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

Snel, M.

CHAPTER 8

Long-term beneficial effect of a 16-week very low calorie diet on pericardial fat in obese type 2 diabetes mellitus patients

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ABSTRACT

Objective. Pericardial fat accumulation has been associated with an increased cardiovascular risk. A very low calorie diet (VLCD) improves the cardiovascular risk profile in patients with type 2 diabetes mellitus (T2DM), by improving the metabolic profile, heart function and triglyceride (TG) stores in (non)adipose tissues. However, long-term effects of a VLCD on pericardial fat volume and tissue-specific TG accumulation have not been documented. The aim of this study was therefore to assess the effects of a 16-week VLCD and of subsequent 14 months follow-up on a regular diet on pericardial fat in relation to other TG stores in obese T2DM patients.

Methods. We included 14 obese patients with insulin-treated T2DM (mean±SEM: age 53±2 years; BMI 35±1 kg/m^2). Pericardial fat and other (non)adipose TG stores were measured using magnetic resonance (MR) imaging and proton spectroscopy before and after a 16-week VLCD and after a 14-month follow-up without dietary interventions.

Results. A 16-week VLCD reduced bodyweight, pericardial fat, hepatic TG content, visceral and subcutaneous abdominal fat volumes to 78, 83, 16, 40 and 53% of baseline values respectively, (all $p<0.05$). After an additional 14 months of follow-up on a regular diet, the reduction in pericardial fat volume sustained, despite a substantial regain in body weight, visceral abdominal fat and hepatic TG content (resp. 90, 83 and 73% of baseline values).

Conclusion. VLCD-induced weight loss in obese T2DM patients is accompanied by a substantial decrease in pericardial fat volume, which is sustained even after subsequent weight regain.

INTRODUCTION

One of the cornerstones of the treatment in type 2 diabetes mellitus (T2DM) patients are lifestyle interventions with diet-induced weight reduction and exercise. A very low calorie diet (VLCD) is safe and effective to induce considerable weight loss and to improve insulin resistance, even in patients with insulin-dependent T2DM (1-3). During short-term weight loss, there is a close relationship between tissue-specific decreases in triglyceride (TG) stores and improvement in insulin sensitivity in obese nondiabetic and diabetic patients. Even a small reduction in weight can induce major reductions in hepatic TG content already within the first two weeks of a diet (4,5) and visceral abdominal fat even within 3 days of a VLCD in patients with T2DM (6). Reductions in hepatic TG content and visceral fat are associated with improvements in hepatic and peripheral insulin sensitivity (7,8). Studies on the long-term effects of a period of VLCD in T2DM have only focused on weight reduction and metabolic effects. The long-term effects of a VLCD on TG accumulation in pericardial fat and other (non)adipose tissues have not been studied.

Pericardial fat is adipose tissue surrounding the heart, which consists of two layers: epicardial and paracardial fat (9). An increased pericardial fat volume has been associated with insulin resistance in non-diabetic obese subjects (10) and T2DM patients (11). Moreover it has been associated with an increased cardiovascular risk (9,12-17). Recently, it has been documented by ultrasound studies that weight loss is associated with a decrease in pericardial fat stores in non-diabetic obese subjects (18,19). These studies also reported a larger decrease in visceral fat volume compared to pericardial fat volume (19). Dietary effects on pericardial fat have been studied only to a limited extent in patients with T2DM.

Therefore, the aim of this study was to assess the effects of a 16-week VLCD and subsequent 14-month follow-up on a regular diet on pericardial fat in relation to other TG stores in obese insulin-dependent patients with T2DM, using magnetic resonance imaging and spectroscopy.

PATIENTS AND METHODS

Patients

Fourteen obese patients (8 men/6 women) with T2DM were studied (mean ± standard error of mean (SEM): age: 53 ± 2 years). Patients were diagnosed with T2DM since 9 ± 1 years and used 81 ± 16 units of insulin per day. Selection criteria were: body mass index (BMI) > 30 kg/m^2, use of at least 20 units of insulin per day with or without oral blood glucose-lowering agents, remaining endogenous insulin secretory capacity (defined as fasting C-peptide levels > 0.8 ng/liter and/or a twofold increase in C-peptide levels upon infusion of 1 mg glucagon) and stable bodyweight during 3 months before inclusion. Exclusion criteria were: smoking,

abnormal stress-electrocardiogram (ECG), renal, hepatic or other endocrine disease and contra-indications for magnetic resonance imaging (MRI).

