Chapter 7

Prolonged Caloric Restriction in Obese Patients with Type 2 Diabetes Mellitus Decreases Myocardial Triglyceride Content and Improves Myocardial Function

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SUMMARY

Objectives: Myocardial triglyceride (TG) content is increased in patients with type 2 diabetes mellitus (DM2) and may reflect altered myocardial function. The purpose of this study was to assess the effects of prolonged caloric restriction in obese patients with DM2 on myocardial TG content and myocardial function.

Materials and methods: Myocardial TG content (1H magnetic resonance (MR) spectroscopy), left ventricular myocardial function (MR imaging), plasma glycated hemoglobin (HbA1c) and body mass index (BMI), were measured in twelve obese, insulin-treated DM2 patients before and after a 16-week very low-calorie diet (VLCD, 450 kcal/day) to achieve substantial weight loss. Insulin was stopped during the VLCD.

Results: BMI decreased from mean ± standard error 35.6 ± 1.2 (baseline) to 27.5 ± 1.3 kg/m² (after the VLCD, \( P < 0.001 \)), associated with an improvement in HbA1c from 7.9 ± 0.4 (baseline) to 6.3 ± 0.3% (after the VLCD, \( P = 0.006 \)). Myocardial TG content decreased from 0.88 ± 0.12 to 0.64 ± 0.14%, respectively (\( P = 0.019 \)), associated with improved diastolic function (reflected by the ratio between the early and atrial filling phase), from 1.02 ± 0.08 to 1.18 ± 0.06, respectively (\( P = 0.019 \)).

Conclusions: Prolonged caloric restriction in obese patients with DM2 decreases BMI and improves glucoregulation associated with decreased myocardial TG content and improved diastolic function. Therefore, myocardial TG stores in obese patients with DM2 are flexible and amendable to therapeutic intervention by caloric restriction.
INTRODUCTION

Obesity and type 2 diabetes mellitus (DM2) are associated with increased deposition of triglycerides (TGs) in non-adipose tissue, like the heart, liver, pancreas and skeletal muscle (1-4). There are indications from animal experiments and human observations, that the increase in myocardial TG content is associated with altered myocardial function. In animal experiments increased myocardial TG content is associated with impaired myocardial function (5;6), via complex routes involving fatty acid derivatives, such as fatty acyl-coenzyme A and diacylglycerol (7-9). In humans myocardial TG content can be measured non-invasively in vivo by hydrogen 1 magnetic resonance spectroscopy ($^{1}$HMRS) (10-14). These studies have documented that increased myocardial TG stores in obese subjects are accompanied by increased left ventricular (LV) mass (13) and changes in LV diastolic function (2).

In healthy subjects myocardial TG stores are not fixed, but vary depending on nutritional conditions. For instance, short-term caloric restriction dose-dependently increases myocardial TG content, whereas a single high-fat meal does not affect myocardial TG stores (12;15). Recently, we reported that the increase in myocardial TG content induced by short-term caloric restriction is associated with impaired diastolic function in healthy normal-weight subjects (15;16). Caloric restriction is an important lifestyle factor in the treatment of obese patients with DM2. However, the effects of caloric restriction on myocardial TG content have not been studied in these patients.

Therefore, the primary aim of the present study was to evaluate the effects of prolonged caloric restriction by using a very low-calorie diet (VLCD) in obese patients with DM2 on myocardial TG content and left ventricular myocardial function in relation to metabolic regulation. In addition, DM2 is associated with ectopic deposition of TGs in the liver (17;18). To assess the tissue-specific effects of caloric restriction we also assessed liver TG content in these obese patients with DM2.

MATERIALS AND METHODS

Patients

We studied 12 obese (mean ± standard error: body mass index (BMI) 35.6 ± 1.2 kg/m$^2$) patients with DM2 (7 men, 5 women). The mean duration of DM2 was 9.6 ± 1.4 years. The age was 48.3 ± 2.8 years. Patients were recruited from the outpatient clinic. All subjects used insulin treatment (mean dosage 93 ± 21 units/day) with or without concomitant use of oral blood glucose-lowering agents. Exclusion criteria were: smoking, an abnormal stress electrocardiogram (ECG), the use of other medication known to influence lipolysis and/ or glucose metabolism, renal, hepatic or other endocrine disease. Furthermore, subjects were excluded if the remaining insulin secretory capacity was insufficient, defined by fasting C-peptide levels < 0.8 ng/l and/
or < twofold increase after glucagon stimulation (1.0 mg iv.). This criterion was included since we documented in a previous study that preservation of the capacity of beta cells to secrete insulin predicts a favourable metabolic response to a VLCD in obese patients with DM2 (19;20). Body weight was stable for at least three months and subjects were instructed not to change lifestyle habits (eating, drinking, and exercise) from screening until the start of the study. The protocol was approved by the institutional ethical committee and all subjects provided written informed consent prior to participation.

