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## The effects of a very low calorie diet and exercise in obese type 2 diabetes mellitus patients

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### Citation

Snel, M. (2011, September 1). *The effects of a very low calorie diet and exercise in obese type 2 diabetes mellitus patients*. Retrieved from <https://hdl.handle.net/1887/17801>

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**Note:** To cite this publication please use the final published version (if applicable).

# CHAPTER 6

## **Prolonged caloric restriction in obese patients with type 2 diabetes mellitus decreases myocardial triglyceride content and improves myocardial function**

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*Journal American College of Cardiology 2008;52:1006–12*



**ABSTRACT**

**Objectives.** Myocardial triglyceride (TG) content is increased in patients with type 2 diabetes mellitus (T2DM) and may reflect altered myocardial function. It is unknown whether myocardial TG content is influenced during a therapeutic intervention. This study sought to assess the effects of prolonged caloric restriction in obese patients with T2DM on myocardial TG content and myocardial function.

**Methods.** Myocardial TG content (magnetic resonance [MR] spectroscopy), myocardial function (MR imaging), plasma hemoglobin A1c, and body mass index (BMI) were measured in 12 obese, insulin-treated T2DM 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.** The BMI decreased from  $35.6 \pm 1.2$  kg/m<sup>2</sup> (baseline, mean  $\pm$  SEM) to  $27.5 \pm 1.3$  kg/m<sup>2</sup> (after the VLCD,  $p < 0.001$ ) and was associated with an improvement in hemoglobin A1c from  $7.9 \pm 0.4\%$  (baseline) to  $6.3 \pm 0.3\%$  (after the VLCD,  $p = 0.006$ ). Myocardial TG content decreased from  $0.88 \pm 0.12\%$  to  $0.64 \pm 0.14\%$ , respectively ( $p = 0.019$ ), and was associated with improved diastolic function (reflected by the ratio between the early and atrial filling phase) from  $1.02 \pm 0.08$  to  $1.18 \pm 0.06$ , respectively ( $p = 0.019$ ).

**Conclusions.** Prolonged caloric restriction in obese T2DM patients decreases BMI and improves glucoregulation associated with decreased myocardial TG content and improved diastolic heart function. Therefore, myocardial TG stores in obese patients with T2DM are flexible and amendable to therapeutic intervention by caloric restriction.

## INTRODUCTION

Obesity and type 2 diabetes mellitus (T2DM) are associated with increased deposition of triglycerides (TG) in nonadipose tissue, such as 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 free fatty acid (FFA) derivatives, such as FFA acyl-coenzyme A and diacylglycerol (7-9).

In humans, myocardial TG content can be measured non-invasively *in vivo* by proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS) (10-14). 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 (15), whereas a single high-fat meal does not affect myocardial TG stores (12). 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 T2DM. However, the effects of caloric restriction on myocardial TG content have not been studied in these patients. Therefore, the primary goal of the present study was to evaluate the effects of prolonged caloric restriction in obese patients with T2DM on myocardial TG content and LV myocardial function in relation to metabolic regulation. In addition, T2DM is associated with ectopic deposition of TG in the liver (17,18). To assess the tissue-specific effects of caloric restriction, we also assessed liver TG content in these obese T2DM patients.

## PATIENTS AND METHODS

### Patients

We studied 12 obese (body mass index (BMI), mean  $\pm$  SEM:  $35.6 \pm 1.2$  kg/m<sup>2</sup>) T2DM patients (7 men, 5 women). The mean duration of T2DM was  $9.6 \pm 1.4$  years. The patients' age was  $48.3 \pm 2.8$  years. Patients were recruited from the outpatient clinic. All subjects used insulin treatment (mean dosage  $93 \pm 21$  IU/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; and 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 <2-fold increase after glucagon stimulation (1.0 mg intravenously). This criterion was included because we documented in a previous study that preservation of the capacity of beta cells to secrete insulin predicts a favorable metabolic response to a very low calorie diet (VLCD) in obese T2DM patients (19,20). Body weight was stable for at least 3 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 before 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 3 sachets of Modifast per day (450 kcal/day, Nutrition & Santé, Antwerp, Belgium), providing about 50 g protein, 50 to 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 h 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 h after the last meal) were repeated. Blood samples were taken after an overnight fast.

