted using Java-based MR user interface software (jMRUI version 2.2, Leuven, Belgium), as described previously.¹⁸ Myocardial triglyceride content relative to water was calculated as (signal amplitude of lipid)/(signal amplitude of water) × 100.^{16,18} ¹H-MR spectroscopy of the liver was performed with an 8 ml voxel positioned in the liver, avoiding gross vascular structures and adipose tissue depots. The twelfth thoracic vertebra was used as a landmark to ensure the same voxel position during both visits. Sixty-four averages were collected with water suppression.¹⁸

Phosphorus magnetic resonance spectroscopy

A 100-mm-diameter surface coil was used to acquire ECG-triggered ^{31}P -MR spectra of the left ventricular anterior wall with subjects in the supine position. Volumes of interest were selected by image-guided spectroscopy with 3D-ISIS. Shimming was performed automatically and tuning and matching of the ^{31}P surface coil was performed manually. Technical details of data acquisition and spectral quantification were similar as described before.¹⁹ Shortly, spectroscopic volume size was typically $7 \times 7 \times 7$ cm. Acquisitions were based on 192 averaged free induction decays, and total acquisition time was 10 min. ^{31}P -MR spectra were quantified automatically in the time domain using prior spectroscopic knowledge and were corrected for partial saturation effects and for the adenosine-triphosphate (ATP) contribution from blood in the cardiac chambers. The phosphocreatine (PCr)/ATP ratios of the spectra were calculated and used as a parameter representing myocardial high-energy phosphate metabolism.²⁰

Positron emission tomography imaging protocol

PET examinations were performed after an overnight fast at a single center (Amsterdam) using an ECAT EXACT HR+ scanner (Siemens/ CTI, Knoxville, TN, USA). Patients received two venous catheters, one in the antecubital vein and one in the vein of the opposite hand, the latter being wrapped into a heated blanket to obtain arterialized blood. During procedures patients were monitored by telemetry and blood pressure was measured at 5-minute intervals. PET was used to measure myocardial blood flow (MBF) with H_2^{15}O , myocardial fatty acid uptake (MFAU), β -oxidation (MFAO) and esterification (MFAE) with ^{11}C -palmitate, and myocardial metabolic rate of glucose uptake (MMRglu) with ^{18}F -2-fluoro-2-deoxy-D-glucose (^{18}F FDG). Perfusion and NEFA metabolism were assessed in the fasting state, whereas MMRglu was measured during a euglycemic-hyperinsulinemic clamp (see below). Following a 10-min transmission scan, H_2^{15}O was injected ($t = 10$ min) and a 10-min dynamic emission scan (40 frames) was acquired. Subsequently, a 30-min dynamic emission scan (34 frames) was performed following ^{11}C -palmitate injection ($t = 35$ min). Hereafter, the clamp was started ($t = 65$ min), as described previously,²¹ to approximate an isometabolic steady state and measure whole-body insulin sensitivity. At steady state (around $t = 155$ min), following a new transmission scan, ^{18}F FDG was injected and a 60-min dynamic emission scan (40 frames) was acquired. Blood samples were collected during ^{11}C -palmitate and ^{18}F FDG scans at predefined time points to measure glucose, NEFA, lactate, lipids and insulin levels. In addition, $^{11}\text{CO}_2$ was measured during the ^{11}C -palmitate scan.⁸

Positron emission tomography image analysis

PET data were quantitatively reconstructed using filtered backprojection applying all appropriate corrections. In order to generate myocardial time-activity curves, regions of

interest (ROIs) were defined on resliced LV short-axis (summed) ^{11}C -palmitate and ^{18}F FDG images and subsequently projected onto the dynamic images. ROIs were drawn as previously described²² and grouped for further analysis. Myocardial segments exposed to liver spill-in were omitted from the analysis of ^{11}C -palmitate data. Additional ROIs were defined in left and right ventricular chambers for ^{11}C -palmitate and H_2^{15}O image-derived input functions (IDIF). A separate ascending aorta ROI was defined for ^{18}F FDG IDIF.

MBF was determined using the standard single-tissue compartment model.²³ ^{11}C -palmitate time-activity curves were analyzed using a three-tissue plasma input kinetic model, which, together with plasma NEFA concentrations, enabled calculation of MFAU, MFAO and MFAE. The ^{11}C -palmitate IDIF was corrected for $^{11}\text{CO}_2$ metabolites and difference between plasma and whole blood concentrations as described elsewhere.⁸ This model is similar to that described by Bergmann et al,²⁴ but with a reduced number of parameters, thereby increasing precision of derived estimates (see appendix). MMRglu was calculated by multiplying the net influx constant for ^{18}F FDG, K_i , by the mean plasma glucose concentration. For determination of K_i , Patlak graphical analysis was used.²⁵

Study endpoints

The primary endpoint was change in parameters of diastolic function, obtained with velocity encoded MR blood flow measurements across the mitral valve, as outlined above, from baseline to follow-up (24 weeks). Secondary efficacy measures included changes in cardiac dimensions, systolic function parameters and myocardial metabolism and perfusion variables as described above, as well as changes in hepatic and myocardial triglyceride content, BMI, blood pressure, glycated hemoglobin, plasma lipids and whole-body insulin sensitivity. Exploratory analyses included changes in the relation of LVEDV and estimates of LV filling pressure, including N-terminal probrain natriuretic peptide (NT-proBNP), the ratio of early diastolic velocity (E) and early diastolic tissue velocity (Ea) and high-energy phosphate metabolism (PCr/ATP ratio). Blood samples for endpoint measurements were analyzed at one central laboratory (Amsterdam).

Statistical analysis

Since at the time of study-design, no data were available regarding the effect of thiazolidinediones on MR-measured cardiac function, we based our sample-size calculations on previous MR studies.²

To detect a subtle 15% (standard deviation [SD] 20%) difference in cardiac diastolic function with 90% power, approximately 80 randomized patients were needed (primary endpoint). The sample-size for the PET measurements was based on available PET studies.⁵ We calculated that 60 randomized patients would be necessary to detect a difference of 20% (SD 25%; estimated drop-out rate 20%) in cardiac metabolism, with 80% power.

Values are reported as mean $\pm$ standard error (SE) or median (interquartile range [IQR]), when non-normally distributed. Between-group comparisons were done with analysis of covariance (ANCOVA) with adjustments for treatment group and baseline values. Within-group changes from baseline were assessed using paired T-tests or Wilcoxon signed-ranks tests. Correlations were calculated by Pearson's or Spearman's correlation analyses, as appropriate. All statistical tests were two-sided, significance was considered at the level of 0.05. Analyses were done with SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA).

