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Effects of Adding Exercise to a 16-Week Very Low-Calorie Diet in Obese, Insulin-Dependent Type 2 Diabetes Mellitus Patients

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Context: Reduction of 50% excess body weight, using a very low-calorie diet (VLCD; 450 kcal/d) improves insulin sensitivity in obese type 2 diabetes mellitus patients.

Objective: The objective of the study was to evaluate whether adding exercise to the VLCD has additional benefits.

Design: This was a randomized intervention study.

Setting: The study was conducted at a clinical research center in an academic medical center.

Subjects: Twenty-seven obese [body mass index 37.2 $\pm$ 0.9 kg/m^2 (mean $\pm$ SEM)] insulin-treated type 2 diabetes mellitus patients.

Intervention: Patients followed a 16-wk VLCD. Thirteen of them simultaneously participated in an exercise program (E) consisting of 1-h, in-hospital training and four 30-min training sessions on a cycloergometer weekly.

Outcome Measures: Insulin resistance was measured by a hyperinsulinemic euglycemic clamp. Insulin signaling, mitochondrial DNA (mtDNA) content, and intramyocellular lipid content was measured in skeletal muscle biopsies.

Results: Baseline characteristics were identical in both groups. Substantial weight loss occurred (-23.7 ± 1.7 kg VLCD-only *vs.* -27.2 ± 1.9 kg VLCD+E, P = NS within groups). The exercise group lost more fat mass. Insulin-stimulated glucose disposal increased similarly in both study groups [15.0 $\pm$ 0.9 to 39.2 $\pm$ 4.7 μmol/min^{-1} $\cdot$ kg lean body mass (LBM^{-1}) VLCD-only *vs.* 17.0 $\pm$ 1.0 to 37.5 $\pm$ 3.5 μmol/min^{-1} $\cdot$ kg LBM^{-1} in VLCD+E], as did phosphorylation of the phosphatidylinositol 3-kinase-protein kinase B/AKT insulin signaling pathway. In contrast, skeletal muscle mtDNA content increased only in the VLCD+E group (1211 $\pm$ 185 to 2288 $\pm$ 358, arbitrary units, $P = 0.016$ *vs.* 1397 $\pm$ 240 to 1196 $\pm$ 179, P = NS, VLCD-only group). Maximum aerobic capacity also only increased significantly in the VLCD+E group (+6.6 $\pm$ 1.7 ml/min^{-1} $\cdot$ kg LBM^{-1} *vs.* +0.7 $\pm$ 1.5 ml/min^{-1} $\cdot$ kg LBM^{-1} VLCD-only, $P = 0.017$).

Conclusion: Addition of exercise to a 16-wk VLCD induces more fat loss. Exercise augments maximum aerobic capacity and skeletal muscle mtDNA content. These changes are, however, not reflected in a higher insulin-stimulated glucose disposal rate. **(*J Clin Endocrinol Metab* 97: 2512–2520, 2012)**

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Abbreviations: AS160, Akt substrate 160; BMI, body mass index; EGP, endogenous glucose production; FFA, free fatty acid; HbA1c, glycosylated hemoglobin; IMCL, intramyocellular lipid accumulation; IR, insulin receptor; LBM, lean body mass; mtDNA, mitochondrial DNA; NOGD, nonoxidative glucose disposal; PI3K, phosphatidylinositol 3-kinase; PKB, protein kinase B; PRAS40, phosphoproline-rich Akt substrate 40; R_a, rate of appearance for glucose and glycerol; R_d, rate of disappearance for glucose and glycerol; VLCD, very low-calorie diet; VO_{2max}, maximum oxygen consumption.

In obese type 2 diabetes mellitus patients, insulin resistance is of pivotal importance. Caloric restriction, increasing physical activity, and cognitive restructuring are the mainstays of the treatment of obesity, especially in the case when type 2 diabetes mellitus is present (1). Diet-induced weight loss in obese type 2 diabetes mellitus improves insulin resistance and phosphatidylinositol 3-kinase (PI3K)-protein kinase B (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 (3). Moderate exercise can enhance peripheral insulin sensitivity even without weight loss (4). Exercise plays an important role in improving mitochondrial capacity and aerobic fitness in healthy individuals (5).

The current study compared the effect of a very low-calorie diet (VLCD) with and without an exercise program in obese, insulin-dependent, type 2 diabetes mellitus 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.

