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

Snel, M.

CHAPTER 3

Adding exercise to a 16-week very low calorie diet in obese, insulin-dependent type 2 diabetes mellitus patients improves metabolic flexibility, VO_{2max} and mitochondrial copy number in skeletal muscle

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Submitted

ABSTRACT

Objective. Loss of 50% overweight using a very low calorie diet (VLCD, 450kcal/day) improves insulin sensitivity in obese type 2 diabetes mellitus patients. This study investigates whether adding exercise to the VLCD has additional benefits.

Methods. Twenty-seven obese (BMI 37.2±0.9kg/m^2 (mean±SEM)) insulin-treated type 2 diabetes mellitus patients followed a 16-week VLCD. Thirteen of them simultaneously participated in an exercise program (E) consisting of one-hour in-hospital training and four 30-minute training sessions on a cyclo-ergometer weekly. Oral glucose-lowering agents and insulin were discontinued 3 weeks prior to and at the start of the VLCD respectively. Anthropometric measurements, hyperinsulinaemic euglycaemic clamp with skeletal muscle biopsies and peak oxygen consumption testing (VO_{2max}) were performed before and after the intervention.

Results. Baseline characteristics were identical in both groups. Substantial weight loss occurred (-23.7±1.7kg VLCD-only vs. -27.2±1.9kg VLCD+E, p=NS). The exercise-group lost more fat mass. Glycemic control improved considerably. Insulin-stimulated glucose disposal increased similarly in both study groups (15.0±0.9 to 39.2±4.7µmol.min^{-1}.$kglbm^{-1}$ VLCD-only vs. 17.0±1.0 to 37.5±3.5 µmol.min^{-1}.$kglbm^{-1}$ in VLCD+E), as did phosphorylation of PI3K-PKB/AKT insulin signaling pathway. In contrast, skeletal muscle mitochondrial DNA (mtDNA) content increased only in the VLCD+E group (1211±185 to 2288±358, arbitrary units p=0.016 vs. 1397±240 to 1196±179, p=NS VLCD-only group). VO_{2max} also only increased significantly in the VLCD+E group (+6.6±1.7 ml.min^{-1}.$kglbm^{-1}$ vs. +0.7 ±1.5 ml.min^{-1}.$kglbm^{-1}$ VLCD-only, p=0.017).

Conclusions. Addition of exercise to a 16-week VLCD induces more fat loss. Exercise augments VO_{2max} and skeletal muscle mtDNA content. These changes are, however, not reflected in a higher insulin-stimulated glucose-disposal rate.

INTRODUCTION

In the obese type 2 diabetes mellitus patients (T2DM) insulin resistance is of pivotal importance. Caloric restriction, increasing physical activity and cognitive restructuring are the mainstays of the treatment of obesity especially in case T2DM is present (1). Diet-induced weight loss in obese T2DM improves insulin resistance and phosphatidylinositol 3-kinase-protein kinase B/AKT (PI3K-PKB/AKT) insulin signaling in the skeletal muscle (2).

Moderate exercise (70% of maximum aerobic capacity) does not play a major role in losing body weight but helps to maintain diet-induced weight loss. Moderate exercise can enhance peripheral insulin sensitivity even without weight loss (3). Exercise plays an important role in improving mitochondrial capacity and aerobic fitness in healthy individuals (4).

The current study compares the effect of a very low calorie diet (VLCD) with and without an exercise program in obese insulin-dependent T2DM patients to elucidate whether the addition of exercise to a VLCD has incremental benefits in terms of weight reduction, glucoregulation, insulin sensitivity, myocyte morphology and mitochondrial capacity.

PATIENTS AND METHODS

Patients

Twenty-seven sedentary (14 males, 13 females) T2DM patients were enrolled in the study. Clinical details are summarized in Table 1. All patients were obese (BMI >30 kg/m^2) and used at least 20 units of insulin per day, with or without oral glucose-lowering medication. In addition, patients had to have residual beta-cell capacity, defined as a fasting plasma C-peptide level greater than 0.8 ng/mL and a 2-fold increase of the basal C-peptide level in response to administration of 1 mg glucagon intravenously (5). The residual beta-cell capacity is necessary to safely stop all glucose-lowering medication.

