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

Snel, M.

CHAPTER 5

Immediate and long-term effects of addition of exercise to a 16-week very low calorie diet on low-grade inflammation in obese, insulin-dependent type 2 diabetes mellitus patients

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Submitted

ABSTRACT

Objective. To assess the short- and long-term effects of addition of exercise to a very low calorie diet (VLCD) on low-grade inflammation in obese patients with type 2 diabetes mellitus (T2DM).

Methods. 27 obese, insulin-dependent T2DM patients followed a 16-week VLCD with (n=13) or without (n=14) exercise (E) and were followed up to 18 months. Anthropometric measurements, metabolic and inflammatory parameters were assessed before, directly after the intervention and at 6 and 18 months follow-up. The same measurements were performed only once in 56 healthy lean and 56 healthy obese controls.

Results. At baseline hsCRP, IL10 and IL8 were significantly elevated in obese T2DM compared to lean healthy controls. After 16 weeks, despite substantial weight loss (-25.4±1.3kg), neither the VLCD nor VLCD+E had an effect on plasma cytokines. At 6 months, in the weight-stabilizing period, measures of low-grade inflammation had decreased substantially and equally in both intervention groups. Despite subsequent weight regain, beneficial effect was sustained upto 18 months in both groups, except for IL1 and hsCRP which had returned to baseline in the VLCD-only group.

Conclusion. Our findings suggest that severe caloric restriction increases cytokine production by adipose tissue macrophages and that the beneficial effects of weight loss become apparent only in the eucaloric state.

INTRODUCTION

Insulin resistant states such as obesity and type 2 diabetes mellitus (T2DM) are associated with chronic low-grade inflammation, as indicated by higher circulating levels of C-reactive protein (CRP), interleukin 6 (IL6) and tumor necrosis factor alpha (TNFα) (1). These pro-inflammatory proteins and cytokines can intervene in intracellular signaling pathways involved in glucose uptake and insulin secretion (2,3). However, the temporal and causal relationship between insulin resistance and elevated markers of inflammation is as of yet unclear.

Both obesity and T2DM are independent risk factors for the development of premature atherosclerosis and ischemic heart disease (1,4). Patients with T2DM have a 2-4 times increased cardiovascular disease (CVD) risk compared to healthy controls (5). This increased cardiovascular risk appears to be associated not only with traditional cardiovascular risk factors such as smoking, gender, hypertension and dyslipidemia but also with the abovementioned markers of inflammation (6,7).

Caloric restriction (CR), weight loss and exercise improve glucoregulation in patients with T2DM and ameliorate the classic CVD risk factors hypertension and dyslipidemia (8). CR and weight loss induce a decline in CRP levels, both in obese, non-diabetic subjects as well as in obese diabetic subjects (9). In addition, weight loss and lifestyle interventions decrease plasma IL6 and TNFα levels in obese non-diabetic subjects (10-13). The effect of exercise on these two markers of inflammation is controversial (14,15). Moreover, the effects of a very low calorie diet (VLCD) on IL6, TNFα and CRP has only been studied after a 3-week intervention (16) in obese patients with T2DM and no long-term follow-up data are available. Therefore, the aim of this study was to assess the effects of a 16-week VLCD (Modifast, 450 kcal/day) on classic cardiovascular risk factors and low-grade inflammation in obese, insulin-dependent T2DM patients. We also assessed whether adding exercise to the VLCD had additional beneficial effects on these outcomes. Patients were re-examined at 6 and 18 months after the start of the 16-week intervention, to evaluate the durability of the effects. In addition, the levels of these inflammatory markers were compared with those obtained in lean and obese healthy controls.

PATIENTS AND METHODS

Patients

Twenty-seven (14 males, 13 females) obese, insulin-dependent T2DM patients (disease duration 9±0.8 years, mean±standard error of the mean (SEM)) were enrolled in the study between June 2006 and June 2007. Clinical characteristics are summarized in Table 1. Exclusion criteria were smoking, known history of CVD and/or other chronic or endocrine disease, weight change within three months prior to the study and silent myocardial ischemia (as

Table 1. Clinical characteristics.