Study design
The study consisted of 2 study occasions separated by a 16-week intervention period during which the subjects used a VLCD to induce substantial weight loss. The VLCD consisted of three sachets Modifast per day (450 kcal/day, Nutrition & Santé, Antwerpen, Belgium), providing about 50 g protein, 50-60 g carbohydrates and 6 g lipids daily. Three weeks before start of the intervention period all oral blood glucose lowering drugs were discontinued and the insulin therapy was intensified. Baseline magnetic resonance (MR) measurements were obtained in the postprandial state (4 hours after the last meal) within 1 week before the start of the VLCD. Baseline blood samples were obtained after an overnight fast. At the start of the VLCD and during the whole intervention period all glucose lowering medication, including insulin, was discontinued. Six of the 12 subjects followed an exercise program, in addition to the VLCD, but were not different with respect to outcome parameters. After 16 weeks, MR measurements (4 hours after the last meal) were repeated. Blood samples were taken after an overnight fast.

1H magnetic resonance spectroscopy of the heart and the liver
All measurements were performed on a 1.5-Tesla Gyroscan ACS-NT MR imaging scanner (Philips Medical Systems, Best, The Netherlands) in the supine position. For 1HMRS measurements, a body coil for radiofrequency transmission and a surface coil (diameter of 17 cm) for signal receiving were used. A point resolved spatially localized spectroscopic pulse sequence was used to acquire single-voxel (8-ml) spectra. For the heart, the voxel was placed in the myocardial septum on four-chamber and short-axis images at end-systole, avoiding contamination with epicardial fat. Data acquisition was double-triggered using ECG triggering and navigator echoes, to minimize breathing artefacts (14). For the liver, voxel sites were matched at the study occasions (by using the twelfth thoracic vertebra as an anatomical landmark), carefully avoiding blood vessels and bile ducts. Water-suppressed spectra with 128 averages were collected to detect lipid signals from the heart, and suppressed spectra with 64 averages were acquired from the liver. Spectral parameters included a repetition time (TR) of at least 3000 ms and an echo time (TE) of 26 ms. 1024 Data points were collected over a 1000-Hz spectral width. Furthermore, unsuppressed spectra with 4 averages were acquired in the same voxel, using the same parameters except for a repetition time of 10000 ms. Spectra were analyzed in the time domain, using the advanced magnetic resonance algorithm in the Java-based MR
user interface software (jMRUI version 2.2 (21)), as described earlier (14). Peak estimates of lipid resonances of myocardial and hepatic TGs at 1.3 parts per million (ppm) and 0.9 ppm were summed and calculated as a percentage of the unsuppressed water signal (%TGs, TGs/water × 100) and used in further analysis.

**Left ventricular function**

Imaging was performed in a single session together with $^1$HMRS measurements, using a body coil for radiofrequency transmission and a 5-element synergy coil for signal receiving. The heart was imaged in the short-axis orientation using an ECG-triggered, sensitivity-encoding balanced steady-state free procession sequence to assess systolic function. Imaging parameters were: field of view = 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. Temporal resolution was 25 to 39 ms (depending on the heart rate). End-diastolic and end-systolic images were identified on all slices and dedicated post processing software (MASS®, Medis, Leiden, The Netherlands) was used to quantify LV ejection fraction, LV mass, cardiac output (CO) and stroke volume as described previously (22). Furthermore, we calculated cardiac index, LV mass index, stroke volume index, end-diastolic index and end-systolic index by dividing the parameter by body surface area. To assess LV diastolic function, an ECG-gated gradient-echo sequence with velocity encoding was performed to measure blood flow across the mitral valve (23). Imaging parameters were: TE = 4.8 ms, TR = 14 ms, flip angle = 20°, slice thickness = 8 mm, field of view = 350 mm$^2$, matrix size = 256 × 256, velocity encoding = 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). Furthermore, the down slope of the E (E deceleration) and an estimation of LV filling pressures (E/Ea) (24) were calculated. During MR imaging, blood pressure and heart rate were measured with an automatic device (Dinamap DPC100X, Freiburg, Germany).