Exclusion criteria were smoking, recent weight change, any other chronic (endocrine) conditions and silent cardiac ischemia. Written informed consent was obtained from all patients. The study was approved by the local ethics committee.

Study design

Throughout the 16-week intervention period no blood glucose-lowering medication (both oral and insulin therapy), was utilized by the patients. All oral blood glucose and lipid lowering medication was discontinued 3 weeks prior to the study (because of the longer half-life of some of the oral glucose-lowering medication). Insulin therapy was intensive during these three weeks to prevent hyperglycemia. One day before the start of the intervention only short-acting insulin was prescribed, and long-acting insulin was omitted to prevent the presence of exogenous insulin during the hyperglycaemic euglycaemic clamp the next day.

After a baseline visit (outlined below), all patients started a 16-week VLCD (Modifast®, Nutrition & Santé, Antwerpen, Belgium). Modifast® provides a total of ~450 kilocalories per day and all necessary vitamins and micronutrients, divided over 3 meals of liquid shakes. Modifast provides about 50 g protein, 50 to 60 g carbohydrate, 7 to 9 g lipid, and 10 g of dietary fibre.

Thirteen of the 27 subjects were randomized to follow an exercise program simultaneously. This exercise program consisted of at least four training sessions per week at home and a one-hour in-hospital training. For the home-training sessions patients had to exercise at least 4 times a week for 30 minutes at 70% of their maximum aerobic capacity on a cyclo-ergometer. The one-hour in-hospital training entailed primarily aerobic exercise, under supervision of a physiotherapist. Patients in the VLCD-only group were instructed not to alter their pattern of physical activity.

During the 16-week intervention period patients visited the outpatient clinic weekly to confirm compliance by questionnaires, providing sachets of Modifast® and reading the heart rate monitor (Polar S610 $_{i}^{tm}$, Polar Electro Oy, Finland), which recorded the duration, heart rate and intensity of every training session. Patients were instructed not to perform physical activity in the last 48 hours before the hyperinsulinaemic euglycaemic clamps.

Hyperinsulinaemic euglycaemic clamp

All studies started after an overnight fast. Height, weight and waist circumference were measured. Lean body mass was assessed by bioelectrical impedance analysis (Bodystat® 1500, Bodystat Ltd., Douglas, Isle of Man, UK).

Metabolic studies were performed as described previously (6). In short, first samples were taken for the measurement of basal levels of glucose, insulin, and background enrichment of [6,6-$^{2}H_{2}$]-glucose and [$^{2}H_{5}$]-glycerol. Basal rates of glucose and glycerol turnover were assessed after 3 hours of continuous infusion of [6,6-$^{2}H_{2}$]-glucose and 1.5 hours of continuous infusion of [$^{2}H_{5}$]-glycerol (Cambridge Isotopes, Cambridge, USA). Subsequently, insulin-stimulated rates of glucose and glycerol turnover were measured after 4.5 hours of a hyperinsulinaemic euglycaemic clamp (Actrapid®, Novo Nordisk Pharma, Alphen aan de Rijn, The Netherlands; rate 40 mU/m^{2}/min). Glucose values were clamped at 5.5 mmol/L via the infusion of a variable rate of 20 % glucose enriched with 3 % [6,6-$^{2}H_{2}$]-glucose.

A physiological and isotopic steady-state was achieved during the last 30 min of both the basal as well as the hyperinsulinaemic period, therefore, the rates of appearance (R_a) and disappearance (R_d) for glucose and glycerol were calculated as the tracer infusion rate divided by the tracer-to-tracee ratio (7). Endogenous glucose production (EGP) during the basal steady-state is similar to the R_a of glucose, whereas EGP during the clamp was calculated as the difference between the rates of glucose appearance and infusion. The hepatic insulin resistance index (HIR) (µmol/min/kg$_{LBM}$/pmol*L) was calculated as the product of EGP and plasma insulin concentration (8). The metabolic clearance rate of insulin (MCR_I) was calculated as the constant infusion rate of insulin divided by the steady-state insulin concentra-

tion corrected for endogenous insulin secretion (basal insulin concentration x [steady state c-peptide/basal c-peptide concentration) (9).

