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

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Hammer S, van der Meer RW, Lamb HJ, de Boer HH, Bax JJ, de Roos A, Romijn JA, Smit JW. Short-term flexibility of myocardial triglycerides and diastolic function in patients with type 2 diabetes mellitus. Am J Physiol Endocrinol Metab 295: E714–E718, 2008. First published July 15, 2008; doi:10.1152/ajpendo.90413.2008.—Short-term caloric restriction increases plasma levels of nonesterified fatty acids (NEFAs) and is associated with increased myocardial triglyceride (TG) content and decreased myocardial function in healthy subjects. Whether this flexibility of myocardial TG stores and myocardial function is also present in patients with type 2 diabetes mellitus (T2DM) is yet unknown. Myocardial TG 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 patients after a 3-day VLCD combined with the antilipolytic drug acipimox. The VLCD induced myocardial TG 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 TG content, diastolic function, and plasma NEFA levels were similar to baseline values. In conclusion, in patients with T2DM, a VLCD increases myocardial TG 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 physiological flexibility of myocardial TG stores and myocardial function in patients with T2DM.

Myocardial TG 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 TG accumulation (6, 21, 30) and a decrease in LV diastolic function in healthy volunteers (6, 30). As patients with uncomplicated T2DM show alterations in myocardial high-energy phosphate metabolism, illustrating the changes in normal myocardial substrate handling (3), we hypothesize that this flexibility is diminished in patients with respect to myocardial TG content and LV diastolic function. Because short-term caloric restriction increases adipose tissue lipolysis, it is a suitable research tool to stress myocardial substrate selection and study the effects on myocardial TG 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 TG 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 coadministration of the antilipolytic drug acipimox (23, 33). Acipimox has been extensively used to decrease plasma fatty acid levels, and it therefore serves as a model to study the effects of fatty acids during the interventions. To study the effects on tissue-specific distribution of ectopic TG pools in patients with T2DM, hepatic TG content was also measured in the three conditions.

MATERIALS AND METHODS

Patients. We included 11 well-controlled male T2DM patients (mean age ± SD 57.6 ± 4.7 yr) in this prospective, crossover 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 TG content of 0.23% in 10 subjects (6). Patient characteristics are shown in table 1. All patients used stable doses of metformin and glimepiride for at least 3 mo. 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 to exclude ischemic heart disease), 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.

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1

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Table 1. Metabolic parameters at baseline, after VLCD, and after VLCD + acipimox

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>VLCD</th>
<th>VLCD + Acipimox</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb A1c, %</td>
<td>6.0 ± 0.2</td>
<td>5.9 ± 0.5</td>
<td>5.9 ± 0.5</td>
</tr>
<tr>
<td>BMI, 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, mIU</td>
<td>6.6 ± 1.3</td>
<td>3.3 ± 0.6</td>
<td>2.3 ± 0.2†</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>2.2 ± 0.4</td>
<td>1.3 ± 0.2†</td>
<td>1.0 ± 0.1†</td>
</tr>
<tr>
<td>Nonesterified 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>

Data are means ± SE. VLCD, very low-calorie diet. *P < 0.001, †P < 0.01, ‡P < 0.05 vs. baseline.

**Study design.** The study consisted of three study conditions. 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 periods.

On the second occasion, the subjects were studied either 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) or after a VLCD for 3 days plus acipimox (VLCD + acipimox). Acipimox (250 mg Neldos; ALTANA Pharma, Hooffdorp, The Netherlands) was administered per os at 6-h intervals during the last 24 h of the 3-day period of VLCD (i.e., 4, 10, 16, and 24 h prior to blood sampling). The sequence of the interventions was randomly assigned to minimize influences caused by the sequence of the interventions. Both VLCD studies were separated by a washout phase of at least 14 days. For all study occasions, patients used their last meal or last sachet of Modifast 4-h prior to blood sampling. Blood samples were taken just before MR evaluation. The duration of the VLCD diet was chosen on the basis of our previous experiments in healthy subjects (30).