**Assays**

Plasma glucose, total cholesterol and TG concentrations were measured on a fully automated P800 analyzer (Roche, Almere, The Netherlands). Insulin was measured on an Immulite 2500 random access analyzer with a chemoluminescence immunoassay (DPC, Los Angeles, CA, USA). Coefficients of variation were < 2% for glucose and < 5% for insulin. Plasma levels of non-esterified fatty acids (NEFAs) were measured by using a commercial kit (NEFA-C; Wako Chemicals, Neuss, Germany). Glycated hemoglobin (HbA1c) levels were measured with an HPLC system (Variant, Biomed, Hercules, CA, USA). Leptin and adiponectin were measured with a radioimmunoassay from Linco Research (St. Charles, MO, USA), with coefficients of variation ranging from 3.0 to 5.1% for leptin and 7 to 9% for adiponectin, and a sensitivity of 0.5 µg/l. The high-sensitive C-reactive protein ELISA came from DSL, Webster, Texas, USA. The sensitivity was 0.03 mg/l and the coefficient of variation was between 3 and 6%.
Statistical analysis

All statistical analyses were performed with SPSS, version 14.0 (SPSS Inc., Chicago, Ill, USA). Statistical comparisons between baseline measurements and measurements after prolonged caloric restriction were made by paired t-tests. Data are shown as mean ± standard error. \( P < 0.05 \) was considered to reflect significant differences.

RESULTS

Metabolic parameters

Caloric restriction reduced BMI from 35.6 ± 1.2 at baseline to 27.5 ± 1.3 kg/m\(^2\) after the intervention period (\( P < 0.001 \), Figure 7.1). Metabolic parameters before and after prolonged caloric restriction are shown in Table 7.1 and Figure 7.2. After 16 weeks of caloric restriction, glycaemic control was significantly improved, as fasting plasma glucose levels decreased from 11.4 ± 0.6 mmol/l at baseline (despite glucose lowering therapy by high dose insulin) to 6.7 ± 0.6 mmol/l after prolonged caloric restriction (only on a VLCD without any glucose lowering therapy for 16 weeks, \( P < 0.001 \)). Furthermore, HbA1c levels decreased from 7.9 ± 0.4 to 6.3 ± 0.3% at baseline and after prolonged caloric restriction respectively, \( P = 0.006 \).

Plasma NEFA levels were 0.92 ± 0.07 mmol/l at baseline and decreased to 0.67 ± 0.05 mmol/l after prolonged caloric restriction (\( P < 0.001 \), Figure 7.2A). Furthermore, liver enzymes, plasma total cholesterol and plasma TG levels were significantly decreased after the VLCD compared to baseline (Table 7.1, Figure 7.2).

Myocardial and hepatic triglyceride content

Typical myocardial \(^1\)HMR spectra of a patient at baseline and after caloric restriction are shown in Figure 7.3. Myocardial TG content decreased from 0.88 ± 0.12 (baseline) to 0.64 ± 0.14% (after the VLCD, \( P = 0.019 \), Figure 7.2C, based on \( n = 11 \) successful myocardial spectral measurements).

![Figure 7.1. Fat stores and body mass index.](image)

Example of a transversal slice at the level of the fifth lumbar vertebrae showing visceral and subcutaneous fat depots, illustrating the effects of 16 weeks of caloric restriction in the same patient (A and B). Body mass index (BMI) is decreased after prolonged caloric restriction (C). Bars represent mean + standard error, * \( P < 0.001 \).
Concomitantly, hepatic TG content decreased from 21.2 ± 4.2 to 3.0 ± 0.9%, respectively (P < 0.001, Figure 7.2D).

**Myocardial systolic and diastolic function**

Systolic blood pressure decreased from 144 ± 8 to 118 ± 6 mmHg at baseline and after substantial weight loss respectively (P < 0.001). Diastolic blood pressure decreased from 81 ± 2 at baseline to 71 ± 2 mmHg after weight loss (P < 0.001). Heart rate was significantly decreased after substantial weight loss (Table 7.2).

During caloric restriction myocardial function improved. Cardiac output decreased significantly from 7971 ± 601 ml/min at baseline to 6508 ± 401 ml/min after prolonged caloric restriction (P = 0.001). Furthermore, LV mass was significantly decreased as well (from 118 ± 7 to 99 ± 6 g respectively, P < 0.001, Figure 7.4A). E/A ratio increased from 1.02 ± 0.08 at baseline to 1.18 ± 0.06 after the VLCD (P = 0.019), reflecting improved diastolic function (Figure 7.4B).