### **<sup>1</sup>H-MRS of the heart and the liver**

All measurements were performed on a 1.5-T Gyroscan ACS-NT MR imaging scanner (Philips Medical Systems, Best, the Netherlands) in the supine position. For <sup>1</sup>H-MRS 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 4-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 artifacts (14). For the liver, voxel sites were matched at the study occasions (by using the 12th 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: repetition time of at least 3,000 ms, echo time 26 ms, and 1,024 data points over 1,000-kHz spectral width. Furthermore, unsuppressed spectra with 4 averages were acquired in the same voxel, using the same parameters except for a repetition time of 10 s. Spectra were analyzed in the time domain, using the

advanced MR algorithm in the Java-based MR user interface software (jMRUI version 2.2, A. van den Boogaart, Katholieke Universiteit Leuven, Leuven, Belgium) (21), as described earlier (14). Peak estimates of lipid resonances of myocardial and hepatic TG at 1.3 and 0.9 ppm were summed and calculated as a percentage of the unsuppressed water signal (%TG, TG/water  $\times$  100) and used in further analysis.

### Left ventricular function

Imaging was performed at 1.5 T in a single session together with spectroscopy 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 precession sequence to assess systolic function. Imaging parameters were: field-of-view = 400  $\times$  320 mm, matrix size = 256  $\times$  256, slice thickness = 10 mm, slice gap = 0 mm, flip angle = 35°, echo time = 1.67 ms, and repetition time = 3.34 ms. Temporal resolution was 25 to 39 ms (depending on the heart rate). End-diastolic and -systolic images were identified on all slices, and dedicated postprocessing software (MASS, V2007-EXP, Leiden University Medical Center, Medis, Leiden, the Netherlands) was used to quantify LV ejection fraction, LV mass, cardiac output, stroke volume, and end-diastolic and -systolic 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: echo time = 5 ms, repetition time = 14 ms, flip-angle = 20°, slice thickness = 8 mm, field of view = 350 mm, matrix size = 256  $\times$  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 (V2006-EXP, Leiden University Medical Center, Medis). Furthermore, the downslope of the early filling phase (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 with an immunoradiometric assay (Biosource, Nivelles, Belgium). Coefficients of variation were <2% for glucose and <5% for insulin. Plasma levels of free fatty acids (FFA) were measured using a commercial kit (FFA-C, Wako Chemicals, Neuss, Germany). The hemoglobin A1c levels were measured with an HPLC system (Variant, Biomed, Hercules, California). Leptin and adiponectin were measured with a radioimmunoassay from Linco Research (St.Charles, Missouri), 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-sensitivity C-reactive protein enzyme-linked immunosorbent assay came from DSL (Webster, Texas). The sensitivity was 0.03 mg/l, and the coefficient of variation was between 3% and 6%.

### Statistical analyses

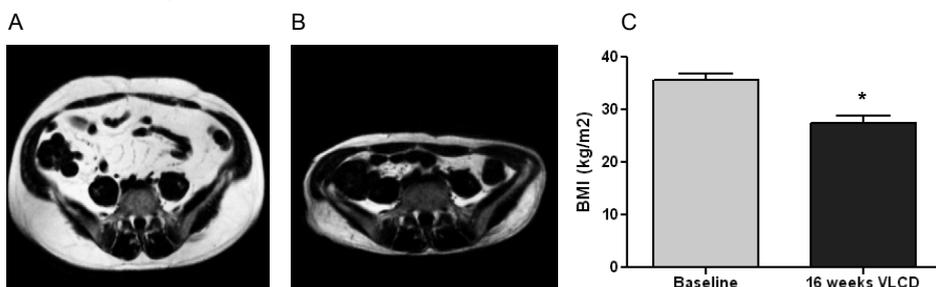
All statistical analyses were performed with SPSS version 14.0 (SPSS Inc., Chicago, Illinois). Statistical comparisons between baseline measurements and measurements after prolonged caloric restriction were made by paired *t* test. Data are shown as mean ± SEM. A value of *p* < 0.05 was considered to reflect significant differences.