Aerobic fitness

Each subject performed an incremental cyclo-ergometer exercise test to determine their maximum oxygen consumption ($VO_{2\ max}$) both before and directly after the intervention period. Exercise intensity was progressively increased while measuring ventilation, oxygen and carbon dioxide concentration of the inhaled and exhaled air. $VO_{2\ max}$ was reached when oxygen consumption remained constant despite an increase in workload.

Indirect calorimetry

Both under basal and hyperinsulinaemic conditions, indirect calorimetry with a ventilated hood (Oxycon Beta, Mijnhardt Jaegher, Breda, The Netherlands) was performed for 30-min. The molar ratio of oxygen consumed to carbon dioxide produced was used to calculate total glucose and lipid oxidation rates as described previously by Simonson and DeFronzo (10). Non-oxidative glucose disposal (NOGD), as a measurement for glycogen storage was calculated by subtracting the glucose oxidation rate from R_d of glucose.

Muscle biopsy (Mitochondria, Insulin Signalling, Oil Red O staining)

Under localised anaesthesia, with 1% lidocaine, muscle biopsies were taken from the vastus lateralis muscle under basal conditions and 30 minutes after the start of the insulin infusion (6) using a modified Bergström needle. Muscle samples were divided into two parts: one frozen in liquid nitrogen for subsequent determination of insulin signaling, whereas the other part was snap-frozen in liquid nitrogen-cooled isopentane and stored at -80°C for determination of intramyocellular lipid accumulation (IMCL).

mtDNA content was assessed using a modification of the quantitative real-time PCR-based method as we described previously (11). Insulin signaling was measured as described previously (12). Tissue sections of basal biopsies were stained with Oil Red O (ORO) combined with a double-immunofluorescence assay (anti-laminin and a monoclonal antibody raised against adult human slow myosin heavy chain) to allow quantification of IMCL as described previously (13).

ASSAYS

Serum insulin was measured with an immunoradiometric assay (Biosource, Nivelles, Belgium). Serum C-peptide levels were measured with a radioimmuno assay (Linco Research, St. Charles MO, USA). HbA1c was measured with a semi automated HPLC machine Primus Ultra 2 (Kordia, Leiden, the Netherlands)

Plasma free fatty acids (FFAs) concentrations were measured by a commercial kit (Wako Chemicals, Neuss, Germany). [6,6-$^2H_2$]-glucose and [$^2H_5$]-glycerol were measured in a single analytical run using gas chromatography-mass spectrometry as described previously (14).

STATISTICAL ANALYSIS

Results are expressed as mean ± standard error (SEM). Paired *t* tests were applied to assess mean differences before and after the intervention within groups, whereas unpaired *t* tests were used to assess differences in means or deltas between groups. Non-parametric tests (Wilcoxon signed-rank test for paired samples, Mann-Whitney for unpaired samples resp.) were performed, when appropriate. Significance level was set at $p < 0.05$. Statistical analyses were performed using SPSS for Windows (release 16.0, SPSS, Inc., Chicago, IL).

RESULTS

Anthropometric measurements

As shown in Table 1, the baseline (pre-intervention) characteristics of the two patient groups (VLCD with exercise (VLCD+E) and VLCD-only) did not differ with respect to both clinical and metabolic parameters. After the 16-week intervention both groups (VLCD+E and VLCD-only) showed significant improvements in clinical and metabolic characteristics.

Similar weight loss was achieved in both patient groups (-27.2±1.9 kg VLCD+E; -23.7±1.6 kg VLCD-only). The VLCD+E group lost significantly more fat mass (-21.8±2.2 kg VLCD+E; -16.6±1.7 kg VLCD-only) and also waist circumference decreased more (-25±1 kg VLCD+E; -19±2 kg VLCD-only) compared to the VLCD-only group (Table 1).