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

RESULTS

Figure 11.1 shows the trial flowchart. At baseline, the study groups were well-matched (Tables 11.1 and 11.2). Glimepiride dose-adjustment was needed in four patients randomized to pioglitazone and in three assigned to metformin. Two patients required metformin back-titration. No clinically evident fluid retention or heart failure occurred during the study.

At 24 weeks, pioglitazone and metformin similarly improved glycemic control from baseline (Table 11.2). Pioglitazone versus metformin significantly increased HDL, whereas metformin decreased total and LDL-cholesterol levels (Table 11.2). Pioglitazone, but not metformin, induced weight-gain relative to baseline (from 91 ± 2 to 94 ± 2 kg; $P = 0.001$ versus 92 ± 2 to 92 ± 3 kg; $P = 0.136$; between-group $P < 0.001$). Both pioglitazone and metformin significantly improved whole-body insulin sensitivity by a median 35.1% and 29.6%, respectively. However, neither treatment affected fasting NEFA levels. Metformin increased, whereas pioglitazone decreased fasting lactate levels (Table 11.2). In both groups, similar decreases in systolic blood pressure and RPP were observed, whereas diastolic blood pressure and heart rate remained unchanged (Table 11.3).

At follow-up, pioglitazone improved diastolic function as measured by E-dec_{peak} ($P = 0.03$), E-dec_{mean} ($P = 0.08$) and the EPFR ($P = 0.07$; Table 11.3). Pioglitazone-treated patients showed an increase in LVEDV ($P = 0.045$), whereas NT-proBNP levels and E/Ea, both estimates of LV filling pressure, remained unchanged (Tables 11.2 and 11.3). In contrast to metformin, pioglitazone shifted the relations of LVEDV and estimates of LV end-diastolic pressure towards improved compliance (Figure 11.2 A/B). Metformin

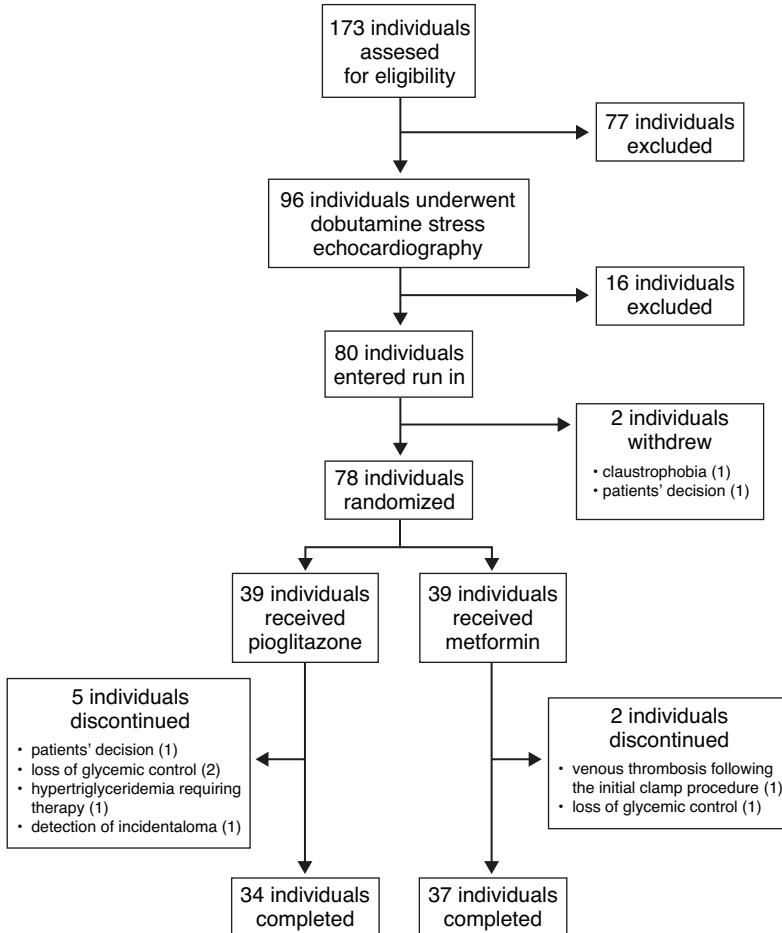


Figure 11.1
Trial profile.

had no significant effect on the diastolic cardiac parameters measured. Comparisons between groups of diastolic function parameters revealed only a significant difference in EPFR, while only a trend was observed for $E\text{-dec}_{\text{mean}}$ (Table 11.3). A significant between group difference in LVSV and LVCI was observed, whereas LVEF remained unaltered in both groups (Table 11.3).

PET examinations were successful in 54 subjects. At follow up, pioglitazone significantly increased, whereas metformin markedly decreased MMRglu from baseline (Figure 11.2 C/D). At 24 weeks, pioglitazone and metformin therapy did not significantly change MFAU from baseline, whereas only metformin significantly reduced MFAO (Figure 11.2 D). MFAE was negligible in both groups. However, increases from baseline were observed after pioglitazone and, to a lesser extent, after metformin therapy (Figures 11.2 C/D). These minor changes measured by PET were not detected by the $^1\text{H-MRS}$

Table 11.1 Patients characteristics at baseline*

	Pioglitazone (n = 39)	Metformin (n = 39)
Age (years)	56.8 ± 1.0	56.4 ± 0.9
Time since diagnosis of diabetes (years)	4 (3 - 6)	3 (1 - 5)
Current smoker (n)	10	7
Body mass index (kg/m ²)	28.2 ± 0.5	29.3 ± 0.6
Waist circumference (cm)	103.8 ± 1.5	104.9 ± 1.8
<i>Concomitant medication</i>		
Statin (n)	19	19
Any antihypertensive medication (n)	19	15
β-blocker (n)	5	2
Diuretic (n)	6	6
ACE inhibitor (n)	9	9
ARB (n)	6	3
Calcium antagonist (n)	1	3

Values are presented as means ± standard error, median (interquartile range) or number of patients (n).

* There were no statistically significant differences between treatment groups.

ACE = angiotensin-converting enzyme; ARB = angiotensin-receptor blocker.