## RESULTS

### Metabolic parameters

Caloric restriction reduced BMI from  $35.6 \pm 1.2$  kg/m<sup>2</sup> at baseline to  $27.5 \pm 1.3$  kg/m<sup>2</sup> after the intervention period (*p* < 0.001) (Figure 1). Metabolic parameters before and after prolonged caloric restriction are shown in Table 1 and Figure 2. After a 16-week VLCD, glycemic control was significantly improved; fasting plasma glucose levels decreased from  $11.4 \pm 0.6$  mmol/l at baseline (despite glucose-lowering therapy by high-dose insulin) to  $6.7 \pm 0.6$  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  $7.9 \pm 0.4\%$  to  $6.3 \pm 0.3\%$  at baseline and after prolonged caloric restriction, respectively, *p* = 0.006). Plasma FFA levels were  $0.92 \pm 0.07$  mmol/l at baseline and decreased to  $0.67 \pm 0.05$  mmol/l after prolonged caloric restriction (*p* < 0.001) (Figure 2A). Furthermore, plasma levels of liver enzymes, total cholesterol, TGs, leptin, and C-reactive protein were significantly decreased after the VLCD compared with baseline, whereas plasma adiponectin levels were increased (Table 1, Figure 2).

**Figure 1.** Fat stores and body mass index. Example of a transverse slice at the level of the 5th lumbar vertebrae showing visceral and subcutaneous fat depots, illustrating the effects of a 16-week very low calorie diet (VLCD) in the same patient (A and B). Body mass index (BMI) is decreased after prolonged caloric restriction (C).



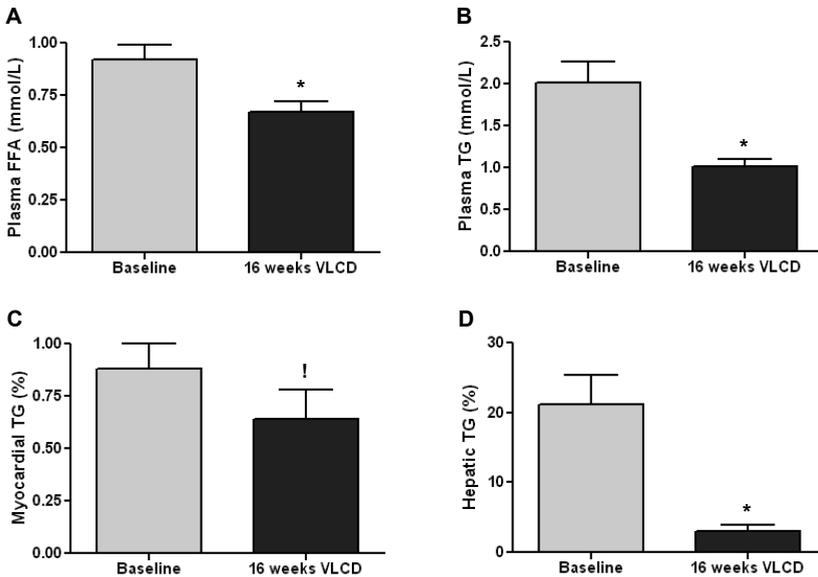
Data are presented as mean ± SEM. \* *p* < 0.001.

**Table 1.** Metabolic response to 16 weeks of caloric restriction in obese patients with type 2 diabetes mellitus.

	baseline	after 16 wks	
Glucose (mmol/l)	11.4 ± 0.6	6.7 ± 0.6	*
HbA1c (%)	7.9 ± 0.4	6.7 ± 0.6	!
Insulin (mU/l)	39 ± 9	10 ± 3	!
AST (mmol/l)	44 ± 5	27 ± 3	!
ALT (mmol/l)	52 ± 12	23 ± 3	!
γGT (mmol/l)	38 ± 5	18 ± 2	!
Total cholesterol (mmol/l)	5.7 ± 0.5	4.8 ± 0.2	!
Free fatty acids (mmol/l)	0.92 ± 0.07	0.67 ± 0.05	*
TG (mmol/l)	2.1 ± 0.3	1.1 ± 0.1	*
Leptin (μg/l)	21.5 ± 4.3	7.6 ± 3.4	*
Adiponectin (μg/l)	5.2 ± 0.7	7.8 ± 1.1	!
hsCRP (mg/l)	18.5 ± 4.2	7.5 ± 2.0	!

Data are mean ± SEM. \*  $p < 0.001$ ; !  $p < 0.01$  versus baseline.

γGT: gamma-glutamyl transferase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; hsCRP: high sensitive c-reactive protein; TG: triglyceride.