	VLCD + exercise	VLCD only	controls obese	controls lean
sex (M/F)	6/8	8/5	28/26	28/26
age (years)	56 ± 2	59 ± 2	56 ± 1	59 ± 2
weight (kg)	114 ± 5 *	113 ± 6 *	112 ± 3 *	73 ± 2
BMI (kg/m^2)	36.4 ± 1.1 *	37.9 ± 1.4 *	37.6 ± 0.7 *	23.8 ± 0.3
waist (cm)	123 ± 3 *	122 ± 3 *	118 ± 2 *	87 ± 1
fat mass (kg)	45.4 ± 3.2 *	49.9 ± 3.6 *	39.2 ± 2.4 *	33.4 ± 2.2
HbA1c (%)	7.8 ± 0.4 * #	7.8 ± 0.3 * #	5.5 ± 0.1 *	5.2 ± 0.0
insulin (IU/day)	77	86	0	0
metformin (number of pts)	10	9	0	0
SU-derivatives (number of pts)	3	1	0	0

Data are presented as mean ± SEM. * significant difference vs. lean controls; # significant difference vs. obese controls.
M: Male; F: female; BMI: body mass index; IU: international units; pts: patients; SU: sulfonylureum.

measured by an incremental cyclo-ergometer cardiac stress test). Patients had to use a minimum dosage of 20 IU insulin/day.

For each T2DM patient, 2 lean control subjects and 2 obese control subjects, matched for age, gender, race, geographical area and in the obese group for BMI were included in the study. In Table 1 baseline characteristics of the lean and obese control groups are summarized.

Patients and healthy controls were recruited via advertisements in local newspapers, and patients also via the outpatient clinics of the departments of Endocrinology/Internal Medicine in the Leiden University Medical Center. Written informed consent was obtained from all subjects. The study was approved by the ethics committee of our center.

Study design

All T2DM patients discontinued their oral glucose-lowering medication three weeks prior to the start of the study. Two days before the start of the study the last dose of long-acting insulin was given and the day before the start of the study the last dose of short-acting insulin was given at the evening meal. The glucose-lowering medication remained discontinued during the 16-week intervention. After the baseline study day (as outlined below) T2DM patients started a 16-week VLCD (450 kcal/day) consisting of three sachets of Modifast® (Nutrition & Santé, Antwerp, Belgium) per day. Modifast® contains all necessary vitamins and micronutrients. In addition, 13 of the 27 patients simultaneously followed an exercise program. This weekly exercise program comprised a minimum of 4 days training at home for 30 minutes at 70% of maximum aerobic capacity on a cyclo-ergometer and a one-hour in hospital training under supervision of a physiotherapist (the attendance rate was 97%).

Patients visited the outpatient clinic weekly, for support, to keep up with the diet, measurement of bodyweight and to check glucoregulation. Compliance with the diet and exercise was confirmed by questioning, counting sachets of Modifast that were supplied weekly and reading the heart rate monitor which was worn during exercise sessions both at home and in the hospital (Polar S610 $_{i}^{tm}$, Polar Electro Oy, Finland). After the intervention a regular diet was slowly reintroduced with the aim of weight maintenance. Thereafter, patients were referred back to their original doctors and treated with routine care. The researchers performed a follow-up visit at 6 and 18 months after the start of the intervention.

At day 0 (start of the study), 16 weeks (end of the intervention), 6 and 18 months (follow-up) patients were studied in the morning after an overnight fast and after 2 days without any exercise. All patients performed all the follow-up visits, so no patients were lost to follow-up. The lean and obese healthy controls were studied only once. Length, weight, BMI and waist circumference were measured according to the World Health Organization recommendations. Blood pressure was measured with an Omron 705IT blood pressure device (Omron Matsusaka Co., Ltd., Japan) and recorded within the limits of 1 mmHg. Fat mass was assessed by bioelectrical impedance analysis (Bodystat® 1500 MDD, Bodystat Ltd., Douglas, Isle of Man, United Kingdom). Fasting blood samples were drawn for the measurement of plasma glucose, insulin, hemoglobin A1c (HbA1c), high-sensitive C-reactive protein (hsCRP), IL1, IL2, IL6, IL8, IL10, TNFα and interferon gamma (INFγ), total cholesterol (TC), high density lipoprotein (HDL)-cholesterol, triglyceride (TG) levels and free fatty acids (FFA). Low density lipoprotein (LDL)-cholesterol was calculated with the Friedewald formula. Ten-year CVD risk was calculated with the United Kingdom Prospective Diabetes Study (UKPDS) risk engine (17). Only at baseline and after the 16-week intervention rate of appearance (Ra) of glycerol and free fatty acid levels were measured. Basal rates of glycerol appearance were measured after a 1.5 hours of continuous infusion of [$^{2}H_{5}$]-glycerol (Cambridge Isotopes, Cambridge, USA) as described previously. A physiological and isotopic steady-state was achieved during the last 30 min therefore, the Ra of glycerol were calculated as the tracer infusion rate divided by the tracer-to-tracee ratio.