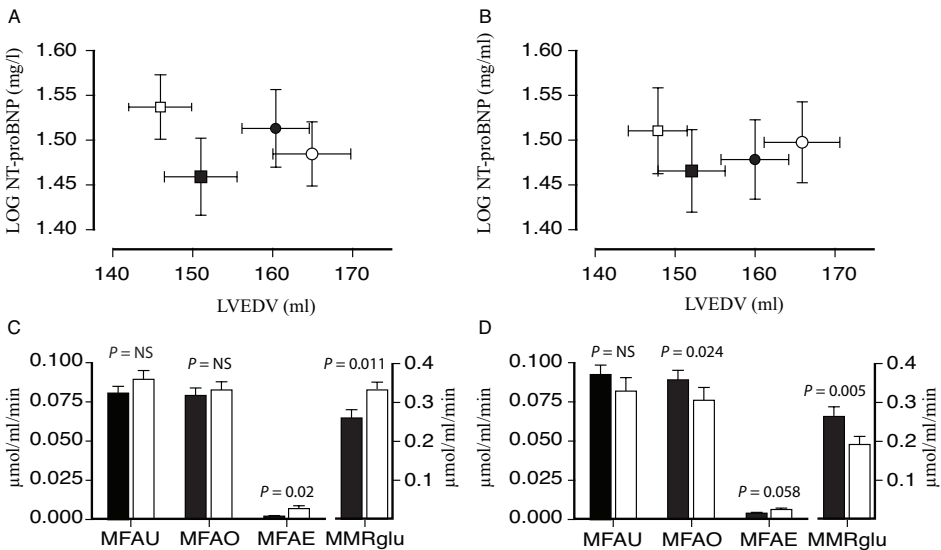


Figure 11.2

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

Table 11.2 Biochemical and metabolic characteristics and whole-body insulin sensitivity at baseline and after 24 weeks

	Pioglitazone		Metformin		P-value (between groups)
	Baseline	24 weeks	Baseline	24 weeks	
<i>Fasting</i>					
Glycated hemoglobin (%)	7.1 ± 0.2	6.5 ± 0.1*	7.0 ± 0.1	6.3 ± 0.1*	0.146
plasma glucose (mmol/l)	8.4 (7.2 - 10.3)	7.6 (6.7 - 9.4)*	8.2 (6.8 - 9.1)	6.8 (5.8 - 7.4)*	0.141
Non-esterified fatty acids (mmol/l)	0.45 (0.41 - 0.59)	0.46 (0.34 - 0.57)	0.53 (0.39 - 0.77)	0.49 (0.39 - 0.56)	0.933
Insulin (pmol/l)	58 (38 - 83)	49 (34 - 70)	80 (31 - 99)	59 (32 - 98)	0.151
Lactate (mmol/l)	1.2 (1.0 - 1.5)	1.0 (0.8 - 1.2)†	1.1 (1.0 - 1.5)	1.5 (1.2 - 1.8)*	0.001
Total cholesterol (mmol/l)	4.5 ± 0.1	4.6 ± 0.2	4.9 ± 0.2	4.5 ± 0.2*	0.042
Low density lipoprotein cholesterol (mmol/l)	2.5 ± 0.1	2.5 ± 0.1	2.9 ± 0.1	2.6 ± 0.2*	0.107
High density lipoprotein cholesterol (mmol/l)	1.07 (0.94 - 1.28)	1.23 (0.99 - 1.46)†	1.13 (0.90 - 1.42)	1.02 (0.86 - 1.26)	0.009
Triglycerides (mmol/l)	1.4 (1.0 - 2.2)	1.4 (0.9 - 2.3)	1.5 (0.9 - 2.1)	1.7 (0.9 - 2.3)	0.596
NT-proBNP (ng/l)	24 (20 - 38)	26 (19 - 40)	32 (18 - 43)	33 (20 - 43)	0.505
<i>During hyperinsulinaemia</i>					
Non esterified fatty acids (mmol/l)	0.07 (0.05 - 0.13)	0.04 (0.02 - 0.05)*	0.09 (0.04 - 0.16)	0.06 (0.03 - 0.14)†	0.036
Insulin (pmol/l)	572 (503 - 620)	521 (447 - 590)†	614 (540 - 710)	520 (472 - 601)*	0.292
Lactate (mmol/l)	1.1 (1.0 - 1.3)	1.1 (1.0 - 1.2)	1.0 (0.9 - 1.3)	1.4 (1.2 - 1.7)*	0.001
M/I value ([mg/kg × min]/[pmol/l])	0.46 (0.28 - 0.73)	0.54 (0.43 - 0.97)†	0.45 (0.19 - 0.80)	0.58 (0.35 - 1.00)†	0.501

Values are mean ± standard error or median (interquartile range).

* $P < 0.001$, † $P < 0.01$, ‡ $P < 0.05$ for within group difference.

M/I value = whole body insulin sensitivity adjusted during the steady state; NT-proBNP = N-terminal probrain natriuretic peptide.

measurements since myocardial triglyceride content remained unchanged in both groups (pioglitazone: 0.77 ± 0.05 vs. $0.82 \pm 0.07\%$; $P = 0.356$; metformin: $0.87 \pm 0.08\%$ vs. $0.89 \pm 0.07\%$, $P = 0.869$, between-group $P = 0.774$). In contrast, pioglitazone but not metformin decreased liver fat content (pioglitazone: 5.9 (2.6 - 17.4) vs. 4.1 (1.9 - 12.3)%; $P < 0.001$; metformin: 7.7 (3.7 - 23.9) vs. 10.7 (5.1 - 22.0)%, $P = 0.209$, between-group $P < 0.001$). PCr/ATP was successfully obtained on both study occasions from 22 patients ($n = 13$ in the pioglitazone group) and was similar at baseline in both groups and not influenced by either treatment (pioglitazone: 2.02 ± 0.06 vs. $1.99 \pm$

Table 11.3 Hemodynamic parameters and cardiac dimensions and function at baseline and after 24 weeks