This study was approved by the local ethics committee. Written informed consent was obtained from all patients and the study was performed in accordance with the Declaration of Helsinki.

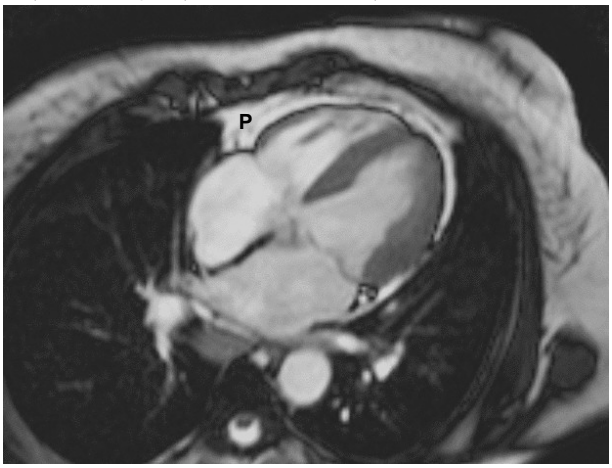
Study procedure

The study procedure during the 16-week VLCD has been described in detail before, but will be summarized here (2). After screening, the patients entered a three week run-in period, in which all oral blood glucose-lowering medication was omitted and insulin therapy was intensified. Within one week before the start of the intervention a baseline magnetic resonance (MR) scan was performed to assess the different fat compartments (see below). The intervention consisted of a very low calorie diet (VLCD), prescribed during 16 weeks, to achieve considerable weight loss. During the VLCD, patients consumed 3 sachets of Modifast Intensive per day (containing 450 kcal, 50 g protein, 50-60 g carbohydrates and 6 g lipids per day, Nutrition & Santé, Antwerpen, Belgium). At the start and, subsequently, during the whole period of the VLCD all glucose-lowering medication including insulin was discontinued. Five of the 14 patients additionally followed an exercise program during the VLCD, however this had no effect on the outcome parameters. At the end of the 16-week VLCD a second MRI-scan was performed. Subsequently, patients received dietary advice and were slowly reintroduced to their diet of choice. Twenty weeks after the start of the study, patients were referred back to their own specialists for regular medical care either in primary care or at the out-patient clinic of our department. Fourteen months after the end of the VLCD a third MRI-scan was performed. The average caloric intake was estimated on basis of the food intake three days before the study days. Blood was drawn on each occasion after an overnight fast.

Pericardial fat

All MR imaging and proton MR spectroscopy (^{1}H-MRS) studies were performed using a 1.5 Tesla whole body MR scanner (Gyroscan ACS-NT15; Philips Medical Systems, Best, The Netherlands) in supine position. All MR-measurements were made in the postprandial state (4 hours after the last meal). Pericardial fat was quantified using electrocardiographically gated breath-holds with a balanced turbo-field echo MR sequence (20). Imaging parameters included: echo time (TE) = 1.60 ms, repetition time (TR) = 3.2 ms, flip-angle = 50°, slice thickness = 10 mm. The whole four chamber view was analyzed. To quantify the pericardial fat volume, contours around the pericardial fat of the ventricles and atria were drawn manually at end systole and multiplied by the thickness of the slice to yield a volume. We used MASS analytical software (Medis, Leiden, the Netherlands) for post processing. Contours were drawn by two independent observers and we used the mean volume of the two observers. A representative image is given in Figure 1.

Figure 1. Pericardial fat (P) as visualized by a four chamber view of the heart on a 1.5 Tesla whole body MR scanner (Gyroscan ACS-NT15; Philips Medical Systems, Best, The Netherlands). To quantify the pericardial fat volume, contours around the pericardial fat of the ventricles and atria were drawn manually at end systole and multiplied by the thickness of the slice to yield a volume.