**Determination of myocardial and hepatic TG content.** All MRI and MR measurements were performed on a 1.5-Tesla Gyroscan ACS-NT MRI scanner (Philips Medical Systems, Best, The Netherlands) in the supine position in the afternoon. Single-voxel (8 ml) spectra were obtained using a body coil for radiofrequency transmission and a circular surface coil (17 cm) 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 (29). In short, an echo time of 26 ms and a repetition time (TR) of 3,000 ms were used. Data points (1,024) were collected using a spectral width of 1,000 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 (with an echo time of 10 s) was measured to be used as an internal standard. For the liver we used the same parameters, except for 64 averages 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 (MRUI version 2.2 (32)), as described earlier (29). Peak estimates of lipid resonances of myocardial and hepatic TG at 1.3 parts per million (ppm) and 0.9 ppm were summed and calculated as a percentage of the unsuppressed water signal (TG/water × 100).

**Evaluation of myocardial systolic and diastolic function.** During MRI, systolic and diastolic blood pressure and heart rate were measured at rest with an automatic device (Dinamap DPC100X, Freiburg, Germany). To assess systolic function, the heart was imaged from apex to base with 12–14 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, a slice thickness 10 mm, slice gap 0 mm, flip angle 35°, echo time 1.67 ms, and TR 3.34 ms. The temporal resolution was 25–39 ms depending on the heart rate. Left ventricular (LV) end diastolic and end systolic contours were drawn using dedicated software (MASS postprocessing software, Medis, Leiden, The Netherlands) as described earlier (18). 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 accurate to assess diastolic function compared with Doppler-derived results (8). Therefore, we measured blood flow across the mitral valve with an ECG-gated gradient-echo sequence with velocity encoding (8, 11). Imaging parameters were as follows: echo time 5 ms, TR 14 ms, flip-angle 20°, slice thickness 8 mm, FOV 350 mm, matrix size 256 × 256 pixels, and velocity encoding 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). The peak slope of the deceleration of the E (E deceleration) and the ratio between the peak filling rate of the E (E-PFR) and A (A-PFR) were calculated (E/A ratio) as measurements for diastolic function. The E/A ratio is load dependent; therefore, the load-independent Ea was measured and an estimation of LV filling pressure was calculated [E/Ea (17)].

**Visceral fat quantification.** Abdominal visceral fat depots were quantified by a turbo spin echo imaging protocol. Imaging parameters were as follows: TE 11 ms, TR 168 ms, flip angle 90°, slice thickness 10 mm. At the level of the fifth lumbar vertebrae, three transverse images were acquired during a breath hold. In postprocessing, visceral fat depots of the slices were calculated by converting the number of pixels to square centimeters multiplied by the thickness of the slices (using MASS analytical software, Medis). The volume of the fat was calculated by summing the volumes of the individual slices.

**Assays.** Plasma concentrations of glucose, total cholesterol (TC), and TG were measured on a fully automated P800 analyzer (Roche, Wako Chemicals, Neuss, Germany).

**Statistical analysis.** Statistical analyses were performed using SPSS version 14.0.2 (SPSS, Chicago, IL). Statistical comparisons between the conditions were made by paired t-tests. P values reflect data compared with baseline unless indicated otherwise. Data are shown as means ± SE, P < 0.05 (two-tailed) was considered significant.

**RESULTS**

**Metabolic changes.** Metabolic changes are listed in Table 1. Plasma NEFA levels increased after the VLCD compared with baseline (from 0.57 ± 0.08 mmol/l to 0.92 ± 0.12, P = 0.019). In contrast, plasma NEFA levels after VLCD + acipimox were unchanged compared with baseline (P = 0.142) but decreased significantly compared with VLCD alone (0.35 ± 0.12 mmol/l, P = 0.006).

**Myocardial and hepatic TG content.** Myocardial TG content at baseline was 0.66 ± 0.09%. After the VLCD, myocardial TG content increased to 0.98 ± 0.16% (P = 0.028), whereas it returned to baseline values after the VLCD + acipimox (to 0.73 ± 0.15%, P = 0.485 vs. baseline; Figs. 1 and 2A). Moreover,
Myocardial TG content was decreased after the VLCD + acipimox compared with the VLCD alone ($P = 0.044$). Myocardial $^1$H-MR spectra could not be obtained in one patient due to technical problems. Hepatic TG content did not change significantly upon both interventions (Table 1).