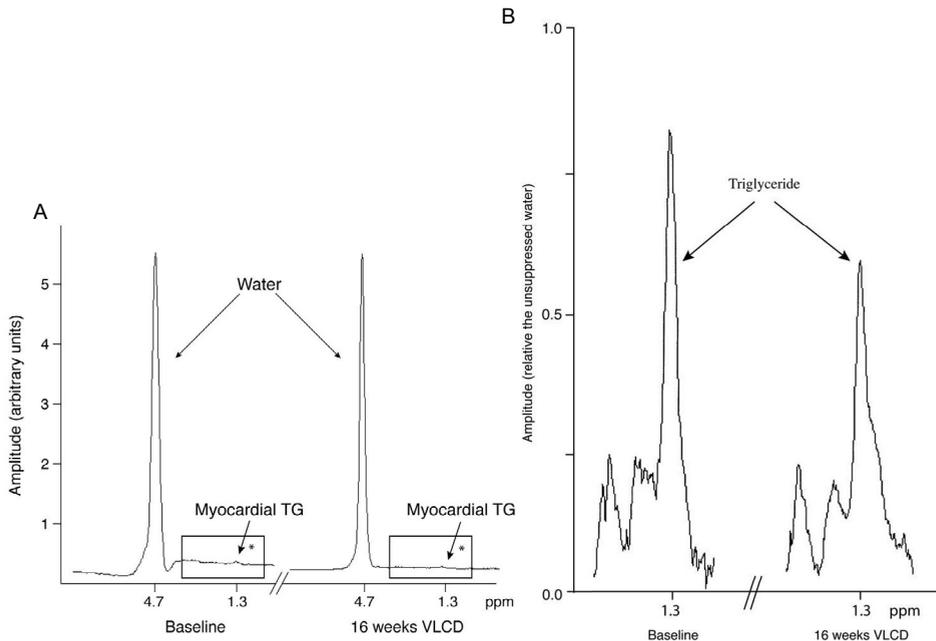
**Figure 2.** Metabolic changes at baseline and after a 16-week very low calorie diet (VLCD). Changes in plasma free fatty acids (FFA) (A), plasma triglyceride (TG) levels (B), and myocardial (C), and hepatic (D) TG content on prolonged caloric restriction.

Data are mean ± SEM. \*  $p < 0.001$ ; !  $p < 0.05$ .

## Myocardial and hepatic TG content

Typical myocardial proton spectra of a patient at baseline and after caloric restriction are shown in Figure 3. Myocardial TG content decreased from  $0.88 \pm 0.12\%$  (baseline) to  $0.64 \pm 0.14\%$  (after the VLCD,  $p = 0.019$ , based on  $n = 11$  successful myocardial spectral measurements) (Figure 2C). Concomitantly, hepatic TG content decreased from  $21.2 \pm 4.2\%$  to  $3.0 \pm 0.9\%$ , respectively ( $p < 0.001$ ) (Figure 2D).

**Figure 3.** Myocardial Proton Spectra. Typical unsuppressed proton spectra of the same patient at baseline and after a 16-week very low calorie diet (VLCD) (A). The starred boxes indicate the part of spectrum where the myocardial lipids resonate, of which the suppressed spectra are shown in (B).



ppm: parts per million; TG: triglyceride.

## Myocardial systolic and diastolic function

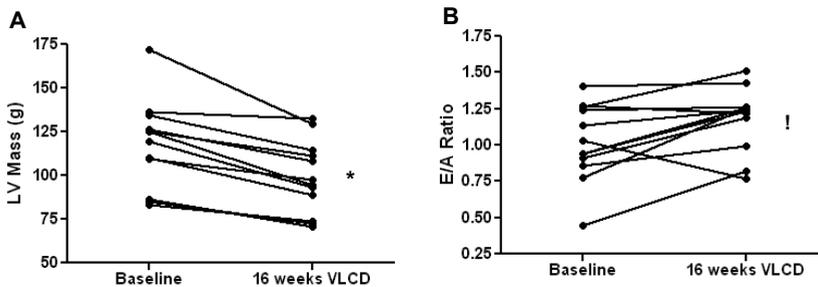
Systolic blood pressure decreased from  $144 \pm 8$  mm Hg to  $118 \pm 6$  mm Hg at baseline and after substantial weight loss, respectively ( $p < 0.001$ ). Diastolic blood pressure decreased from  $81 \pm 2$  mm Hg at baseline to  $71 \pm 2$  mm Hg after weight loss ( $p < 0.001$ ). Heart rate was significantly decreased after substantial weight loss (Table 2). During caloric restriction, myocardial function improved. Cardiac output decreased significantly from  $7,971 \pm 601$  ml/min at baseline to  $6,508 \pm 401$  ml/min after prolonged caloric restriction ( $p = 0.001$ ). Furthermore, LV mass was significantly decreased as well (from  $118 \pm 7$  g to  $99 \pm 6$  g, respectively,  $p < 0.001$ ) (Figure 4A). The E/A ratio increased from  $1.02 \pm 0.08$  at baseline to  $1.18 \pm 0.06$  after the VLCD ( $p = 0.019$ ), reflecting improved diastolic function (Figure 4B).