Materials and Methods

Settings and participants

Twenty-seven sedentary (14 males, 13 postmenopausal females) type 2 diabetes mellitus patients were enrolled in the study. Clinical details are summarized in Table 1. All patients were obese [body mass index (BMI) > 30 kg/m^2] and used at least 20 IU of insulin per day, with or without oral glucose-lowering medication. In addition, patients had to have residual β-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 iv (6). The residual β-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.

Design overview and intervention

Throughout the 16-wk intervention period, no blood glucose-lowering medication (both oral and insulin therapy) was used by the patients. All oral blood glucose and lipid lowering medication was discontinued 3 wk before the study (because of the longer half-life of some of the oral glucose lowering medication; insulin therapy was increased incrementally during these 3 wk 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 hyperinsulinemic euglycemic clamp the next day.

After a baseline visit (outlined below), all patients started a 16-wk VLCD (Modifast; Nutrition & Santé, Antwerpen, Belgium). Modifast provides a total of approximately 450 kcal/d and all necessary vitamins and micronutrients, divided over three meals of liquid shakes. Modifast provides about 50 g protein, 50–60 g carbohydrate, 7–9 g lipid, and 10 g of dietary fiber.

Thirteen of the 27 subjects were randomized to follow an exercise program simultaneously. This exercise program con-

TABLE 1. Clinical characteristics, body composition, and fasting plasma levels before and after a 16-wk VLCD ± exercise in obese insulin-dependent type 2 diabetes mellitus patients

	VLCD only		VLCD + exercise	
	Baseline	After 16 wk	Baseline	After 16 wk
Sex (M/F)	6/8		8/5	
Age (yr)	56.1 ± 2.4		53.0 ± 2.5	
Weight (kg)	112.7 ± 5.6	89.0 ± 4.3[a]	113.5 ± 5.1	86.3 ± 4.2[a]
BMI (kg/m^2)	37.9 ± 1.4	30.0 ± 1.1[a]	36.4 ± 1.1	27.7 ± 1.0[a]
Waist (cm)	122 ± 3	103 ± 3[a]	123 ± 3	98 ± 3[a,b]
Fat mass (kg)	49.9 ± 3.6	33.2 ± 2.8[a]	45.4 ± 3.2	23.5 ± 2.2[a,b]
Systolic blood pressure (mm Hg)	161 ± 4	140 ± 4[a]	145 ± 5	132 ± 5[a]
Diastolic blood pressure (mm Hg)	87 ± 3	78 ± 2[a]	81 ± 3	75 ± 3
HbA1c (%)	7.8 ± 0.3	6.7 ± 0.3[a]	7.8 ± 0.4	6.3 ± 0.4[a]
Fasting plasma glucose (mmol/liter)	12.1 ± 0.5	7.7 ± 0.6[a]	10.9 ± 0.7	6.6 ± 0.8[a]
Fasting insulin (mU/liter)	24.4 ± 4.3	12.6 ± 2.0[a]	25.1 ± 2.2	8.8 ± 0.8[a]
Fasting c-peptide (nmol/liter)	2.9 ± 0.3	2.2 ± 0.2	3.5 ± 0.3	2.0 ± 0.1[a]
TC (mmol/liter)	6.1 ± 0.4	5.5 ± 0.3	5.4 ± 0.4	4.5 ± 0.3[a]
TG (mmol/liter)	2.3 ± 0.2	1.5 ± 0.2[a]	2.5 ± 0.5	1.2 ± 0.1[a]
HDL (mmol/liter)	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.0	1.2 ± 0.1
LDL (mmol/liter)	4.4 ± 0.4	3.7 ± 0.3[a]	3.6 ± 0.3	3.0 ± 0.3[a]
Metformin (number of patients)	9	0	10	0
SU derivative (number of patients)	1	0	3	0
Average insulin (IU/d)	86.2	0	77.2	0

Data are presented as mean ± SEM. M, Male; F, female; SU, sulfonylurea; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

[a] $P < 0.001$ within the group.

[b] $P < 0.05$ between the groups.

sisted of at least four training sessions per week at home for 30 min and a 1-h in-hospital training. The 1-h in-hospital training entailed primarily aerobic exercise under supervision of a physiotherapist. The average amount of training at home on a cycloergometer was 5.25 times/wk with a length on average of 35.7 min/training. Patients exercised at an individual set target heart rate, which equals 70% of their maximum aerobic capacity during the incremental cycloergometer exercise test. After 8 wk the target heart rate was adjusted by the physiotherapist. Patients in the VLCD-only group were instructed not to alter their pattern of physical activity.

