Magnetic resonance of the diabetic heart
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 ± SE, baseline] to 0.98 ± 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 lipoapoptosis 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 \(^1\)\(^2\)\(^-\)\(^4\)\(^,\)\(^13\)\(^-\)\(^14\) 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 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 ± SD age: 57.6 ± 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.\textsuperscript{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.

\textbf{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.\textsuperscript{14}

\textbf{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 (Gyroscan 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.\textsuperscript{18} In short, an echo time (TE) of 26 ms and a repetition time (TR) of
Short-term flexibility of myocardial triglycerides and diastolic function in patients with type 2 diabetes mellitus

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. 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 × 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 procession sequence with breath-holds (1 for each slice). Imaging parameters included a field-of-view (FOV) of 400 × 320 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. 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. 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. Therefore, we measured blood flow across the mitral valve with an ECG-gated gradient-echo sequence with velocity encoding (Venc). Imaging parameters were: TE = 5 ms, TR = 14 ms, flip-angle = 20°, slice thickness = 8 mm, FOV = 350 × 350 mm, matrix size = 256 × 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).

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 ± 0.09%. After the VLCD myocardial triglyceride content increased to 0.98 ± 0.16% (\( P = 0.028 \)), whereas it returned to baseline values after the VLCD+acipimox (0.73 ± 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

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>VLCD</th>
<th>VLCD + acipimox</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycated hemoglobin (%)</td>
<td>6.0 ± 0.2</td>
<td>25.8 ± 0.8*</td>
<td>25.9 ± 0.9*</td>
</tr>
<tr>
<td>Body mass index [kg/m²]</td>
<td>26.6 ± 0.9</td>
<td>25.8 ± 0.8*</td>
<td>25.9 ± 0.9*</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>6.0 ± 0.4</td>
<td>5.2 ± 0.3‡</td>
<td>4.9 ± 0.2†</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>6.6 ± 1.3</td>
<td>3.3 ± 0.6ª</td>
<td>2.3 ± 0.2ª</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>2.2 ± 0.4</td>
<td>1.3 ± 0.2ª</td>
<td>1.0 ± 0.1ª</td>
</tr>
<tr>
<td>Non-esterified fatty acids (mmol/l)</td>
<td>0.57 ± 0.08</td>
<td>0.92 ± 0.12‡</td>
<td>0.35 ± 0.12</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.5 ± 0.4</td>
<td>4.7 ± 0.2</td>
<td>4.5 ± 0.3</td>
</tr>
<tr>
<td>Visceral adipose tissue (ml)</td>
<td>375 ± 55</td>
<td>295 ± 35‡</td>
<td>303 ± 39</td>
</tr>
<tr>
<td>Hepatic triglyceride content (%)</td>
<td>16.4 ± 1.4</td>
<td>14.2 ± 1.0</td>
<td>14.2 ± 1.2</td>
</tr>
</tbody>
</table>

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.
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 ± 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 ml/s² × 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 ml/s² × 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.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>VLCD</th>
<th>VLCD+acipimox</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>115 ± 5</td>
<td>114 ± 6</td>
<td>110 ± 5</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>73 ± 2</td>
<td>69 ± 3</td>
<td>66 ± 2</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>64 ± 3</td>
<td>63 ± 2</td>
<td>64 ± 3</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>55 ± 1</td>
<td>58 ± 2</td>
<td>55 ± 2</td>
</tr>
<tr>
<td>Cardiac index (l/min/m²)</td>
<td>2.8 ± 0.1</td>
<td>2.7 ± 0.1</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>E peak filling rate (ml/s)</td>
<td>415 ± 27</td>
<td>342 ± 30</td>
<td>380 ± 19</td>
</tr>
<tr>
<td>A peak filling rate (ml/s)</td>
<td>415 ± 16</td>
<td>394 ± 33</td>
<td>395 ± 17</td>
</tr>
<tr>
<td>E/Ea</td>
<td>8.5 ± 0.8</td>
<td>9.1 ± 1.0</td>
<td>9.9 ± 0.9</td>
</tr>
</tbody>
</table>

Values are mean ± standard error.

† P < 0.01, ‡ 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, 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...
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
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, 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 clinical 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, 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. 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. 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.
REFERENCES