Glucose and lipid metabolism

After the intervention fasting plasma glucose, insulin and HbA_{1C} levels improved substantially and similarly in both intervention groups. During the clamp pre- and post-intervention steady state plasma glucose concentrations were similar in the two intervention groups (VLCD+E 5.4±0.4 vs. 5.4±0.5 mmol/L; VLCD-only 5.5±0.7 vs. 5.6±0.6 mmol/L, pre- and post-intervention resp.; both NS) Steady state plasma insulin during the clamp was significantly lower after the intervention but similar in both groups, as a result of an increase in clearance of exogenous insulin after weight loss (Table 2).

There was a similar reduction in basal EGP in both patient groups. The HIR index diminished both under basal and hyperinsulinaemic conditions after the intervention to a similar extent in both patient groups. Also peripheral insulin sensitivity improved considerably; glucose R_d increased with ~150% in both patient groups. The R_a of glycerol as a measure of the rate

Table 1. Clinical characteristics, body composition and fasting plasma levels before and after a 16-week VLCD +/- exercise in obese insulin-dependent type 2 diabetes mellitus patients.

	VLCD only			VLCD + exercise		
	baseline	after 16 wks		baseline	after 16 wks	
sex (M/F)	6/8			8/5		
age (years)	56.1 ± 2.4			53.0 ± 2.5		
weight (kg)	112.7 ± 5.6	89.0 ± 4.3	*	113.5 ± 5.1	86.3 ± 4.2	*
BMI (kg/m^2)	37.9 ± 1.4	30.0 ± 1.1	*	36.4 ± 1.1	27.7 ± 1.0	*
waist (cm)	122 ± 3	103 ± 3	*	123 ± 3	98 ± 3	* $
fat mass (kg)	49.9 ± 3.6	33.2 ± 2.8	*	45.4 ± 3.2	23.5 ± 2.2	* $
systolic bloodpressure (mmHg)	161 ± 4	140 ± 4	*	145 ± 5	132 ± 5	*
diastolic bloodpressure (mmHg)	87 ± 3	78 ± 2	*	81 ± 3	75 ± 3	
HbA1c (%)	7.8 ± 0.3	6.7 ± 0.3	*	7.8 ± 0.4	6.3 ± 0.4	*
fasting plasma glucose (mmol/L)	12.1 ± 0.5	7.7 ± 0.6	*	10.9 ± 0.7	6.6 ± 0.8	*
fasting insulin (mU/L)	24.4 ± 4.3	12.6 ± 2.0	*	25.1 ± 2.2	8.8 ± 0.8	*
fasting C-peptide (nmol/L)	2.9 ± 0.3	2.2 ± 0.2		3.5 ± 0.3	2.0 ± 0.1	*
metformin (number of patients)	9	0		10	0	
SU-derivative (number of patients)	1	0		3	0	
average insulin (IU/day)	86.2	0		77.2	0	

Data are presented as mean±SEM. * $p<0.001$ within the group; $ $p< 0.05$ between the groups.
M: male F: female; kg: kilogram; cm: centimeter; IU: international units.

of lipolysis in basal conditions was somewhat higher after VLCD+E (whereas VLCD-only did not affect this measure), but the capacity of insulin to suppress lipolysis improved only by VLCD+E (Table 2).

Insulin signaling

Both patient groups had a higher insulin receptor (IR) expression after the intervention. IR expression increased further in the VLCD+E compared to the VLCD-only. Phosphorylation of proline rich substrate 40 (PRAS40) was similarly increased in both groups after the intervention, in basal as well as in hyperinsulinaemic conditions. AKT substrate 160 (AS160) phosphorylation showed a similar trend, but this increase was not statistically significant (Figure 1).

Glucose and lipid oxidation rates

Basal lipid oxidation and NOGD increased significantly post-intervention only in the VLCD+E group (Table 2). Before the intervention the switch between glucose and lipid oxidation was lost, post-intervention this improved in both groups. The basal lipid oxidation and insulin-mediated suppression of lipid oxidation improved more in the VLCD+E group.

Table 2. Metabolic parameters before and after a 16-week VLCD +/- exercise in obese insulin-dependent type 2 diabetes mellitus patients.