During the 16-wk 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$_i$; 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 h before the hyperinsulinemic euglycemic clamps.

Hyperinsulinemic euglycemic 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 (7). In short, first samples were taken for the measurement of basal levels of glucose, insulin, and background enrichment of [6,6-$^2H_2$]glucose and [$^2H_5$]glycerol. Basal rates of glucose turnover were assessed after 3 h of continuous infusion of [6,6-$^2H_2$]glucose and 1.5 h of continuous infusion of [$^2H_5$]glycerol (Cambridge Isotopes, Cambridge, MA). Subsequently, insulin-stimulated rates of glucose turnover were measured after 4.5 h of a hyperinsulinemic euglycemic 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/liter via the infusion of a variable rate of 20% glucose enriched with 3% [6,6-$^2H_2$]glucose.

A physiological and isotopic steady state was achieved during the last 30 min of both the basal as well and the hyperinsulinemic 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 (8). 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 metabolic clearance rate of insulin was calculated as the constant infusion rate of insulin divided by the steady-state insulin concentration corrected for endogenous insulin secretion (basal insulin concentration × (steady state c-peptide/basal c-peptide concentration) (9).

Aerobic fitness

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

Indirect calorimetry

Both under basal and hyperinsulinemic conditions, indirect calorimetry with a ventilated hood (Oxycon β; 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). Nonoxidative glucose disposal (NOGD), as a measurement for glycogen storage, was calculated by subtracting the glucose oxidation rate from the R_d of glucose.

Muscle biopsy (mitochondria, insulin signaling, Oil Red O staining)

Under localized anesthesia, with 1% lidocaine, muscle biopsies were taken from the vastus lateralis muscle under basal conditions and 30 min after the start of the insulin infusion (7) 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).

Mitochondrial DNA (mtDNA) content was assessed using a modification of the quantitative real-time PCR-based method we described previously (11). For determination of insulin signaling, homogenates of the muscle biopsies were prepared and analyzed by Western blotting as described previously (2).The antibodies used were: anti-phosphoproline-rich Akt substrate 40 (PRAS40)-Thr24, anti-phospho-Akt substrate 160 (AS160) (both from Biosource International, Camarillo, CA), and antiinsulin receptor β-subunit (Santa Cruz Biotechnology, Santa Cruz, CA). Protein expression and phosphorylation levels were normalized by reprobing the stripped filters with antibodies for PRAS40 (Biosource International). Tissue sections of basal biopsies were stained with Oil Red O combined with a double-immunofluorescence assay (antilaminin and a monoclonal antibody raised against adult human slow myosin heavy chain) to allow quantification of IMCL as described previously (12). The percentage of type 1 fibers was determined by area.

Assays

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

Plasma free fatty acid (FFA) 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 (13).

Statistical analysis

The major end point of the trial was insulin sensitivity. We performed the power calculation based on the expected results in insulin sensitivity. In a previous study from our group (2), we observed a 2.7 (μ2)-fold improvement in insulin stimulated glucose disposal with a SD of 1.4 (σ) after a VLCD leading to a weight loss of 50% of overweight (time of diet 2–7 months, mean 3 months). Another study (3) found a 1.1 (μ1) fold improvement

in insulin stimulated glucose disposal after a 16-wk aerobic exercise intervention in overweight type 2 diabetic women. Using these values, we calculated the group size at 13.

Results are expressed as mean ± SE (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. Nonparametric tests (Wilcoxon signed rank test for paired samples, Mann-Whitney for unpaired samples, respectively) 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 (before the 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-wk 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 with the VLCD-only group (see Table 1).

Glucose and lipid metabolism

After the intervention, fasting plasma glucose, insulin, and HbA1c levels improved substantially and similarly in both intervention groups. During the clamp pre- and postintervention steady-state plasma glucose concentrations were similar in the two intervention groups (VLCD+E, 5.4 ± 0.4 *vs.* 5.4 ± 0.5 mmol/liter; VLCD-only, 5.5 ± 0.7 *vs.* 5.6 ± 0.6 mmol/liter, before and after the intervention, respectively; both P = 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 (see Table 2).