	Pioglitazone		Metformin		P-value (between groups)
	Baseline	24 weeks	Baseline	24 weeks	
<i>Hemodynamics</i>					
Systolic blood pressure (mmHg)	130 ± 2	125 ± 2*	126 ± 2	121 ± 2*	0.486
Diastolic blood pressure (mmHg)	77 ± 1	74 ± 1	74 ± 1	73 ± 1	0.971
Heart rate (beats/min)	65 ± 1	63 ± 1	65 ± 1	64 ± 1	0.904
Rate pressure product (beats/min) × mmHg	8508 ± 256	7853 ± 195*	8206 ± 215	7744 ± 193†	0.771
<i>Cardiac function and dimensions</i>					
LV mass (g)	108 ± 2	105 ± 3	107 ± 3	103 ± 3	0.542
LV end-systolic volume (ml)	66 ± 3	66 ± 3	60 ± 2	59 ± 2	0.911
LV end-diastolic volume (ml)	160 ± 4	166 ± 5*	152 ± 4	148 ± 4	0.003
LV stroke volume (ml)	94 ± 3	99 ± 3*	92 ± 3	89 ± 2	0.001
Ejection fraction (%)	59 ± 1	60 ± 1	61 ± 1	60 ± 1	0.533
Cardiac index ((l/min) × m ²)	2.9 ± 0.1	2.9 ± 0.1	2.9 ± 0.1	2.7 ± 0.1	0.008
E peak filling rate (ml/s)	422 ± 15	440 ± 14	409 ± 14	407 ± 13	0.047
E deceleration peak (ml/s ² × 10 ³)	3.5 ± 0.2	3.8 ± 0.2*	3.5 ± 0.2	3.5 ± 0.2	0.106
E deceleration mean (ml/s ² × 10 ³)	2.3 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	0.064
E/A peak flow	1.07 ± 0.05	1.09 ± 0.05	1.01 ± 0.04	1.01 ± 0.03	0.348
E/Ea	9.2 (7.4 - 11.4)	9.1 (6.6 - 12.0)	9.3 (6.3 - 12.3)	10.3 (8.3 - 11.8)	0.254

Values are means ± standard error.

* $P < 0.05$, † $P < 0.01$ for within group difference.

LV = left ventricular; E = early diastolic filling phase; A = diastolic atrial contraction; E/Ea = estimate of the left ventricular filling pressure.

0.11; $P = 0.792$; metformin: 2.19 ± 0.10 vs. 2.04 ± 0.07 , $P = 0.552$, between-group $P = 0.976$). PCr/ATP of the remaining participants could not be collected at both baseline and follow-up, or appeared of insufficient spectral quality (Cramér-Rao standard deviation (rCRSD) >20%). MBF did not change significantly after treatment (between-group $P = 0.254$).

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

DISCUSSION

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

Thiazolidinedione-related improvements in cardiac diastolic function were reported by some,^{26,27} but not by others.^{28,29} These contrasting findings may be due to differences in study populations, severity and duration of diabetes, co-morbid conditions including pre-existent cardiac dysfunction and CAD, medication use and the use of echocardiography versus MRI in small populations. The relatively normal cardiac function at baseline may explain the seemingly modest, but clinically relevant, improvements in our patients: the pioglitazone-related increase in LVEDV at similar estimates of LV filling pressure is compatible with an improved LV diastolic compliance.^{30,31} These data are in line with earlier findings in diabetic rats, showing that pioglitazone improved diastolic function by reducing myocardial collagen content and by favorably affecting matrix remodeling.^{12,32} Only pioglitazone increased LVSV, as reported by others, possibly due to a decrease in peripheral resistance.³³ Metformin tended to decrease LVCI, compatible with the reported metformin-related effects on cardiac sympathovagal balance.³⁴

The present study is timely in the light of the ongoing debate regarding the safety profile of thiazolidinediones.^{35,36} During this short-term trial we observed no cardiac events nor heart failure. In the PROactive population, the majority of which had a history of CAD, pioglitazone use was associated with an increased risk of heart failure. Our data indicate that when pioglitazone is used in patients with uncomplicated T2DM without cardiac ischemia, it may reverse the process of cardiac concentric remodeling, which is among the hallmarks of diabetic cardiomyopathy, by shifting the LV end-diastolic pressure-volume relation towards improved compliance. However, it is conceivable that in patients with compromised hearts, in particular those with (ischemic) dilated cardiomyopathy, pioglitazone may actually promote the risk of overt heart failure.

Both treatments induced significant, albeit differential changes in cardiac substrate metabolism. Pioglitazone increased MMRglu which may be due to the simultaneous reduction of competing substrates, in particular NEFA, but also by direct enhancement of myocardial insulin signaling and expansion of the available pool and translocation of GLUT-4 receptors in the heart.²⁹ Metformin significantly decreased MMRglu and MFAO and these changes were paralleled by an increase in plasma lactate, whereas NEFA levels decreased during hyperinsulinemia and remained unchanged during fasting. Others

showed a trend towards MMRglu decline but no changes in lactate levels in 9 patients after 26-week metformin therapy.³⁷ The normal human heart may be regarded as a metabolically flexible omnivore that utilizes the most energy-efficient substrate available. Although NEFA are the preferential substrate due to the highest ATP yield, during stress, increased workload and ischemia, the heart can switch to energetically more favorable substrates, including glucose and lactate.³ Inasmuch as myocardial lactate uptake is directly proportional to circulating lactate levels,³⁸ cardiac lactate utilization is likely to be increased in metformin-treated patients. However, this assumption may need confirmation by PET using a lactate-tracer.³⁸ Interestingly, metformin therapy was previously shown to increase skeletal muscle lactate oxidation by 25% in T2DM.³⁹

Unexpectedly and contrary to findings from animal studies, we found no association between treatment-related cardiac functional and metabolic changes. Few and partly conflicting data exist regarding NEFA uptake/ utilization in the human (pre)diabetic heart and its relation to cardiac function.^{40,41} Based on animal studies, it was proposed that the diabetic heart primarily relies on the abundantly supplied NEFA in the presence of myocardial insulin resistance.⁴² Chronically elevated NEFA utilization may lead to impaired β -oxidation, accumulation of toxic intermediates, production of reactive oxygen species and mitochondrial dysfunction and finally, to cardiac functional abnormalities. Because glucose- relative to NEFA-oxidation requires less oxygen per mole of ATP produced, therapies enhancing myocardial glucose utilization, including insulin and thiazolidinediones, have been advocated in T2DM patients with cardiac ischemia. However, it is unknown whether enforced myocardial glucose use is beneficial at all occasions.