	VLCD only		VLCD+ exercise	
	baseline	after 16 wks	baseline	after 16 wks
basal EGP (μmol/kg_{LBM}/min)	17.7 ± 0.7	15.1 ± 0.6 *	17.1 ± 0.7	14.1 ± 0.4 *
clamp EGP (μmol/kg_{LBM}/min)	4.8 ± 0.7	1.2 ± 0.6 *	3.1 ± 0.8	1.8 ± 0.6
suppression EGP (%)	-73.7 ± 3.4	-93.1 ± 3.2 *	-82.0 ± 4.5	-87.6 ± 4.1
basal HIR (μmol/kg_{LBM}/min/pmol*L)	3009 ± 419	1242 ± 187 *	2644 ± 267	983 ± 136 *
clamp HIR (μmol/kg_{LBM}/min/pmol*L)	3492 ± 558	737 ± 335 *	2135 ± 572	1134 ± 392 *
glucose R_d (μmol/kg_{LBM}/min)	15.5 ± 1.2	38.6 ± 4.6 *	16.6 ± 1.2	41.8 ± 3.6 *
clamp insulin (mU/L)	102.4 ± 9.0	86.6 ± 7.3 *	102.0 ± 5.6	79.4 ± 5.5 *
metabolic clearance rate insulin (ml/m^2/min)	8.9 ± 1.6	17.9 ± 4.2 *	7.3 ± 1.3	16.2 ± 2.0 *
basal glucose ox. (μmol/kg_{LBM}/min)	10.9 ± 1.5	6.1 ± 1.4 *	15.8 ± 1.8	3.6 ± 1.1 * $
clamp glucose ox. (μmol/kg_{LBM}/min)	16.2 ± 2.1	17.4 ± 1.8	16.9 ± 2.2	12.9 ± 1.6
increase glucose ox. (μmol/kg_{LBM}/min)	5.2 ± 1.6	11.3 ± 1.9 *	1.0 ± 1.3	9.3 ± 1.2 *
basal NOGD (μmol/kg_{LBM}/min)	6.8 ± 1.4	9.1 ± 1.4	1.4 ± 1.7	10.5 ± 1.2 * $
clamp NOGD (μmol/kg_{LBM}/min)	0.0 ± 2.4	21.3 ± 4.2 *	0.0 ± 2.1	29.0 ± 2.7 *
basal R_a glycerol (μmol/kg_{FM}/min)	11.3 ± 1.2	11.3 ± 1.3	12.9 ± 1.0	15.9 ± 1.1 *
clamp R_a glycerol (μmol/kg_{FM}/min)	5.9 ± 0.9	5.8 ± 1.0	7.2 ± 1.3	7.9 ± 1.3
suppression R_a glycerol (μmol/kg_{FM}/min)	-5.4 ± 0.7	-5.5 ± 0.8	-5.6 ± 0.8	-7.9 ± 0.9 *
basal FFA levels (mmol/L)	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.0
clamp FFA levels (mmol/L)	0.3 ± 0.0	0.2 ± 0.1	0.3 ± 0.0	0.1 ± 0.0
basal lipid ox. (μmol/kg_{LBM}/min)	7.0 ± 0.4	6.6 ± 0.3	4.9 ± 0.4	6.9 ± 0.3 * $
clamp lipid ox. (μmol/kg_{LBM}/min)	4.6 ± 0.6	3.6 ± 0.5	4.7 ± 0.5	4.6 ± 0.5
suppression lipid ox. (μmol/kg_{LBM}/min)	-2.4 ± 0.6	-2.9 ± 0.6	-0.2 ± 0.4	-2.4 ± 0.4 *

Data are presented as mean±SEM.* $p<0.001$ within the group; $ $p< 0.05$ between the groups.
EGP: endogenous glucose production; LBM: lean body mass; HIR: hepatic insulin resistance index; Rd: rate of disappearance; Ra: rate of appearance, FM: fat mass; FFA: free fatty acid; ox: oxidation; NOGD: non-oxidatieve glucose disposal.