Assays

Plasma glucose, TC, HDL-cholesterol and TG concentrations were measured with a fully automated P-800 module (Roche, Almere, the Netherlands). Insulin was detected with an immunometric assay on an automated Immulite 2500 (Siemens, Breda, the Netherlands). Glycosylated hemoglobin (HbA1c) levels were measured with a high-performance liquid chromatography (HPLC) system (Variant, Biomed, Hercules, California, USA). hsCRP levels were determined with an enzyme-linked immonosorbent assay (DSL, Webster, Texas, USA). Plasma free fatty acids (FFAs) concentrations were measured by a commercial kit (Wako Chemicals, Neuss, Germany). Plasma levels of the various cytokines were assessed using precoated 96-well multispot plates from Meso Scale Discovery (MSD; Gaithersburg, Maryland, USA), an

enzyme linked immunosorbent assay (ELISA) based electrochemiluminiscence assay. Briefly, plates were incubated with plasma or calibrator (25 µl/well) for 2 hours. After washing (3x, PBS with 2% Tween 20), detection antibody labeled with MSD SULFO-TAG (1 µg/mL) was added and incubated for another 2 hours. Wells were washed and reading buffer was added. Plates were immediately read using the SECTOR Imager 2400 and cytokine concentrations were determined by a non-linear standard curve fit.

Statistical analyses

A general linear model for repeated measurements analyzed differences within the patient groups at the various time points. Differences between both intervention groups were analyzed by calculating a delta between two time points. The deltas were subsequently compared using the two-tailed Student's *t*-test for unpaired data or, when appropriate, by non-parametric tests for independent samples. Differences between all groups (the two intervention groups and the two healthy controls) were analyzed using a one-way ANOVA, LSD post-hoc tests were used in case of a significant F-ratio. Data are presented as mean±SEM. Data analyses were performed using SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL, USA). A p-value <0.05 was considered statistically significant.

RESULTS

Effect on bodyweight and glucoregulation

The VLCD with exercise (VLCD+E) and VLCD-only groups did not differ with respect to clinical and metabolic parameters at baseline (Table 1). Moreover, gender, age, weight, BMI and waist circumference of the obese control group were well matched with the T2DM patients. The lean and obese control groups had significantly lower levels of fasting glucose and HbA1c as compared to the two patient groups. Medication use of the patients can be found in Table 1.

Bodyweight and glucoregulation improved significantly (baseline vs. after the 16 week intervention bodyweight: VLCD+exercise 114±5 vs. 86±4 p<0.0001; VLCD-only 113±6 vs. 89±4 p<0.0001 HbA1c: VLCD+exercise 7.8±0.4 vs. 6.3±0.4 p<0.0001; VLCD-only 7.8±0.3 vs. 6.7±0.3 p<0.0001) and to an equal extent in both intervention groups directly after the 16-week VLCD despite the cessation of all glucose-lowering medication. The VLCD+E group lost significantly more fat mass and waist circumference compared to the VLCD-only group (delta VLCD+exercise vs. delta VLCD fat mass -21.8±2.7 vs. -16.6±2.7 p=0.020; waist -24.8±1.5 vs. -19.3±1.7 p=0.049). At 6 and 18 months, both groups had gained weight. However, weight, fat mass and waist circumference were still significantly lower compared to baseline values in both groups (Table 2). Nevertheless at 18 months, glucoregulation had deteriorated in both intervention groups, mostly because patients were not restarted on insulin therapy (Table 3).

Table 2. Clinical, metabolic characteristics and cholesterol levels before, directly after 6, and 18 months after a 16-week VLCD only or VLCD with exercise in obese insulin-dependent T2DM patients and comparisons with (obese and lean) healthy controls.