Similarly, rosiglitazone increased MMRglu in T2DM patients with CAD, without affecting echocardiographically measured function.²⁹ Additionally, indirect stimulation of myocardial glucose metabolism by acute deprivation of NEFA by acipimox in heart failure patients resulted in depressed cardiac work and efficiency.¹⁵ These findings support the notion that compromised hearts may lose their flexibility to respond to imposed changes in substrate availability. In contrast, it is likely that the myocardium of patients with uncomplicated T2DM of short duration still possesses sufficient metabolic flexibility and oxidative capacity to benefit from NEFA as the preferential myocardial substrate. Forced glucose utilization in these patients will not necessarily lead to improved cardiac function. As changes in myocardial metabolism and function in the pioglitazone-treated patients were not related, it is not clear if the improvement in diastolic function originates from altered metabolism. Pioglitazone decreased liver but not myocardial triglyceride content, indicating differential regulation of various body lipid compartments.

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

In conclusion, only pioglitazone improved diastolic function, whereas both pioglitazone and metformin altered myocardial substrate metabolism, likely due to treatment-specific changes in plasma substrate levels. Treatment with pioglitazone in patients with uncomplicated well-controlled T2DM and absence of cardiac ischemia might be beneficial to the heart, as demonstrated by the improved diastolic function and LV compliance.

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APPENDIX

For ^{11}C -palmitate kinetic analysis, a modification of the three tissue plasma input model as proposed by Schelbert ¹ and Bergmann ² was used which is shown in Figure 11.X. The first tissue compartment is the cytosol and the third is the mitochondrion. The second compartment describes the slow turnover pool of esterified ^{11}C -palmitate. A total of three rate constants between the compartments were used. The first, k_{p1} , simply reflects myocardial perfusion and capillary permeability for ^{11}C -palmitate. This perfusion phase is followed by two elimination phases. The first elimination phase is considered to represent beta-oxidation, k_{13} , and it is clinically the most important. The parameter k_{12} mainly reflects esterification into a slow turnover pool.³ Spill-over of activity from the left ventricle pool into the myocardium was also included in the model.

The model is an optimized trade-off between detail in tracer physiology and accuracy of parameter estimation. To this end, back-diffusion of unaltered ^{11}C -Palmitate (k_{1p}) was omitted since it mathematically cannot be estimated independently from the parallel oxidation-path (k_{13} , k_{3p}). Furthermore, the transfer rate from the esterification pool back to the cell k_{21} was fixed to zero based on earlier findings that this rate is orders of magnitude smaller than the influx k_{12} in the pool.^{3,4} Finally, k_{3p} was fixed equal to k_{13} , based on the assumption that no $^{11}\text{CO}_2$ is accumulated in the cell.

As input to the model, ^{11}C -palmitate concentrations were determined by correcting venous whole-blood samples for plasma/ whole blood concentration ratios and $^{11}\text{CO}_2$ levels.

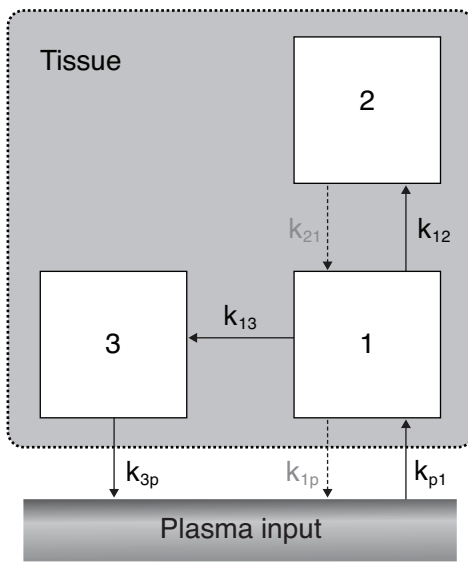


Figure 11.X

Modified Bergmann model for myocardial ^{11}C -palmitate kinetics.

Oxidation and esterification were described using mathematical indices MFAO and MFAE that can directly be calculated from the model ^{3,4}: $MFAO = C_{NEFA} \times k_{p1} \times k_{13} / (k_{12} + k_{13})$, $MFAE = C_{NEFA} \times k_{p1} \times k_{12} / (k_{12} + k_{13})$, where C_{NEFA} is the plasma fatty acid concentration [mmol/ml]. The total fatty acid utilization MFAU was defined as the sum of MFAO and MFAE.

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Summary and conclusions

Samenvatting en conclusies

List of publications

Acknowledgements

Curriculum vitae

Summary and conclusions

The aim of this thesis was to obtain more insight into the influence of myocardial steatosis on left ventricular function in healthy volunteers and in patients with type 2 diabetes mellitus using magnetic resonance imaging and spectroscopy techniques.

Chapter 1 provides general background information about myocardial steatosis which may play a role in the development of diabetic cardiomyopathy.

Chapter 2 is a review on the current status of cardiovascular molecular imaging. Magnetic resonance techniques that have already shown potential are discussed. Metabolic magnetic resonance imaging can be performed by ^{31}P -magnetic resonance spectroscopy, ^{23}Na -magnetic resonance spectroscopy, and ^1H -magnetic resonance spectroscopy; some examples are illustrated. Furthermore, a concise overview is given of several non-specific and specific contrast agents for cardiovascular molecular magnetic resonance imaging, such as gadolinium-based contrast agents, iron oxide contrast agents and fibrin-targeted contrast agents.

Chapter 3 describes the reproducibility of myocardial triglyceride content assessment by ^1H -magnetic resonance spectroscopy with and without the use of respiratory motion compensation based on navigator echoes. In 20 healthy volunteers, both techniques were used to acquire ^1H -magnetic resonance spectroscopic data of the myocardial septum. This study shows that respiratory navigator gated and electrocardiographically triggered ^1H -magnetic resonance spectroscopy of the human heart to assess myocardial triglycerides performed substantially better in terms of spectral resolution and reproducibility than electrocardiographically triggered ^1H -magnetic resonance spectroscopy without respiratory motion correction. Therefore, respiratory motion correction is essential for reproducible assessment of myocardial triglyceride content.

Chapter 4 evaluates the influence of a short-term very low calorie diet in healthy subjects on myocardial and hepatic triglyceride accumulation and on myocardial function. Fourteen healthy male volunteers underwent magnetic resonance scanning in the afternoon on two different occasions. Before both visits, they were instructed to follow one of two different dietary regimes for 3 days before the measurements. In the first regime, each subject used his normal diet, and this dietary condition was used for the collection of baseline data. During the second regime, all subjects consumed a very low calorie diet

consisting of 471 kcal, 50.2 g carbohydrates, and 6.9 g fat (0.94 g saturated fat) per day. The short-term very low calorie diet raised plasma non-esterified fatty acid levels and induced significant accumulation of myocardial triglycerides, paralleled by a decrease in left ventricular diastolic function, but without alterations in myocardial high-energy phosphate metabolism. In addition, hepatic triglyceride content was significantly reduced by a short-term very low calorie diet. Therefore, this study documents diet-dependent physiological variations in myocardial and hepatic triglyceride content and in left ventricular diastolic function in healthy subjects.