**Table 2.** Effects of 16 weeks of caloric restriction on systolic and diastolic function in obese patients with type 2 diabetes mellitus.

	baseline	after 16 wks	
Systolic blood pressure (mm Hg)	144 ± 8	118 ± 6	*
Diastolic blood pressure (mm Hg)	81 ± 2	71 ± 2	*
Heart rate (beats/min)	78 ± 3	61 ± 2	*
LVEF (%)	57 ± 2	58 ± 2	
Stroke volume (ml)	102 ± 6	103 ± 8	
Stroke volume index (ml/m <sup>2</sup> )	45 ± 2	51 ± 3	!
Cardiac output (ml/min)	7971 ± 601	6508 ± 401	!
Cardiac Index (l/min/m <sup>2</sup> )	3.5 ± 0.2	3.2 ± 0.2	
LV Mass (g)	118 ± 7	99 ± 6	*
LV mass index (g/m <sup>2</sup> )	53 ± 3	49 ± 3	!
ED volume (ml)	177 ± 8	177 ± 11	
ED index (ml/m <sup>2</sup> )	79 ± 3	88 ± 4	!
ES volume (ml)	76 ± 4	74 ± 5	
ES index (ml/m <sup>2</sup> )	34 ± 2	37 ± 2	
E deceleration (ml/s <sup>2</sup> × 10 <sup>-3</sup> )	4.04 ± 0.50	4.30 ± 0.42	
E/A peak ratio	1.02 ± 0.08	1.18 ± 0.06	!
E/Ea	11.9 ± 1.2	11.4 ± 1.5	

Data are mean ± SEM. \*  $p < 0.001$ ; !  $p < 0.05$  versus baseline.

A: atrial filling phase; E: early filling phase; E/Ea: estimated left ventricular filling pressure;  
ED: end-diastolic; ES: end-systolic; LV: left ventricular; LVEF: left ventricular ejection fraction.

**Figure 4.** Changes in myocardial function. Intraindividual changes in left ventricular (LV) mass (A) and the ratio between the early filling phase and the atrial filling phase (E/A ratio) after a 16-week very low calorie diet (VLCD) (B).

\*  $p < 0.001$ ; !  $p < 0.05$ .

## DISCUSSION

This study shows 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 T2DM. The data prove that myocardial TG stores in obese patients with T2DM are flexible and amendable to therapeutic intervention by caloric restriction.

Myocardial TG accumulation is the net result of excessive FFA uptake in relation to oxidative FFA 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 that was higher in subjects suffering from obesity and T2DM (25). Recently, McGavock et al. (2) documented that in patients with T2DM myocardial TG content is increased, and suggested that myocardial TG accumulation precedes overt changes in systolic function. Therefore, myocardial TG content may be an interesting marker for the risk of nonischemic heart disease, and a potential surrogate marker for assessing 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 on 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, such as BMI and blood pressure.

Others reported beneficial effects of weight loss on cardiac function after bariatric surgery (27) or VLCD (28). Moreover, we found a decrease in heart rate, which is beneficial because 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 data (30). The 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). The 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). Because of the dramatic changes in body size, some of the indexed values for LV dimensions were changed after the intervention period. The LV mass index decreased, whereas the end-diastolic index was increased. The decrease in LV mass can directly influence LV filling pressures, and consequently, parameters of LV 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 the E/A ratio may

be improved elastic properties of the LV, in line with results from animal models, documenting the relationship between myocardial TG accumulation and myocardial function (5,6). One of the alternative mechanisms may be that changes in plasma FFAs 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).

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 nonadipose tissues, including the liver and heart.

There are limitations to this study. First, the study is descriptive and does not establish a causal relationship 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 controls, and the magnitude of the metabolic and functional changes is illustrative because it indicates dynamic features of myocardial TGs and diastolic function.

In conclusion, prolonged caloric restriction in obese T2DM patients decreases BMI and improves glucoregulation associated with decreased myocardial TG content and improved diastolic heart function. Therefore, myocardial TG stores in obese patients with T2DM are flexible and amendable to therapeutic intervention by caloric restriction.

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