**DISCUSSION**

This study shows that in well-controlled patients with T2DM short-term caloric restriction increases myocardial TG content by $\sim 48\%$. This increase in myocardial TG content is accompanied by a decrease in myocardial diastolic function. A VLCD combined with acipimox has no effects on myocardial TG 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 reesterification of fatty acids in T2DM patients. As patients with T2DM have altered myocardial metabolism (3), the short-term flexibility of myocardial TG...
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 have different effects. 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 they influence myocardial TG content and diastolic function (31).

During caloric restriction, elevated plasma levels of NEFAs increase hepatic VLDL-TG production (13), which is an important supplier of fatty acids to the myocardium (5, 22). During the VLCD with acipimox, no changes were observed in myocardial TG content. This supports the notion that there is a relationship between increased fatty acid fluxes from the adipose tissue and myocardial TG stores, although we cannot exclude the possibility of a direct effect of acipimox. This appears, however, unlikely, as the antilipolytic effects would lead to an increase, rather than a decrease, in myocardial TG 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 TG accumulation after the VLCD. Although our results cannot be extrapolated to the long-term implications of chronic (hyper- or eucaloric) exposure to elevated NEFA levels in obesity and T2DM, the data suggest that, in general, interventions aiming to decrease plasma lipids or pathological elevated myocardial TG content seem promising. Accordingly, it was recently shown in insulin-treated T2DM patients that adding pioglitazone to insulin therapy decreased myocardial TG stores (35). We used MR velocity mapping to assess blood flow across the mitral valve, E-PFR, A-PFR, and their ratio (E/A) obtained with MR velocity mapping are measurements that are highly correlated to the same parameters when obtained with echocardiography (8). Early deceleration is an MR reflection of the early deceleration time that is used in echocardiography. Therefore, the observed changes in parameters of diastolic function as observed in the present study would likewise be observed when the study was performed with ultrasound. The flow measurements can be affected by changes in preload. Furthermore, systemic effects of acipimox include vasodilatation (19). However, MR-estimated LV filling pressures were unaffected after the interventions; 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 (12). Furthermore, the results are in accord with results obtained in animal models of obesity documenting the association between myocardial TG accumulation and myocardial function (1, 24, 34). Alternatively, caloric restriction and increased plasma NEFA levels may change myocardial calcium handling and thereby influence diastolic function (7, 9, 36). A causal relationship between myocardial TG stores and diastolic function cannot, therefore, be derived from the present data. Acipimox is not suitable for long-term administration regarding the rebound effects on plasma levels of NEFA (28); however, the present data warrant future studies in a clinical setting to study the effects of therapeutic interventions on myocardial TG content and myocardial function. We believe that the differences observed in diastolic function are too small to reflect relevant clinical 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 (16), although this cannot be concluded from the present data.

We also studied the effects of a VLCD on hepatic TG content in the patients with T2DM. Hepatic TG content was increased six- to sevenfold at baseline compared with our previous observations in healthy subjects (6, 30). In contrast to myocardial TG content, hepatic TG content was not significantly affected by the short-term VLCD with or without acipimox. We postulate that the duration of the VLCD is too short to induce reductions in hepatic TG content in T2DM subjects with hepatic steatosis, since a prolonged VLCD in obese T2DM subjects induces major reductions in hepatic TG content (14). Nonetheless, the present study documents that the heart and the liver have different responses 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 patients and patients were their own controls, the number of patients in the study was 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 TG content with data obtained using positron emission tomography (PET) on fatty acid and glucose uptake, 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 TG 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 physiological flexibility of myocardial TG stores and myocardial function in patients with T2DM.

### Table 2. MR parameters at baseline, after VLCD, and after VLCD + acipimox

<table>
<thead>
<tr>
<th>Variable</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>68±2</td>
</tr>
<tr>
<td>Heart rate, beats/min</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·m–2</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±2</td>
<td>342±3</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>

Data are means ± SE. MR, magnetic resonance; E, early diastolic wave; A, atrial diastolic wave; LVEF, left ventricular ejection fraction; E/Ea, estimated LV filling pressure. *P < 0.01, †P < 0.05 vs. baseline.
REFERENCES