		VLCD + exercise	VLCD only	controls	
				obese	lean
weight (kg)	baseline	114 ± 5 *	113 ± 6 *	112 ± 3 *	73 ± 2
	16 weeks	86 ± 4 !	89 ± 4 !		
	6 months	90 ± 5 ! ?	93 ± 5 ! ?		
	18 months	98 ± 5 ! ?	103 ± 5 ! ?		
BMI (kg/m^2)	baseline	36.4 ± 1.1 *	37.9 ± 1.4 *	37.6 ± 0.7 *	23.8 ± 0.3
	16 weeks	27.7 ± 1.0 !	30.0 ± 1.1 !		
	6 months	28.9 ± 1.3 ! ?	31.1 ± 1.2 ! ?		
	18 months	31.6 ± 1.2 ! ?	34.7 ± 1.3 ! ?		
waist (cm)	baseline	123 ± 3 *	122 ± 3 *	118 ± 2 *	87 ± 1
	16 weeks	98 ± 3 !	103 ± 3 !		
	6 months	101 ± 3 !	105 ± 3 !		
	18 months	107 ± 4 ! ?	114 ± 3 ! ?		
fat mass (kg)	baseline	45.4 ± 3.2 *	49.9 ± 3.6 *	39.2 ± 2.4 *	33.4 ± 2.2
	16 weeks	23.5 ± 2.2 !	33.2 ± 2.8 !		
	6 months	29.5 ± 2.8 ! ?	35.2 ± 2.9 ! ?		
	18 months	35.4 ± 2.6 ! ?	44.2 ± 3.0 ! ?		
systolic bloodpressure (mmHg)	baseline	145 ± 5	161 ± 4	149 ± 3	141 ± 3
	16 weeks	132 ± 5 !	140 ± 4 !		
	6 months	136 ± 4 !	142 ± 4 !		
	18 months	146 ± 5 ?	157 ± 4 ?		
diastolic bloodpressure (mmHg)	baseline	81 ± 3	87 ± 3	87 ± 1	82 ± 1
	16 weeks	75 ± 2	78 ± 2 !		
	6 months	77 ± 3	83 ± 2 ?		
	18 months	82 ± 2 ?	89 ± 3 ?		
heart rate (beats/min)	baseline	79 ± 2 *	82 ± 3 *	70 ± 2	67 ± 2
	16 weeks	64 ± 3 !	69 ± 3 !		
	6 months	65 ± 3 !	70 ± 3 !		
	18 months	70 ± 4	76 ± 3		
HbA1c (%)	baseline	7.8 ± 0.4 * #	7.8 ± 0.3 * #	5.5 ± 0.1 *	5.2 ± 0.0
	16 weeks	6.3 ± 0.4 !	6.7 ± 0.3 !		
	6 months	6.1 ± 0.4 !	6.7 ± 0.3 !		
	18 months	7.5 ± 0.6	8.2 ± 0.5 ?		
fasting glucose (mmol/L)	baseline	10.9 ± 0.7 * #	12.1 ± 0.5 * #	5.5 ± 0.1 *	4.9 ± 0.1
	16 weeks	6.6 ± 0.8 !	7.7 ± 0.6 !		
	6 months	7.4 ± 0.7 !	8.4 ± 0.8 !		
	18 months	9.2 ± 1.0	12.2 ± 1.1 ?		

Table 2. (continued)