**DISCUSSION**

This study demonstrates that prolonged caloric restriction decreases BMI and considerably improves glucoregulation, associated with decreased myocardial TG content and beneficial effects on blood pressure and myocardial function in insulin-treated obese patients with DM2. The data prove that myocardial TG stores in obese patients with DM2 are flexible and amendable to therapeutic intervention by caloric restriction.

### Table 7.1. Metabolic response to 16 weeks of caloric restriction in obese patients with type 2 diabetes mellitus.

<table>
<thead>
<tr>
<th>Fasting plasma concentrations</th>
<th>Baseline</th>
<th>After 16 weeks of caloric restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>11.4 ± 0.6</td>
<td>6.7 ± 0.6*</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.9 ± 0.4</td>
<td>6.7 ± 0.6†</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>39 ± 9a</td>
<td>10 ± 3†</td>
</tr>
<tr>
<td>AST (mmol/l)</td>
<td>44 ± 5</td>
<td>27 ± 3†</td>
</tr>
<tr>
<td>ALT (mmol/l)</td>
<td>52 ± 12</td>
<td>23 ± 3‡</td>
</tr>
<tr>
<td>γGT (mmol/l)</td>
<td>38 ± 5</td>
<td>18 ± 2†</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.7 ± 0.5</td>
<td>4.8 ± 0.2‡</td>
</tr>
<tr>
<td>Non-esterified fatty acids (mmol/l)</td>
<td>0.92 ± 0.07</td>
<td>0.67 ± 0.05*</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>2.1 ± 0.3</td>
<td>1.1 ± 0.1*</td>
</tr>
<tr>
<td>Leptin (µg/l)</td>
<td>21.5 ± 4.3</td>
<td>7.6 ± 3.4*</td>
</tr>
<tr>
<td>Adiponectin (mg/l)</td>
<td>5.2 ± 0.7</td>
<td>7.8 ± 1.1†</td>
</tr>
<tr>
<td>hs-CRP (mg/l)</td>
<td>18.5 ± 4.2</td>
<td>7.5 ± 2.0†</td>
</tr>
</tbody>
</table>

*P < 0.001, †P < 0.01 and ‡P < 0.05 vs baseline. Data are mean ± standard error. HbA1c = glycated hemoglobin, AST = aspartate aminotransferase, ALT = alanine aminotransferase, γGT = gamma-glutamyl transferase, TG = triglyceride, hs-CRP = high-sensitive C-reactive protein, a insulin (short acting) was stopped >12 hours.
Myocardial TG accumulation is the net result of excessive fatty acid uptake in relation to oxidative fatty acid requirements. In animal experiments this increased myocardial TG pool is associated with impaired myocardial function (5;6). In human studies, myocardial TG accumulation is also associated with impaired myocardial function.

For instance, a post mortem study in obese patients with severe metabolic dysregulation and heart failure documented myocardial lipid accumulation, which was higher in subjects suffering from obesity and DM2 (25). Recently, McGavock et al. documented that in patients with DM2 myocardial TG content is increased, and suggested that myocardial TG accumulation precedes overt changes in systolic function (2). Therefore, myocardial TG content may be an interesting marker for the risk of non-ischemic heart disease, and a potential surrogate marker to assess the effects of metabolic interventions on the heart. In rodents, the restoration of myocardial TG metabolism is associated with improvements in cardiac function (6;26), in accordance with our findings. Nonetheless, the improvement in myocardial function upon caloric restriction in the present study cannot merely be ascribed to the decreased myocardial TG stores, because there were also major alterations in other factors that affect cardiac mass and function like BMI, and blood pressure.

Figure 7.2. Metabolic changes in at baseline and after 16 weeks caloric restriction. Changes in plasma NEFAs (A), plasma TGs (B), and myocardial (C) and hepatic (D) TGs upon prolonged caloric restriction. VLCD = very low-calorie diet, TGs = triglycerides, NEFAs = non-esterified fatty acids. Bars represent mean ± standard error, * P < 0.001, ‡ P < 0.05.
Others reported beneficial effects of weight loss on cardiac function after bariatric surgery (27) or a VLCD (28). Moreover, we found a decline in heart rate, which is beneficial as heart rate is independently associated with increased mortality (29). In addition to this decreased heart rate, we observed a decrease in cardiac output and LV mass, in line with previously reported

**Figure 7.3. Myocardial ¹H magnetic resonance spectra.**

Typical unsuppressed ¹H magnetic resonance spectra of the same patient at baseline and after 16 weeks of caloric restriction (A). The starred boxes indicate the part of the spectrum where the myocardial triglycerides (TGs) resonate, of which the suppressed spectra are shown in B.