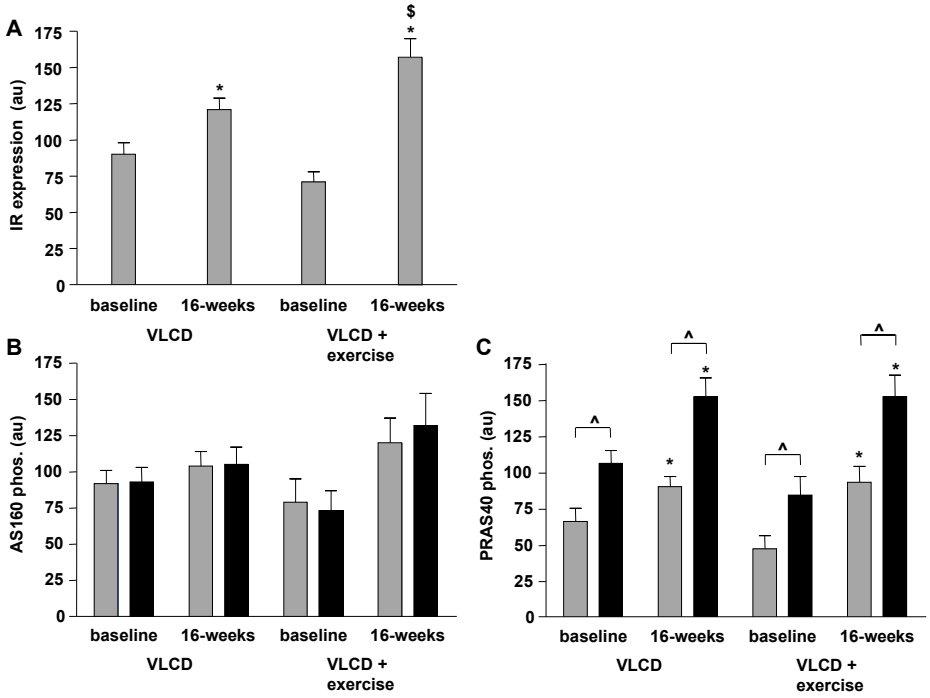
Maximal aerobic capacity

At baseline, VO_{2max} was similar in both groups. Participants in the VLCD-only group had a non-significant change (0.7±1.5 mg/kg_{LBM}/min difference from baseline) in $VO2_{max}$. In contrast there was a significant increase in VO_{2max} in the VLCD+E group (6.6±1.7 mg/kg_{LBM}/min difference from baseline) (Figure 2A).

Mitochondria

Muscle mtDNA content was not affected following a VLCD-only (1398±240 vs. 1127±180 au, pre- and post-intervention resp.). In contrast, the VLCD+E group showed a significant increase following the intervention (1211±185 vs. 2288±359 au, pre- and post-intervention resp.; $p<0.05$) (Figure 2B).

Figure 1. A. Insulin receptor (IR) expression at baseline and after 16 weeks of intervention; B. basal (grey bars) and insulin stimulated (black bars) Akt substrate 160 (AS160) phosphorylation (phos); C. basal (grey bars) and insulin stimulated (black bars) Proline rich Akt substrate 40 (PRAS40) phosphorylation.



* p<0.05 within the group compared to baseline; $ p< 0.05 between the groups; ^ p<0.05 basal vs. insulin stimulated.
au: arbitrary units.