		VLCD + exercise	VLCD only	controls obese	controls lean
fasting insulin (mU/L)	baseline	25 ± 2 * #	24 ± 4 * #	14 ± 1 *	5 ± 0
	16 weeks	9 ± 1 !	13 ± 2 !		
	6 months	9 ± 2 !	11 ± 2 ! ?		
	18 months	13 ± 2 !	17 ± 6		
HOMA-IR	baseline	12.3 ± 1.3 * #	12.9 ± 2.3 * #	3.4 ± 0.3 *	1.1 ± 0.1
	16 weeks	2.5 ± 0.2 !	4.3 ± 0.8 !		
	6 months	2.7 ± 0.5 !	4.3 ± 1.0 !		
	18 months	4.7 ± 0.8 ! ?	9.0 ± 3.1		
TC (mmol/L)	baseline	5.4 ± 0.4	6.1 ± 0.4	6.0 ± 0.1	6.0 ± 0.1
	16 weeks	4.5 ± 0.3 !	5.5 ± 0.3		
	6 months	4.4 ± 0.4 !	4.7 ± 0.3 ?		
	18 months	4.3 ± 0.3 !	5.5 ± 0.4		
TG (mmol/L)	baseline	2.5 ± 0.5 * #	2.3 ± 0.2 * #	1.6 ± 0.1 *	1.1 ± 0.1
	16 weeks	1.2 ± 0.1 !	1.5 ± 0.2 !		
	6 months	1.2 ± 0.2 !	1.5 ± 0.2 !		
	18 months	1.9 ± 0.3 ?	2.8 ± 0.6 ?		
HDL (mmol/L)	baseline	1.1 ± 0.0 * #	1.2 ± 0.1 * #	1.4 ± 0.0 *	1.8 ± 0.1
	16 weeks	1.2 ± 0.1	1.2 ± 0.1		
	6 months	1.4 ± 0.1 ! ?	1.5 ± 0.1 ?		
	18 months	1.2 ± 0.1	1.4 ± 0.1 !		
Chol:HDL ratio	baseline	4.9 ± 0.4 *	5.3 ± 0.4	4.2 ± 1.4 *	3.5 ± 1.4
	16 weeks	4.0 ± 0.3 !	4.8 ± 0.4 !		
	6 months	3.0 ± 0.2 !	3.1 ± 0.2 !		
	18 months	3.7 ± 0.3 !	4.1 ± 0.3 !		
LDL (mmol/L)	baseline	3.6 ± 0.3	4.4 ± 0.4	3.8 ± 0.1	3.7 ± 0.1
	16 weeks	3.0 ± 0.3 !	3.7 ± 0.3 !		
	6 months	2.4 ± 0.2 ! ?	2.8 ± 0.2 ! ?		
	18 months	2.5 ± 0.7 !	3.5 ± 1.0		
FFA (mmol/L)	baseline	0.9 ± 0.1	1.0 ± 0.1		
	16 weeks	0.9 ± 0.0	1.0 ± 0.1		
Ra glycerol (µmol/kgFFM/min)	baseline	12.9 ± 1.0	11.3 ± 1.2		
	16 weeks	15.9 ± 1.1 !	11.3 ± 1.3		

Data are presented as mean ± SEM. ! significant difference vs. baseline values within the group; ? significant difference vs. 16 weeks values within the group; * significant difference vs. lean controls; # significant difference vs. obese controls.

BMI: Body mass index; HOMA-IR: homeostatic model assessment of insulin resistance; TC: total cholesterol; TG: triglyceride; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; FFA: free fatty acids; Ra: rate of appearance; FFM: fat free mass.

Table 3. Use of medication before, directly after 6 and 18 months after a 16-week VLCD only or VLCD with exercise in obese insulin-dependent T2DM patients.

	VLCD + exercise				VLCD only			
	before start of the study	16 weeks	6 months	18 months	before start of the study	16 weeks	6 months	18 months
insulin (IU/day)	77	0	0	0	86	0	2	36
metformin (*)	10	13	13	12	9	14	13	12
SU-derivatives (*)	3	0	1	4	1	0	5	4
statine (*)	9	13	13	12	9	14	13	10
0/1 antihypertensiva (*)	6	8	8	6	3	6	7	7
2 antihypertensiva (*)	3	3	3	4	6	4	3	1
3 of more antihypertensiva (*)	4	2	2	3	5	4	4	6
exercise (min/week)	34	180	132	192	24	0	75	45

IE: international units; SU: sulfonylureum; * number of patients.

Effect on lipids and blood pressure

After the 16-week VLCD ± exercise intervention, systolic blood pressure, TG, cholesterol /HDL-cholesterol ratio and LDL-cholesterol decreased significantly (Table 2), whereas TC improved only in the VLCD+E group. At 18 months the effects on blood pressure and TG were completely abolished in both intervention groups while the beneficial effect on the cholesterol/HDL-cholesterol ratio was maintained. The decline in LDL-cholesterol was sustained only in the VLCD+E group.

Ten year coronary heart disease risk estimates (UKPDS risk score) showed a non-significant improvement in both intervention groups (baseline vs. 18 months follow up: 17.4±2.9% to 11.0±1.8% VLCD+E group and 24.7±4.3% to 19.2±3.5% VLCD-only group).

Effect on low-grade inflammation

Directly after the 16-week intervention hsCRP levels declined only in the VLCD+E group (p=0.011) (Table 4). All plasma cytokines in both intervention groups were equal or even higher compared to baseline values immediately after the intervention. On the contrary, at 6 months hsCRP was lower in both the VLCD-only group (p=0.005) and the VLCD+E group (p=0.005). Moreover, all pro-inflammatory cytokines (including TNFα and IL6) were significantly lowered in both groups. At 18 months, hsCRP and all measured pro-inflammatory cytokines were still reduced compared to baseline in the VLCD+E group (p=0.038). In the VLCD-only group, hsCRP (p=0.065) and IL1 (p=0.410) returned to baseline whereas other plasma cytokine levels remained lowered.