Visceral and subcutaneous fat

Abdominal fat was quantified with a turbo spin echo imaging (6). Imaging parameters were: TE = 11ms, TR =168ms, flip angle = 90°, slice thickness = 10mm. Three transverse images were obtained during one breath hold, at the level of the 5th lumbar vertebrae. With post-processing software (MASS analytical software, Medis, Leiden, The Netherlands), the volumes of the visceral and subcutaneous fat depots were quantified. The number of pixels were converted to square centimeters and multiplied by the slice thickness. The total fat volume was calculated by summing the fat volumes of the three individual slices.

Hepatic magnetic resonance spectroscopy

Hepatic ^{1}H-MRS was performed as previously described (21). The bodycoil was used for radio-frequency transmission and a 17 cm diameter circular coil for signal receiving. An 8 ml voxel was placed in the liver, carefully avoiding large vascular structures. To ascertain the same position of the voxel at all study days, we used the twelfth thoracic vertebrae as marker. A TR of 3000 ms and TE of 26 ms were used. A spectrum with water suppression to detect small TG peaks and a spectrum without water suppression as internal standard were obtained. Sixty-four averages were collected without water suppression. The spectra were fitted using Java-base MR user interfase software (jMRUI version 2.2), as previously described (21). The percentage of hepatic TG signals was calculated as: (signal amplitude of hepatic triglycerides)/ (signal amplitude of water) x 100.

Assays

Serum insulin and C-peptide were measured with an automated immunoluminometric assay (ILMA) on an Immulite 2500 analyzer of Siemens Diagnostics (Breda, The Netherlands, intra-assay coefficient of variant (CV) 6-7.5%, inter-assay CV 7-8.2% respectively). Serum glucose, cholesterol and triglycerides were measured using a Modular P800 chemistry analyzer of Roche Diagnostics (Mannheim, Germany, total CV for glucose, cholesterol and triglyceride <2%). Plasma FFA concentrations were measured by a commercial kit (FFA-C; Wako Chemicals, Neuss, Germany).

Statistical analysis

Data are expressed as mean values ± standard error of the mean (SEM) or as median (interquartile range) if not normally distributed. Differences between the three measurements were analyzed with a general linear model for repeated measures, with time as within-subject factor. LSD post-hoc tests were used in case of a significant F-ratio. The plots were created with GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, USA). Statistical analyses were performed using SPSS 17.0 (SPSS Inc.Chicago, Illinois, USA). A P-value of 0.05 or less was considered statistically significant.

RESULTS

Caloric intake and weight gain

The VLCD reduced caloric intake from 2471±115 kcal/day at baseline to 450 kcal/day, which resulted in a 22% weight reduction compared to baseline ($P<0.05$, Table 1). After discontinuation of the VLCD, after an additional 14 months of follow-up, the estimated caloric intake was 1889±62 kcal/day, $P<0.05$ vs. baseline). During follow-up patients regained weight to 90% of baseline ($P<0.05$ vs. baseline, Table 1).

Antidiabetic medication, glycemic control and lipids

At baseline, before start of the VLCD, all patients used insulin, 8 patients used metformin and 3 patients a sulfonylureum (SU)-derivate. During the 16 week-VLCD all patients had stopped insulin therapy and oral glucose-lowering medication. Directly after the intervention metformin was prescribed to all patients and 3 patients were prescribed an additional SU-derivate. After an additional 14 months of follow-up (patients were treated by their specialists according to the standard guidelines), four patients had restarted insulin therapy, 12 patients used metformin and 4 patients used a SU-derivate.