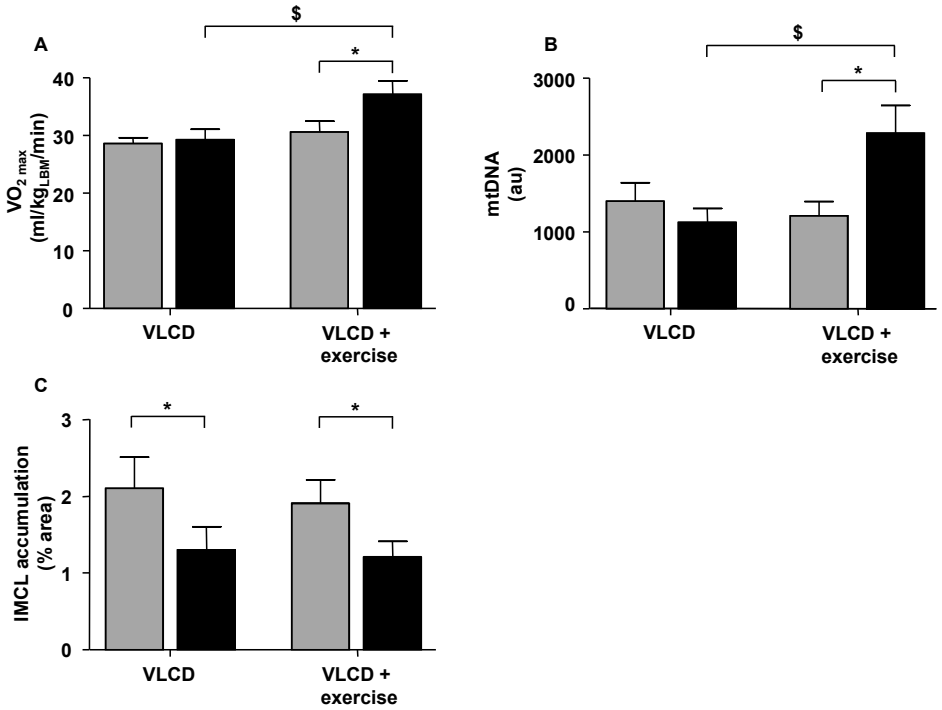
Muscle morphology and IMCL

In both intervention groups there was a similar decline in IMCL accumulation in the skeletal muscle fibers after the 16-week intervention (Figure 2C). The percentage of type 1 fibers in the skeletal muscle increased, whereas the percentage of type 2 fibers decreased significantly and similarly in both groups (VLCD+E type 1 and 2: 52.4±3.0% and 47.6±3.0% vs. 58.4±3.2% and 41.6±3.2% pre- and post-intervention resp.; both p<0.05; VLCD-only type 1 and 2: 51.8±3.0% and 48.2±3.0% vs. 60.7±4.1% and 39.3±4.1%, pre- and post-intervention resp.; both p<0.05).

DISCUSSION

A 16-week VLCD in obese insulin-dependent T2DM patients with or without moderate intense exercise resulted in substantial weight reduction and decrease of waist circumference. Exercise did not result in extra weight loss. However, it induced a greater loss of fat mass

Figure 2. A: Maximum aerobic capacity (VO_{2max}); B: mitochondrial DNA (mtDNA) content of the skeletal muscle; C: intramyocellular lipid accumulation (IMCL) as relative fraction of cell area containing lipid droplets at baseline (grey bars) and after (black bars) a 16-week VLCD +/- exercise in obese insulin-dependent type 2 diabetes mellitus patients.



* p<0.05 within the group; $ p< 0.05 between the groups.
LBM: lean body mass, au: arbitrary units.

and thus conservation of lean body mass. Glucoregulation improved to the same extent in both groups despite the cessation of all glucose-lowering agents including insulin. Insulin sensitivity of the liver, adipose tissue and skeletal muscle improved similarly in both groups, which is in accordance with the observed similar improvement in insulin signaling and decrease in IMCL in skeletal muscle. Also a significant increase in type 1 oxidative muscle fibers was observed in both groups. Maximal aerobic capacity and mitochondrial copy number increased only in the exercise group while these parameters remained unchanged in the VLCD-only group.

The current study confirms that diet-induced weight reduction improves glucoregulation in obese patients with T2DM by ameliorating both hepatic and peripheral insulin resistance. The high dosage used during insulin infusion almost completely suppressed EGP in both groups. Since plasma insulin clearance was increased by weight loss, insulin infusion resulted in lower circulating insulin concentrations during clamp steady state after the intervention. Thus, for a more accurate comparison of hepatic insulin sensitivity before and after the intervention the HIR was used which showed improved hepatic insulin resistance in both groups.

The current study failed to show additional effects of 16-weeks moderate intense exercise on both peripheral and hepatic insulin sensitivity. One possible explanation for this lack of an additional effect of exercise might be that the magnitude of caloric restriction and the achieved weight loss masked the potential additional effect of exercise.