There was a similar reduction in basal EGP in both patient groups. Also, peripheral insulin sensitivity improved considerably; glucose R_d increased with approximately 150% in both patient groups. The R_a of glycerol as a measure of the rate 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 was also improved only by VLCD+E (see Table 2).

TABLE 2. Metabolic parameters before and after a 16-wk VLCD ± exercise in obese, insulin-dependent type 2 diabetes mellitus patients

	VLCD only		VLCD + exercise	
	Baseline	After 16 wk	Baseline	After 16 wk
Basal EGP (μmol/kg LBM per minute)	17.7 ± 0.7	15.1 ± 0.6[a]	17.1 ± 0.7	14.1 ± 0.4[a]
Clamp EGP (μmol/kg LBM per minute)	4.8 ± 0.7	1.2 ± 0.6[a]	3.1 ± 0.8	1.8 ± 0.6
Suppression EGP (%)	−73.7 ± 3.4	−93.1 ± 3.2[a]	−82.0 ± 4.5	−87.6 ± 4.1
Glucose R_d (μmol/kg LBM per minute)	15.5 ± 1.2	38.6 ± 4.6[a]	16.6 ± 1.2	41.8 ± 3.6[a]
Glucose infusion rate (μmol/kg LBM per minute)	10.2 ± 1.8	37.5 ± 5.1[a]	13.0 ± 1.8	40.3 ± 3.6[a]
Clamp insulin (mU/liter)	102.4 ± 9.0	86.6 ± 7.3[a]	102.0 ± 5.6	79.4 ± 5.5[a]
Metabolic clearance rate insulin (ml/m^2 · min)	113.5 ± 7.8	130.1 ± 9.1[a]	109.7 ± 6.3	145.0 ± 17.4[a]
RQ basal	0.79 ± 0.01	0.76 ± 0.01	0.84 ± 0.01	0.74 ± 0.01[a,b]
RQ clamp	0.84 ± 0.02	0.87 ± 0.02	0.84 ± 0.02	0.83 ± 0.02
Basal glucose ox. (μmol/kg LBM per minute)	10.9 ± 1.5	6.1 ± 1.4[a]	15.8 ± 1.8	3.6 ± 1.1[a,b]
Clamp glucose ox. (μmol/kg LBM per minute)	16.2 ± 2.1	17.4 ± 1.8	16.9 ± 2.2	12.9 ± 1.6
Increase glucose ox. (μmol/kg LBM per minute)	5.2 ± 1.6	11.3 ± 1.9[a]	1.0 ± 1.3	9.3 ± 1.2[a]
Basal NOGD (μmol/kg LBM per minute)	6.8 ± 1.4	9.1 ± 1.4	1.4 ± 1.7	10.5 ± 1.2[a,b]
Amp NOGD (μmol/kg LBM per minute)	0.0 ± 2.4	21.3 ± 4.2[a]	0.0 ± 2.1	29.0 ± 2.7[a]
Basal R_a glycerol (μmol/kg FM per minute)	11.3 ± 1.2	11.3 ± 1.3	12.9 ± 1.0	15.9 ± 1.1[a]
Clamp R_a glycerol (μmol/kg FM per minute)	5.9 ± 0.9	5.8 ± 1.0	7.2 ± 1.3	7.9 ± 1.3
Suppression R_a glycerol (μmol/kg FM per minute)	−5.4 ± 0.7	−5.5 ± 0.8	−5.6 ± 0.8	−7.9 ± 0.9[a]
Basal FFA levels (mmol/liter)	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.0
Clamp FFA levels (mmol/liter)	0.3 ± 0.0	0.2 ± 0.1	0.3 ± 0.0	0.1 ± 0.0
Basal lipid ox. (μmol/kg LBM per minute)	7.0 ± 0.4	6.6 ± 0.3	4.9 ± 0.4	6.9 ± 0.3[a,b]
Clamp lipid ox. (μmol/kg LBM per minute)	4.6 ± 0.6	3.6 ± 0.5	4.7 ± 0.5	4.6 ± 0.5
Suppression lipid ox. (μmol/kg LBM per minute)	−2.4 ± 0.6	−2.9 ± 0.6	−0.2 ± 0.4	−2.4 ± 0.4[a]

Data are mean ± SEM. FM, Fat mass; RQ, respiratory quotient; ox., oxidation.

[a] $P < 0.05$ within the group.

[b] $P < 0.05$ between the groups.