The VLCD significantly improved glycemic control, reflected in decreased fasting plasma glucose and HbA1c levels, even though all patients had stopped the use of insulin and oral blood glucose-lowering medication during the whole VLCD period (Table 1). At 18 months

Table 1. Metabolic changes and changes in fat distribution at baseline, after a 16-week VCLD and after an additional 14 months of follow-up on a regular diet.

	baseline	16 week VLCD		18 months		
Weight (kg)	107 ± 4	83 ± 4	*	96 ± 4	*	!
Body Mass Index (kg/m^2)	35.3 ± 1.1	27.5 ± 1.1	*	31.7 ± 1.1	*	!
Fasting plasma concentrations						
HbA1c (%)	8.4 ± 0.3	7.0 ± 0.4	*	7.7 ± 0.5		
Fasting glucose (mmol/L)	11.9 ± 0.6	7.8 ± 0.8	*	10.4 ± 1.0		!
Fasting insulin (mmol/L)	23.5(14.3-29.3)	10.0(6.8-12.0)	*	6.5(5.0-14.3)		
Fasting C-peptide (mmol/L)	2.8 ± 0.3	2.1 ± 0.3		1.0 ± 0.1	*	!
Total cholesterol (mmol/L)	6.0 ± 0.4	5.3 ± 0.3		5.2 ± 0.4		
Triglycerides (mmol/L)	2.3 ± 0.2	1.2 ± 0.1	*	2.8 ± 0.6		!
HDL (mmol/L)	1.13 ± 0.05	1.25 ± 0.09	*	1.29 ± 0.07	*	
LDL (mmol/L)	4.4 ± 0.4	3.7 ± 0.3	*	3.2 ± 0.2	*	
Fat distribution						
Hepatic TG content (%)	22.8 ± 3.9	3.6 ± 1.0	*	13.4 ± 2.2	*	!
Subcutaneous abdominal fat (ml)	1194 ± 105	701 ± 108	*	978 ± 89	*	!
Visceral abdominal fat (ml)	553 ± 37	228 ± 46	*	456 ± 51	*	!
Visceral/subcutaneous fat	0.52 ± 0.07	0.33 ± 0.04	*	0.53 ± 0.09		!
Pericardial fat (ml)	39 ± 4	31 ± 2	*	32 ± 2	*	

Data are mean ± SEM or median (interquartile range). * $p<0.05$ vs. baseline, ! $p<0.05$ 18 months vs. 16 weeks.
HbA1c: glycated hemoglobin; HDL: high density lipoprotein; LDL: low density lipoprotein, TG: triglyceride.

however, HbA1c and fasting plasma glucose returned to baseline values, albeit with less intensive anti-diabetic medication.

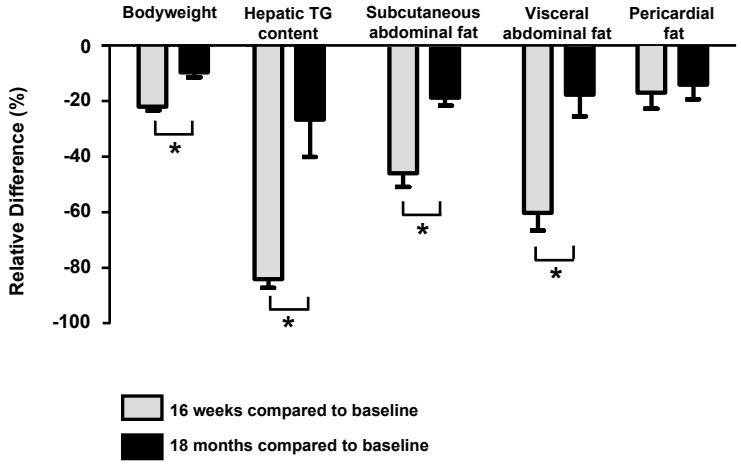
Total plasma cholesterol levels did not change after the VLCD. HDL-cholesterol levels were higher and LDL cholesterol levels lower after the VLCD which was sustained after 18 months of follow-up, despite the above described weight regain (Table 1).

Pericardial fat and other fat compartments

After the 16-week VLCD, pericardial fat significantly decreased by 17% (from 39±4 ml at baseline to 31±2 ml after 16 weeks). Fourteen months after discontinuation of the VLCD, pericardial fat volume remained stable, despite the considerable weight regain and deterioration of glycemic control.