Chapter 5 extends the findings described in chapter 4 by assessing the influence of complete caloric restriction on myocardial and hepatic triglyceride accumulation and on myocardial function. Ten healthy subjects, who also participated in the study described in chapter 4 additionally underwent 3-days of starvation (only ingestion of water was allowed). Magnetic resonance spectroscopy and imaging showed that complete starvation further increased myocardial triglyceride content, paralleled by further decrease of left ventricular diastolic function. Complete starvation did not influence hepatic triglyceride content. Therefore, this study shows that progressive caloric restriction induced a dose dependent increase in myocardial triglyceride content accompanied by a dose dependent decrease in left ventricular diastolic function in lean healthy men. Hepatic triglyceride content showed a differential response to progressive caloric restriction, indicating that redistribution of endogenous triglyceride stores is tissue specific.

Chapter 6 describes the adaptations of the liver and the heart to a short-term high-energy, high-fat diet in healthy volunteers. This study was designed to further elucidate the understanding of the response of triglyceride depositions in non-adipose tissues to differences in dietary calorie and fat intake. Fifteen healthy male volunteers underwent magnetic resonance scanning in the afternoon on two different occasions. Before both visits, they were instructed to follow one of two different dietary regimes for 3 days before the measurements. In the first regime, each subject used his normal diet. Mean intake was approximately 2100 kcal/day. The calories were approximately divided as follows: carbohydrates 40%, fat 35%, protein 25%. This reference diet was used for the collection of baseline data. During the second regime, each subject used a high-fat, high-energy diet consisting of the same intake as the reference diet, complemented with 800ml of cream every day.

During the high-fat, high-energy diet, total energy intake was approximately 4732 kcal/day with the calories divided as follows: carbohydrates 20%, fat 69%, protein 11%. The short-term high-fat, high-energy diet resulted in major increases in plasma triglyceride and non-esterified fatty acid concentrations accompanied by an increase in hepatic triglyceride content, whereas it did not influence myocardial triglyceride content or left

ventricular function. These observations indicate differential, tissue-specific distribution of triglycerides and/ or fatty acids among non-adipose organs during high-fat, high-energy containing diets.

Chapter 7 reveals that myocardial triglyceride content is an independent predictor of left ventricular diastolic function in the physiologically ageing heart. In 43 healthy male subjects varying in age from 20-66 years, ¹H-magnetic resonance spectroscopy and imaging were performed. In these volunteers, age was significantly correlated to myocardial triglyceride content. Furthermore, myocardial triglyceride content correlated to the age-related decline in left ventricular diastolic function, independently of body mass index, blood pressure, and biochemical blood markers.

Although a causal relationship between increased myocardial triglyceride content and reduced left ventricular function could not be demonstrated by the techniques used, this chapter supports the evidence that the processes underlying myocardial triglyceride accumulation might be harmful to the human heart. Therefore, physiological triglyceride accumulation may become important in the ageing heart by increasing susceptibility to lipotoxicity in otherwise healthy individuals.

Chapter 8 describes the effects of a short-term very low calorie diet in 11 patients with well-controlled type 2 diabetes mellitus without evidence of myocardial ischemia. The very low calorie diet was consumed both with and without co-administration of the anti-lipolytic drug acipimox.

In this patient group, short-term caloric restriction increased plasma non-esterified fatty acid levels. In addition, myocardial triglyceride content increased by approximately 48%. This increase in myocardial triglyceride content was accompanied by a decrease in left ventricular diastolic function. A very low calorie diet combined with acipimox had no effects on plasma non-esterified fatty acid levels, myocardial triglyceride content, or left ventricular function. This supports the notion that there is a relationship between increased fatty acid fluxes from adipose tissue, myocardial triglyceride stores, and left ventricular function.

Chapter 9 shows the potential value of an integrated cardiovascular magnetic resonance imaging protocol for comprehensive evaluation of aortic and cardiac function in patients with type 2 diabetes mellitus. Fourteen patients with well-controlled, uncomplicated type 2 diabetes mellitus, and 16 age and gender matched healthy subjects were included. Aortic stiffness was determined by assessing aortic pulse wave velocity and aortic distensibility using magnetic resonance imaging. In addition, left ventricular systolic and diastolic function were assessed.

The main finding in this chapter is that aortic stiffening was significantly increased in patients compared to healthy subjects. In addition, aortic distensibility correlated with left ventricular diastolic function. These observations are similar to those found using other imaging modalities in previous studies that required larger sample sizes due to their lower reproducibility as compared to magnetic resonance imaging techniques.

In **Chapter 10**, myocardial triglyceride content and left ventricular function were measured in 38 patients with type 2 diabetes mellitus patients and 28 healthy age- and body mass index matched volunteers by proton magnetic resonance spectroscopy and imaging, respectively. In the patient group, myocardial triglyceride content was significantly higher than in healthy volunteers. Systolic function did not significantly differ from that in healthy subjects, whereas indices of diastolic function, including left ventricular E/A ratio and E peak deceleration, were significantly impaired in patients suffering from type 2 diabetes mellitus. Multivariable analysis indicated that myocardial triglyceride content was associated with E/A ratios and E peak decelerations, independently of age, heart rate, body mass index, and diastolic blood pressure.

In the future, therapeutic strategies that target reduction of cardiac lipid overexposure might be beneficial to prevent diabetic cardiomyopathy.

Chapter 11 evaluates the effects of pioglitazone treatment in patients with uncomplicated type 2 diabetes mellitus. For this purpose, 78 males with type 2 diabetes mellitus without evidence of cardiac ischemia were randomly assigned to pioglitazone (30 mg daily) or metformin (2000 mg daily) and matching placebo during 24 weeks. The primary endpoint was change from baseline in left ventricular diastolic function relative to myocardial metabolic changes measured by magnetic resonance imaging, proton magnetic resonance spectroscopy and positron-emission tomography using ^{18}F -2-fluoro-2-deoxy-D-glucose and ^{11}C -palmitate. Furthermore, whole-body insulin sensitivity and myocardial and hepatic triglyceride content were assessed.