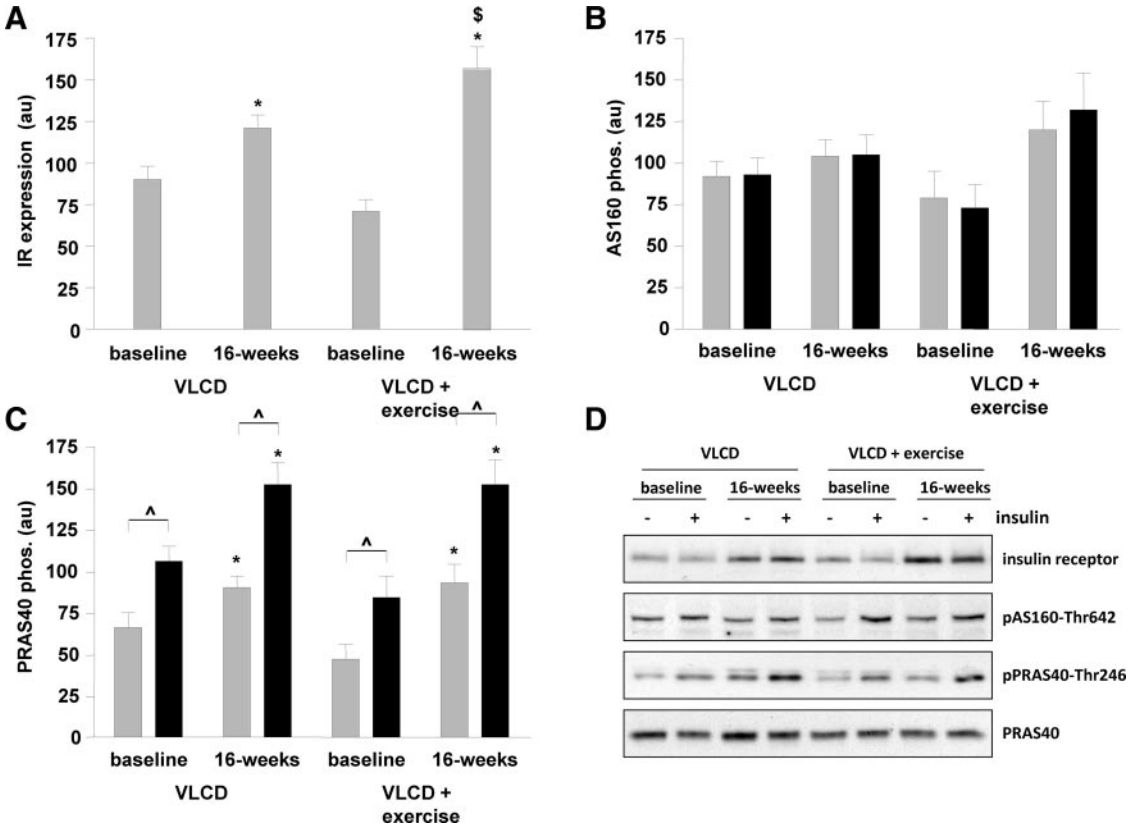

FIG. 1. A, Quantification of the IR expression at baseline and after 16 wk of intervention. B, Basal (*gray bars*) and insulin-stimulated (*black bars*) AS160 phosphorylation (phos). C, Basal (*gray bars*) and insulin-stimulated (*black bars*) PRAS40 phosphorylation. D, Representative blots of IR expression, AS160, and PRAS40 phosphorylation. *, $P < 0.05$ within the group compared with baseline; $, $P < 0.05$ between the groups; ^, $P < 0.05$ baseline *vs.* 16 wk. au, Arbitrary units. Protein expression and phosphorylation levels were corrected for PRAS40 expression and expressed as mean ± SEM.

Insulin signaling

Both patient groups had a higher insulin receptor (IR) expression after the intervention. IR expression increased further in the VLCD+E compared with the VLCD-only. Phosphorylation of PRAS40 was similarly increased in both groups after the intervention in basal as well as in hyperinsulinemic conditions. AS160 phosphorylation showed a similar trend, but this increase was not statistically significant (see Fig. 1).

Glucose and lipid oxidation rates

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

Maximal aerobic capacity

At baseline, VO_{2max} was similar in both groups. Participants in the VLCD-only group had a nonsignificant change [0.7 ± 1.5 mg/kg lean body mass (LBM) per minute 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 per minute difference from baseline) (see Fig. 2A).