Table 4. Low-grade inflammation before, directly after 6 and 18 months after a 16-week VLCD only or VLCD with exercise in obese insulin-dependent T2DM patients and comparisons with (obese and lean) healthy controls.

		VLCD + exercise	VLCD only	controls	
				obese	lean
hsCRP	baseline	5.1 ± 0.9 *	5.5 ± 1.1 *	4.5 ± 0.7 *	1.7 ± 0.5
	16 weeks	2.8 ± 0.7 !	4.9 ± 2.0 *		
	6 months	1.6 ± 0.4 ! #	2.2 ± 0.5 !		
	18 months	2.2 ± 0.7 !	3.3 ± 0.5		
IFNγ	baseline	2.6 ± 0.9	2.3 ± 0.2	2.7 ± 0.5	1.8 ± 0.4
	16 weeks	2.5 ± 0.4	2.0 ± 0.1		
	6 months	1.0 ± 0.3 ! ?	1.6 ± 0.3 !		
	18 months	2.0 ± 0.9 !	1.6 ± 0.3 !		
IL10	baseline	10.2 ± 2.9 * #	6.7 ± 1.8 *	4.2 ± 0.6	3.5 ± 0.7
	16 weeks	15.2 ± 6.9 * #	6.7 ± 2.0		
	6 months	7.4 ± 3.7 * ?	3.6 ± 1.9 ! ?		
	18 months	5.4 ± 2.2 !	3.8 ± 1.5 ! ?		
IL1	baseline	0.5 ± 0.1	0.5 ± 0.1	0.2 ± 0.0	0.4 ± 0.1
	16 weeks	0.4 ± 0.1	0.8 ± 0.2 * #		
	6 months	0.2 ± 0.1 !	0.3 ± 0.1 ! ?		
	18 months	0.1 ± 0.0 ! ?	0.4 ± 0.1		
IL2	baseline	0.6 ± 0.1	1.0 ± 0.2 *	0.6 ± 0.1	0.3 ± 0.1
	16 weeks	0.7 ± 0.1	1.2 ± 0.2 * #		
	6 months	0.1 ± 0.0 ! ?	0.3 ± 0.1 ! ?		
	18 months	0.2 ± 0.1 ! ?	0.3 ± 0.1 ! ?		
IL6	baseline	2.0 ± 0.3	2.4 ± 0.4	3.1 ± 0.6	1.6 ± 0.4
	16 weeks	1.9 ± 0.2	2.2 ± 0.4		
	6 months	1.0 ± 0.1 ! ?	1.1 ± 0.2 ! ?		
	18 months	1.1 ± 0.2 ! ?	1.2 ± 0.2 ! ?		
IL8	baseline	6.3 ± 0.7 * #	7.5 ± 1.3 * #	2.6 ± 0.2 *	2.1 ± 0.1
	16 weeks	5.9 ± 0.5 * #	7.0 ± 0.9 * #		
	6 months	2.8 ± 0.4 ! ?	2.2 ± 0.3 ! ?		
	18 months	2.8 ± 0.3 ! ?	2.9 ± 0.4 ! ?		
TNFα	baseline	7.6 ± 1.3	6.6 ± 0.5	6.2 ± 0.6	5.8 ± 0.5
	16 weeks	7.2 ± 0.5 * #	7.0 ± 0.7		
	6 months	4.2 ± 0.5 ! ?	4.5 ± 0.6 ! ?		
	18 months	4.4 ± 0.5 ! ?	4.5 ± 0.6 ! ?		

Data are presented as mean ± SEM. ! significant difference vs. baseline values within the group; ? significant difference vs. 16 weeks values within the group; * significant difference vs. lean controls; # significant difference vs. obese controls.
hsCRP: high sensitive c-reactive protein; IFNγ: interferon gamma; IL: interleukin; TNFα: tumor necrosis factor alpha

Parameters of low-grade inflammation in healthy controls vs. patients

At baseline, both patient groups and the healthy obese controls had significantly higher hsCRP and IL8 levels compared to healthy lean controls, also patients had higher levels of the presumed anti-inflammatory cytokine IL10. Directly after the 16-week intervention hsCRP declined only in the VLCD+E group and not to the level of healthy lean controls. It was only 2 months after the cessation of both interventions (i.e. at 6 months), when patients were on a eucaloric diet, that parameters of chronic inflammation improved to the levels of healthy lean controls, with the exception of IL8 in the VLCD+E group (Table 4). At 18 months pro-inflammatory markers were still reduced and comparable to healthy lean controls in both patient groups.