A preferential loss of visceral fat compared to subcutaneous fat was observed after the 16-week VLCD (40% and 55% resp. of baseline values (both $P< 0.05$, Figure 2)). After an additional 14 months of follow-up, both visceral and subcutaneous fat volumes increased to 83% and 82% resp. of baseline values (both $P<0.05$ compared to baseline, Figure 2). Accordingly, the ratio between visceral and subcutaneous fat decreased significantly from 0.52 to 0.33 after 16

Figure 2. Relative changes in bodyweight, hepatic triglyceride (TG) content, subcutaneous and visceral abdominal fat and pericardial fat after 16 weeks of a very low calorie diet (grey box) and after 14 months of follow-up on a regular diet (black box) compared to baseline.



* p<0.05 for decrease in weight/fat between respectively 16 weeks and 18 months compared to baseline.

weeks, but returned to a ratio of 0.53 after an additional follow-up of 14 months. Therefore, the preferential loss of visceral fat had disappeared.

Due to technical problems abdominal fat and hepatic TG content could not be assessed in 1 patient at 18 months. The VLCD decreased hepatic TG content to 16% of baseline levels. However, 14 months after discontinuation, hepatic TG content increased to 73% of baseline values (P<0.05 vs. baseline, Figure 2).

DISCUSSION

This study is the first to assess the long-term effects of caloric restriction on pericardial fat and other fat compartments in patients with T2DM using state of the art MR imaging and spectroscopy. Our data reveal differential short- and long-term effects of a 16-week VLCD and 14 months follow-up on weight, pericardial fat and other fat compartments. In general, we found a substantial regain in (non)adipose TG compartments with as clear exception pericardial fat.

We found that the beneficial effects of a 16-week VLCD on weight and metabolic parameters partially deteriorated 14 months after reinstating a regular diet, which is in line with previous studies (22-25). However, in those studies, effects on fat compartments were not studied. Interestingly, despite the fact that the relative reduction in pericardial fat volume after the VLCD was the smallest of all fat compartments, this was the only fat compartment which did not expand during the additional follow-up of 14 months. Previous studies on caloric restriction in non-diabetic subjects, also reported a larger decrease in visceral fat volume

as compared to pericardial fat volume (19). Likewise, in a short-term caloric restriction study in overweight women, cross-sectional, but no longitudinal associations between pericardial fat volume and other fat compartments and metabolic parameters were found (26). However, in these studies, no long-term effects after reinstating a regular diet were assessed. We previously found that treatment with pioglitazone in patients with T2DM, increased pericardial fat, but did not change visceral fat volumes (20). The results of the previous and present studie(s) clearly suggest that pericardial fat and visceral fat volumes are differentially influenced by diet and drugs (like thiazolidinediones), and cannot be considered as metabolically identical compartments. As pericardial fat volume has been associated with cardiovascular risk in cross-sectional studies (11,13,27), it can be hypothesized that our finding is compatible with a sustained beneficial effect of a VLCD on cardiovascular risk.

We observed a preferential loss of visceral fat compared to subcutaneous fat volume immediately after the 16-week VLCD. After 14 months of follow-up this preferential loss of visceral fat was no longer present. This short-term effect can be explained by a higher susceptibility of visceral fat to lipolysis than subcutaneous fat via a diminished response to insulin (28) and a greater sensitivity to norepinephrine (29,30).

The weight reduction of 22% immediately after the VLCD observed in our study was associated with a major reduction of 84% in hepatic TG content. Reductions in hepatic TG content are strongly associated with improved hepatic insulin sensitivity and lipoprotein metabolism through different mechanisms, including effects of fatty acids metabolites and inflammatory intermediates on insulin receptor signaling and VLDL synthesis (8). Therefore, the regain in hepatic TG content after 14 months on a regular diet is likely related to the deterioration in fasting plasma glucose and TG levels, although remarkably, beneficial effects of the VLCD on HDL- and LDL cholesterol were preserved after long-term follow-up.

In conclusion, diet-induced weight loss and subsequent regain of weight during regular diet induces tissue-specific variations in (non)adipose TG stores in T2DM patients. Whereas visceral fat volume and hepatic TG content largely parallel changes in weight and metabolic parameters, sustained effects on pericardial fat are observed. These observations are most likely the consequence of differences in sensitivity to regulatory mechanisms, controlling TG accumulation in different (non)adipose tissue compartments.

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