The substantial increase in peripheral insulin sensitivity is in accordance with the increased level of insulin receptor expression and improvement in the PI3K-PKB/AKT insulin signaling pathway in skeletal muscle cells as reflected by increased PRAS40 phosphorylation. These data confirm the results of other studies showing that diet-induced weight loss (2) or the combination with exercise improve peripheral insulin sensitivity (15,16) and the insulin signaling pathway (17) in obese patients with or without T2DM. In our study, insulin receptor expression increased even further with the addition of exercise. However, this was not accompanied by further improvement of glucoregulation, as evidenced by similar baseline and insulin induced levels of PRAS40 and AS160 phosphorylation in both groups.

A low oxidative capacity leads to accumulation of lipids in the skeletal muscle. IMCL content is elevated in the skeletal muscle in obese T2DM patients and associated with insulin resistance. Not IMCLs per se, but IMCL derivatives, such as diacylglycerol (DAG) and long chain fatty acid-CoA are known to activate protein kinase C, which in turn, phosphorylates the serine residue of insulin receptor substrate 1 (IRS1). Serine-phosphorylated IRS1 is unable to activate PI3K and leads to disruption in the PI3K-PKB/AKT insulin signaling cascade (18). The improvement in insulin signaling found in our study might partially be explained by the observed decrease in IMCL content in both intervention groups. IMCL content in the skeletal muscle is not only increased in obese and insulin resistant subjects, it is also high in endurance trained athletes; here IMCLs represent a physiological role as readily available energy source. In literature, exercise in obese non-diabetic and obese T2DM subjects, either increased (19), decreased (15,20) or led to unchanged (21,22) IMCL accumulation. In our group of patients a possible exercise-induced increase in IMCL could be hidden by the strong effect of caloric restriction and weight loss.

Prospective studies have shown that a low ability to oxidize fat is a risk factor for weight gain, obesity and insulin resistance (23). Impaired muscle fatty acid oxidation, reflected by reduced number of mitochondria, could be considered as a primary defect causing IMCL accumulation in T2DM patients (24). T2DM patients are characterized by low basal fat oxidation and increased lipogenesis rates (25). The current study showed that only a VLCD with exercise increased the reliance on lipid oxidation and lipolysis during fasting after the intervention.

It has been shown that the reduced muscle mitochondrial content and functional capacity in obese subjects are reversible with moderate weight loss (10%) combined with moderate-intensity regular physical activity (16,21). This suggests that sedentary behavior might be responsible for the reduction in mitochondrial capacity in obese T2DM patients. Indeed, here we showed that only combining VLCD with exercise increases mitochondrial copy number. However, the increase in mitochondrial copy number in the VLCD with exercise group was

not associated with a further decrease in IMCL and a greater increase in glucose disposal rate as compared to VLCD-only group.

We found an identical and significant increase in type 1, and decrease in type 2 muscle fibers in both intervention groups. A low capacity to oxidize fat due to a low percentage of type 1 (oxidative) muscle fibers might lead to obesity and T2DM, although a causal relation has not been established. To the best of our knowledge this is the first study which shows a significant increase in type 1 oxidative muscle fibers in T2DM patients after weight loss. Type 2 muscle fibers are responsible for generating strength and power, the decrease in type 2 muscle fibers could well be a reflection of reduced weight-bearing, since both groups lost similar but excessive amounts of weight after the intervention.

Even moderately-intense regular exercise leads to improved fitness in obese T2DM patients as shown in the present and previous studies (20,21,26), whereas aerobic capacity did not improve in the group receiving dietary advice without exercise program.

In conclusion, diet-induced weight loss improves insulin sensitivity and glucoregulation, decreases intramyocellular lipids, and improves insulin signaling in skeletal muscle. Adding exercise to the diet leads to additional loss of fat mass and conservation of lean body mass, an increased number of intramyocellular mitochondria and an improvement of maximum aerobic capacity. However, despite all these beneficial metabolic effects, exercise does not reinforce insulin action in this setting, perhaps because a VLCD per se ameliorates insulin resistance to a maximal extent in obese patients with T2DM.

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