DISCUSSION

This study shows that a 16-week VLCD or a 16-week VLCD+E have equal effects on lowering body weight and improving glucoregulation and dyslipidemia. Remarkably, this led not to an immediate lowering of markers of low-grade inflammation with the exception of a decrease in hsCRP in the VLCD+E group. Surprisingly, at 6 months all measured cytokines and hsCRP were significantly lower and comparable to the values in healthy lean controls. These effects were sustained after 18 months of follow-up. The direct effect of the 16-week intervention on the cardiovascular risk factors hypertension and dyslipidemia was not lasting; only cholesterol-HDL ratio and LDL-cholesterol were still lower compared to baseline at 18 months in the VLCD+E group.

The effect of dietary interventions on hsCRP levels has mostly been studied in obese non-diabetic subjects. Weight loss was clearly associated with a decrease in hsCRP in these subjects and is related to the amount of weight loss (9). Exercise had no, or only a minimal effect on hsCRP levels in either obese non-diabetic or obese diabetic patients (14,15,18). We showed that hsCRP levels decreased immediately after the intervention in the VLCD+E group. In the VLCD-only group, hsCRP levels declined just at six months when energy intake was eucaloric and weight loss still preserved. Interestingly, another study in obese T2DM patients, using a diet of ~1600 kcal for 8 weeks leading to 5-6 kg weight loss, also showed no improvement in hsCRP directly after the intervention but did so at 12 months follow-up (19). These findings could suggest that a VLCD combined with exercise is lowering activation of the acute phase response by the liver, while VLCD-only does not. The exact mechanism by which diets and exercise lower hsCRP levels needs further investigation.

In obese non-diabetic patients, diet, exercise or combined interventions have controversial effects on plasma IL6 and TNFα levels (10-13,20-22). Studies performed in obese T2DM patients are scarce. In one study, 7 obese T2DM women received a VLCD for 3 weeks which decreased plasma IL6 levels but had no effect on TNFα levels (16). We also observed no direct

effect of a VLCD of longer duration (16 weeks) on IL6 and TNFα levels but at 6 and 18 months both were improved compared to baseline in the two intervention groups alike. It should be noted that the effects of acute and chronic exercise are different (23). Acute exercise can elicit a pro-inflammatory response whereas chronic exercise is thought to mediate an anti-inflammatory effect. To purely study the chronic effects of exercise we performed our measurements after 2 days of abstinence of exercise. At 16 weeks no effects of a VLCD+E on plasma TNFα and IL6 levels were observed but at 6 and 18 months TNFα and IL6 levels were decreased compared to baseline. In several other exercise studies the effects on IL6 were equivocal and there were no effects on TNFα (15,22).

The effects on the presumed anti-inflammatory cytokine IL10 are paradoxical. We found elevated levels in both patient groups compared to healthy lean controls at baseline and a slow decrease in IL10 levels during the intervention with near-normalization at 18 months. On the contrary, a 4-week aerobic exercise training (3 times/week 60 minutes, level of intensity not specified) had no effect on IL10 levels in twenty obese T2DM patients (14) whereas a 6-month aerobic exercise intervention (4 times/week 45-60 minutes) significantly increased IL10 levels in 60 obese T2DM patients (24). Perhaps patient characteristics (obese vs. obese diabetics on insulin therapy), differences in intervention and/or assays used can explain the discrepancy found.

We could not find articles discussing the effects of a VLCD±exercise on the other cytokines measured (IL1, IL2 and INFγ). All these cytokines are pro-inflammatory cytokines. We hypothesize that this is the reason why these cytokines show the same trend as IL6 and TNFα.