Both therapies similarly improved glycemic control and whole-body insulin sensitivity. In addition, pioglitazone had beneficial cardiac effects in this patient group, as evidenced by improved left ventricular compliance and increased myocardial glucose uptake. Moreover, pioglitazone induced a reduction in hepatic triglyceride content, whereas metformin did not affect myocardial function or hepatic triglyceride content. Therefore, pioglitazone may have beneficial cardiac effects in patients with type 2 diabetes mellitus without myocardial ischemia as evidenced by improved left ventricular compliance.

Samenvatting en conclusies

Het doel van dit proefschrift was om meer inzicht te verkrijgen in de invloed van hartspiervervetting op de linker ventrikel functie van gezonde vrijwilligers en van patiënten met type 2 diabetes mellitus met behulp van kernspinresonantie technieken (magnetic resonance spectroscopy [MRS] en imaging [MRI]).

Hoofdstuk 1 geeft algemene achtergrondinformatie over hartspiervervetting. Deze vervetting lijkt een rol te spelen bij de pathogenese van diabetische cardiomyopathie.

Hoofdstuk 2 bespreekt de huidige stand van zaken in cardiovasculaire beeldvorming met betrekking tot het in beeld brengen van moleculaire processen (moleculaire imaging). MRS en MRI, technieken die een belangrijke rol kunnen spelen op het gebied van moleculaire imaging, worden beschreven. Metabole MRS technieken zijn ^{31}P -MRS, ^{23}Na -MRS en ^1H -MRS; enkele toepassingen van deze technieken worden toegelicht. Tevens wordt een overzicht gegeven van verschillende aspecifieke en specifieke contrastmiddelen die ontwikkeld zijn voor cardiovasculaire moleculaire imaging zoals contrastmiddelen op basis van gadolinium of ijzeroxide en van contrastmiddelen die met fibrine een verbinding aangaan.

Hoofdstuk 3 beschrijft de reproduceerbaarheid van ^1H -MRS om hartspiervervetting te bepalen, met en zonder het gebruik van compensatie voor de beweging van het hart die ontstaat door de ademhaling. Beide technieken werden uitgevoerd bij 20 gezonde vrijwilligers om ^1H -MR spectra van het interventriculaire septum te verkrijgen. Deze studie laat zien dat ^1H -MRS van het hart met behulp van compensatie voor beweging die wordt veroorzaakt door de ademhaling een significant betere spectrale resolutie en reproduceerbaarheid oplevert dan ^1H -MRS zonder deze compensatie. Compensatie voor beweging veroorzaakt door de ademhaling is dus noodzakelijk voor reproduceerbare bepalingen van hartspiervervetting met behulp van proton spectroscopie.

Hoofdstuk 4 evalueert de effecten van een kortdurend zeer laag calorisch dieet in gezonde vrijwilligers op hartspier- en leververvetting en op hartfunctie. Op twee verschillende momenten werd een MRI/ MRS scan gemaakt van 14 gezonde mannelijke vrijwilligers. Voor ieder bezoek gebruikten de vrijwilligers gedurende 3 dagen een bepaald dieet. Na het eerste dieet werden de uitgangswaarden van de verschillende studie parameters bepaald. Dit dieet bestond uit het normale dieet van de vrijwilliger. Het tweede

dieet bestond uit een zeer laag calorisch dieet van 471 kcal, 50.2 gram koolhydraten en 6.9 gram vet (0.94 gram verzadigd vet) per dag. Als gevolg van het kortdurende zeer laag calorisch dieet stegen zowel de vrije vetzuren in het bloed als het vetgehalte van de hartspeer. Tegelijkertijd nam de diastolische linker ventrikel functie af, terwijl er geen veranderingen werden gezien in het hoogenenergetisch fosfaat metabolisme. Tevens nam het vetgehalte van de lever af tijdens het kortdurende zeer laag calorisch dieet. Uit deze studie blijkt dat er bij gezonde personen fysiologische variaties bestaan in zowel de vetgehalten van hart en lever als in diastolische linker ventrikel functie.

Hoofdstuk 5 vult de bevindingen uit hoofdstuk 4 aan en beschrijft de invloed van volledige calorierestrictie op het vetgehalte van de hartspeer en de lever en op hartfunctie. Tien gezonde vrijwilligers, die ook hadden deelgenomen aan de studie beschreven in hoofdstuk 4, gebruikten nog een derde dieet gedurende 3 dagen dat bestond uit volledig vasten (alleen het gebruik van water was toegestaan). Met behulp van MRS en MRI technieken werd aangetoond dat volledig vasten het vetgehalte in de hartspeer verder verhoogde, waarbij de diastolische linker ventrikel functie verder afnam. Het vetgehalte in de lever werd niet beïnvloed door volledig vasten. Deze studie laat zien dat toenemende calorierestrictie in gezonde mannen een dosis-afhankelijke toename van het vetgehalte van het hart induceert, terwijl de diastolische functie dosis-afhankelijk afneemt. Het vetgehalte van de lever liet een andere reactie op toenemende calorierestrictie zien dan het vetgehalte van het hart, wat impliceert dat redistributie van endogene triglyceriden weefsel-specifiek is.

Hoofdstuk 6 beschrijft de reacties van de lever en het hart op een kortdurend hoogenenergetisch, hoog vet dieet in gezonde vrijwilligers. Deze studie is opgezet om de reactie van vetdeeltjes in hart- en levercellen op verschillende hoeveelheden calorieën en vet in de voeding te vergroten. Op twee verschillende momenten werd een MRI/ MRS scan gemaakt van 15 gezonde mannelijke vrijwilligers. Voor ieder bezoek gebruikten de vrijwilligers gedurende 3 dagen een bepaald dieet. Het eerste dieet bestond uit het normale dieet van de vrijwilliger. Tijdens dit dieet was de gemiddelde energie-inname ongeveer 2100 kcal/dag. De calorieën werden ongeveer voor 40% geleverd door koolhydraten, voor 35% door vetten en voor 25% door eiwitten. Na dit dieet werden de uitgangswaarden van de verschillende studie parameters bepaald. Tijdens het tweede dieet gebruikte iedere vrijwilliger een hoogenenergetisch, hoog vet dieet, dat bestond uit het normale dieet van de vrijwilliger, aangevuld met 800 ml slagroom per dag. Tijdens dit hoogenenergetisch, hoog vet dieet was de gemiddelde energie-inname ongeveer 4732 kcal/dag, waarbij de calorieën voor ongeveer 20% door koolhydraten werden geleverd, voor 69% door vetten en voor 11% door eiwit. Het hoogenenergetisch, hoog vet dieet induceerde forse stijgingen van het vetgehalte in het bloed en in de lever, maar

had geen invloed op hartspiervervetting of op hartfunctie. Deze bevindingen wijzen op weefsel-specifieke verdeling van vetten in hart en lever gedurende een hoogenergetisch, hoog vet dieet.