VLCD = very low-calorie diet, ppm = parts per million.
data (30). LV ejection fraction was normal and did not change after the intervention period, in accordance with previous data showing that normal LV ejection fraction was unchanged 3 months after weight loss in obese subjects (31). LV mass is predictive of cardiovascular morbidity and mortality and can be decreased by improvements in blood pressure (32). In addition, the decrease we found in LV mass is influenced by the substantial weight loss (33) and possibly by the improvements in insulin sensitivity (34). Due to the dramatic changes in body size, some of the indexed values for LV dimensions were changed after the intervention period. LV mass index decreased, whereas end-diastolic index was increased.

The decrease in LV mass can directly influence left ventricular filling pressures, and, consequently, parameters of left ventricular diastolic function (35). However, the presently used estimation of LV filling pressures (E/Ea) showed no changes after prolonged caloric restriction. Therefore, an alternative explanation for the increase in E/A ratio may be improved elastic properties of the LV, in line with results from animal models, documenting the relation between myocardial TG accumulation and myocardial function (5;6). One of the alternative mechanisms may be that changes in plasma fatty acids change the calcium homeostasis in the myocardium (36) which influences LV diastolic function (37). Furthermore, the present improvements in the inflammatory parameter C-reactive protein may influence myocardial function as well (38).

Our study has some limitations. First, the study is descriptive and does not establish a causal relation between myocardial TG accumulation and myocardial function, although the results are in accordance with data obtained in different animal models of obesity and, additionally, show the metabolic flexibility of the diabetic heart. Second, the sample size is relatively small. However, the patients are their own control and the magnitude of the metabolic and functional changes is illustrative as it indicates dynamic features of myocardial TG and diastolic function.

### Table 7.2. Intra-individual effects of 16 weeks of caloric restriction on systolic and diastolic function in obese patients with type 2 diabetes mellitus.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>After 16 weeks of caloric restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>144 ± 8</td>
<td>118 ± 6*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>81 ± 2</td>
<td>71 ± 2*</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>78 ± 3</td>
<td>61 ± 2*</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>57 ± 2</td>
<td>58 ± 2</td>
</tr>
<tr>
<td>Stroke volume (ml)</td>
<td>102 ± 6</td>
<td>103 ± 8</td>
</tr>
<tr>
<td>Stroke volume index (ml/m²)</td>
<td>45 ± 2</td>
<td>51 ± 3‡</td>
</tr>
<tr>
<td>Cardiac output (ml/min)</td>
<td>7971 ± 601</td>
<td>6508 ± 401†</td>
</tr>
<tr>
<td>Cardiac Index (l/min/m²)</td>
<td>3.5 ± 0.2</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>LV Mass (g)</td>
<td>118 ± 7</td>
<td>99 ± 6*</td>
</tr>
<tr>
<td>LV mass index (g/m²)</td>
<td>53 ± 3</td>
<td>49 ± 3‡</td>
</tr>
<tr>
<td>ED volume (ml)</td>
<td>177 ± 8</td>
<td>177 ± 11</td>
</tr>
</tbody>
</table>

* P < 0.001, † P < 0.01 and ‡ P < 0.05 vs baseline. Data are mean ± standard error.

LVEF = left ventricular ejection fraction, LV = left ventricular, ED = end-diastolic, ES = end-systolic, E = early filling phase, A = atrial filling phase, E/Ea = estimated LV filling pressure.
In addition to the decrease in myocardial TG content, the VLCD dramatically decreased hepatic TG content, associated with improvements in plasma lipid profile, and liver enzymes. Moreover, insulin sensitivity was markedly increased after substantial weight loss in accordance with previous studies (19;20;39;40). The improvement in hepatic TG content indicates that there is a general reduction in ectopic deposition of TG in non-adipose tissues, including liver and the heart.

**CONCLUSIONS**

In conclusion, prolonged caloric restriction in obese patients with DM2 decreases BMI and improves gluoregulation associated with decreased myocardial TG content and improved LV diastolic function. Therefore, myocardial TG stores in obese patients with DM2 are flexible and amendable to therapeutic intervention by caloric restriction.

*Figure 7.4. Changes in myocardial function.*
Intra-individual changes in left ventricular (LV) mass (A) and the ratio between the early filling phase and the atrial filling phase (E/A ratio) upon progressive caloric restriction (B), *P* < 0.001, ‡ *P* < 0.05.
REFERENCES