The mechanisms by which CR, weight loss and exercise decrease plasma pro-inflammatory cytokines and increase anti-inflammatory cytokines are largely unknown. One putative mechanism is the decrease in visceral fat mass. Visceral adipose tissue (which consists of fat cells, adipose tissue macrophages (ATMs) and stromal vascular fraction) releases several so-called adipocytokines among which IL1, IL6 and TNFα that in turn stimulate CRP-production by the liver (25). Indeed, waist circumference, an indirect measure of visceral fat mass was decreased significantly as compared to baseline at all measured time points. Furthermore, the decrease in waist circumference was larger in the VLCD+E group, which indeed exhibited the greatest although non-significant beneficial effects on plasma cytokines. However, we could not find a correlation between the change in waist circumference and the change in plasma cytokines. The lack of an immediate effect of a VLCD+/-E on low-grade inflammation has been observed by others. In 22 obese women undergoing a 4 week VLCD (800 kcal/day) followed by a 2-month hypocaloric diet (-600 kcal of estimated energy requirements) a euglycaemic hyperinsulinaemic clamp with adipose tissue biopsies for adipocyte and macrophage gene expression and markers were performed (26). It appeared that adipocyte genes involved in metabolism were downregulated during the VLCD and upregulated during weight stabilization (hypocaloric diet) whereas macrophage genes involved in inflammatory pathways were upregulated during the VLCD (with increased plasma inflammatory markers) and downregulated during the

hypocaloric diet (with decreased plasma inflammatory markers). This is compatible with our findings of lower levels of inflammation after the 16 week-intervention during the weight stabilization period, and has recently been further investigated in mice (27). CR led to a gradual decrease in body weight and fat mass in previously high-fat fed mice due to a decrease in adipocyte size not number. However, ATM content increased during the first 3 days of the diet (and these were lipid-loaded macrophages) to gradually decrease thereafter. This rise and fall of ATM content was closely correlated with plasma free fatty acid levels and adipose tissue lipolysis. Total lipolysis is the sum of basal lipolysis (which is elevated in adiposity and greater in visceral vs. subcutaneous adipose tissue) and demand lipolysis (which is driven by hormones in response to nutritional demands). The authors speculate that in early weight loss, adipocyte size is the same and therefore basal lipolysis remains high. However, demand lipolysis increases with an increase in local free fatty acids that subsequently attract ATMs. These ATMs phagocytose excess lipids and might even secrete antilipolytic factors followed by a decrease of the free fatty acid-driven cytokine production. We only measured rate of appearance of glycerol and plasma free fatty acids in the patients before and after 16 weeks of a VLCD+/-E. Indeed, at 16 weeks free fatty acids levels were equal as compared to the start of the diet and rate of appearance of glycerol, which was high at baseline, had not changed in the VLCD-only group whereas it was further increased in the VLCD+E group. This suggests that our patients were still having a high (demand) lipolytic rate at 16 weeks (while still on the VLCD) with probably still elevated levels of ATMs, explaining the lack of decrease in plasma cytokines at 16 weeks. We presume that when a eucaloric diet was reinstituted the demand lipolysis decreased with lower local free fatty acid levels, a decrease in ATMs and subsequent decrease in plasma cytokines. This hypothesis, already tested in mice (27), should be further investigated in obese humans during various stages of CR with adipose tissue biopsies. The set-up will be limited however, by the fact that it is difficult to obtain visceral fat biopsies.

A limitation of the present study is the relatively small sample size. Nonetheless, our results are in line with the existing literature. Furthermore we measured the control population once only. The reason for this is that the lean control subjects were not able to perform a 16 week VLCD. Another limitation is the lack of fat biopsies. Fat biopsies could have provided more information for example with regard to the amount of macrophages in the fat mass. A possible confounder in the data could be that all patients in who insulin therapy was reinstated at 18 months came from the VLCD-only group, this could mean higher advanced glycated end products in this group. However fasting glucose levels and HbA1c levels were similar in both groups at 18 months, other advanced glycated end products have not been measured.

In conclusion, a 16-week VLCD with or without exercise does not directly decrease pro-inflammatory plasma cytokine levels despite a large decrease in body weight and waist circumference and an improvement in dyslipidemia and glucoregulation. The beneficial effects on chronic inflammation became apparent only when patients were on a eucaloric regular diet and were sustained up till 18 months after the start of the diet despite bodyweight re-

gain and deterioration of glucoregulation. Our findings are compatible with the hypothesis that demand lipolysis activation during CR leads to local free fatty acid accumulation with attraction of ATMs and hence an increased production of cytokines (27). When nutritional status is eucaloric and demand lipolysis decreases the amount of ATMs will decrease along with a reduction in cytokine production. This hypothesis should be confirmed in humans with adipose tissue biopsies during various amounts of calorie restriction. The initial question was whether exercise has additional beneficial effects on low-grade inflammation. This appears to be true only for hsCRP. Nonetheless, since chronic inflammation is associated with cardiovascular risk this study again shows the importance of weight reduction and exercise for the modification of these risk factors.

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