Hoofdstuk 7 toont aan dat de mate van hartspiervervetting samenhangt met diastolische linker ventrikel functie tijdens fysiologische veroudering. Bij 43 gezonde mannelijke vrijwilligers, variërend in leeftijd van 20-66 jaar, werd ¹H-MRS en MRI uitgevoerd. Leeftijd was significant geassocieerd met hartspiervervetting in deze groep. Tevens was hartspiervervetting gecorreleerd met achteruitgang in diastolische linker ventrikel functie, onafhankelijk van de body mass index, bloeddruk en biochemische bloedwaarden. Een causaal verband tussen hartspiervervetting en afgenomen linker ventrikel functie kan met de technieken die in dit hoofdstuk gebruikt zijn niet worden aangetoond. Desalniettemin ondersteunt dit hoofdstuk de hypothese dat de onderliggende processen van hartspiervervetting schadelijk zijn voor het hart. Hartspiervervetting als gevolg van het ouder worden is een mogelijke verklaring voor fysiologische achteruitgang van linker ventrikel functie op oudere leeftijd .

Hoofdstuk 8 beschrijft de effecten van een kortdurend zeer laag calorisch dieet bij 11 patiënten met goed gereguleerde type 2 diabetes mellitus zonder onderliggende ischemie van de hartspier. Iedere patiënt gebruikte het zeer laag calorisch dieet; zowel met als zonder de toevoeging van het geneesmiddel acipimox. Acipimox verlaagt de bloedspiegel van vrije vetzuren.

In de geselecteerde patiëntengroep waren de vrije vetzuren in het bloed gestegen na het zeer laag calorisch dieet. Tegelijkertijd steeg het vetgehalte in de hartspier met ongeveer 48%. Parallel aan deze stijging in vetgehalte van de hartspier nam de diastolische linker ventrikel functie af. Het zeer laag calorisch dieet, gecombineerd met acipimox, had geen effecten op het gehalte vrije vetzuren in het bloed, op het vetgehalte in de hartspier of op de diastolische linker ventrikel functie. Dit experiment ondersteunt de hypothese dat er een relatie bestaat tussen verhoogde lipolyse in het vetweefsel, hartspiervervetting en hartfunctie.

Hoofdstuk 9 laat de waarde zien van een MRI protocol waarmee zowel de functie van de aorta als de hartfunctie kan worden bepaald van patiënten met type 2 diabetes mellitus. Veertien patiënten met goed gereguleerde, ongecompliceerde type 2 diabetes mellitus en 16 gezonde vrijwilligers van dezelfde leeftijd en geslacht werden bestudeerd. De stijfheid van de aorta werd bepaald met behulp van MRI door het meten van de "pulse wave velocity" en de distensibiliteit van de aorta. In aanvulling hierop werd de systolische en diastolische functie van de linker ventrikel bepaald.

De belangrijkste bevinding in dit hoofdstuk is dat de aorta van patiënten significant stijver is in vergelijking met de aorta van gezonde vrijwilligers. Tevens correleerde de distensibiliteit van de aorta met diastolische linker ventrikel functie. Vanwege de goede reproduceerbaarheid van MRI bevestigen deze bevindingen de resultaten die zijn behaald in eerdere studies met grotere aantallen patiënten waarin minder accurate meetmethodes werden gebruikt.

In **Hoofdstuk 10** werden hartspiervervetting en hartfunctie van 38 patiënten met type 2 diabetes mellitus en 28 gezonde vrijwilligers met een gelijke leeftijd en Quetelet index bepaald met ^1H -MRS en MRI. Bij patiënten was er significant meer hartspiervervetting zichtbaar in dan bij gezonde vrijwilligers. De systolische linker ventrikel functie van patiënten was niet significant verschillend van die van gezonde vrijwilligers, terwijl verschillende maten van diastolische linker ventrikel functie, waaronder de E/A ratio en E deceleratie, significant lager waren bij patiënten met type 2 diabetes mellitus. Statistische analyse toonde aan dat hartspiervervetting geassocieerd was met E/A en E deceleratie, onafhankelijk van de leeftijd, de hartslag, de Quetelet index en diastolische bloeddruk.

Deze parameters kunnen in de toekomst mogelijk gebruikt worden voor het vervolgen van therapie effecten.

Hoofdstuk 11 bestudeert de effecten van behandeling met pioglitazon bij patiënten met ongecompliceerde type 2 diabetes mellitus. Achtenzeventig patiënten met type 2 diabetes mellitus zonder aanwijzingen voor ischemie van het hart werden behandeld met pioglitazon (30 mg per dag) of metformine (2000 mg per dag) en een placebo gedurende 24 weken. Het primaire eindpunt van de studie was de verandering van diastolische linker ventrikel functie ten opzichte in relatie tot metabole veranderingen in de hartspeer. Om dit te meten werden MRI, MRS en PET technieken gebruikt. Ook werden de insuline gevoeligheid, leververvetting en hartspiervervetting van de patiënten bepaald. Beide therapieën verbeterden de glucoseregulatie en insuline gevoeligheid in dezelfde mate. Pioglitazon had tevens een gunstig effect op het hart, wat tot uitdrukking kwam in een betere linker ventrikel compliantie en verhoogde glucose opname door de hartspeer. Pioglitazon therapie induceerde ook een afname van de leververvetting. Metformine had geen effect op linker ventrikel functie of leververvetting. Concluderend: bij patiënten met type 2 diabetes mellitus heeft pioglitazon therapie mogelijk gunstige effecten op het hart.

List of publications

PAPERS

Van der Meer RW, Rijzewijk LJ, De Jong HWAM, Lubberink M, Romijn JA, Bax JJ, De Roos A, Kamp O, Paulus WJ, Heine RJ, Lammertsma AA, Smit JWA, Diamant M.
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Hepatic and myocardial triglyceride content, assessed by ^1H -MRS, have a short term flexibility, but show opposite changes during short term low calorie diet in healthy subjects.
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Moderated poster

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Curriculum vitae

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