Mitochondria

Muscle mtDNA content was not affected after a VLCD-only (1398 ± 240 *vs.* 1127 ± 180 arbitrary units before and after the intervention, respectively). In contrast, the VLCD+E group showed a significant increase after the intervention (1211 ± 185 *vs.* 2288 ± 359 arbitrary units before and after the intervention, respectively; $P < 0.05$) (see Fig. 2B).

Muscle morphology and IMCL

In both intervention groups, there was a similar decline in IMCL accumulation in the skeletal muscle fibers after the 16-wk intervention (see Fig. 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 types 1 and 2: 52.4 ± 3.0 and 47.6 ± 3.0% *vs.* 58.4 ± 3.2% and 41.6 ± 3.2% before and after the intervention, respectively; both $P < 0.05$; VLCD-only types 1 and 2: 51.8 ± 3.0 and 48.2 ± 3.0% *vs.*

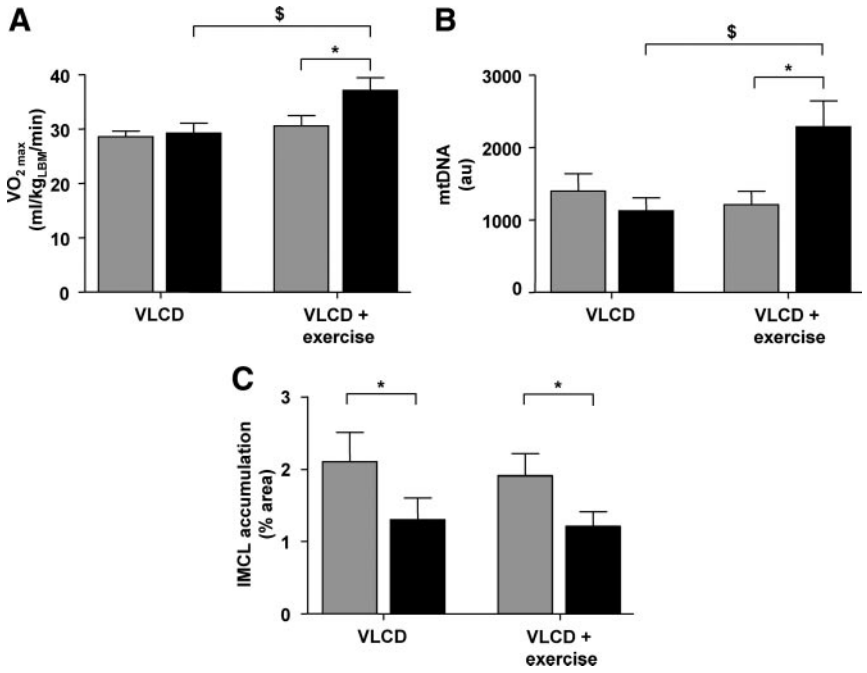

FIG. 2. A, VO_{2max}. B, mtDNA content of the skeletal muscle. C, IMCL as a relative fraction of the cell area containing lipid droplets at baseline (*gray bars*) and after (*black bars*) a 16-wk VLCD ± exercise in obese, insulin-dependent type 2 diabetes mellitus patients. *, $P < 0.05$ within the group; $, $P < 0.05$ between the groups. au, arbitrary units.

60.7 ± 4.1 and 39.3 ± 4.1%, before and after the intervention, respectively; both $P < 0.05$).

Discussion

A 16-wk VLCD in obese insulin-dependent diabetes mellitus type 2 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 and thus conservation of LBM. 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 of IMCL in skeletal muscle. In addition, 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, whereas these parameters remained unchanged in the VLCD-only group.

The current study confirms that diet-induced weight reduction improves glucoregulation in obese patients with type 2 diabetes mellitus by ameliorating both hepatic and peripheral insulin resistance. The current study failed to show additional effects of 16 wk 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. Another explanation, at the level of EGP, might be the high dosage of insulin infusion used during the clamp. This almost completely suppressed EGP in both groups already at baseline. Therefore, a potential better insulin supressibility of EGP in the VLCD+E could not be detected.

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 (14, 15) and the insulin signaling pathway (16) in obese patients with or without type 2 diabetes mellitus. 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.

NOGD decreased during the hyperinsulinemic euglycemic clamp before the intervention and increased during the clamp after the 16-wk intervention. In healthy subjects, NOGD increases, along with glucose Rd, during hyperinsulinemia (17). In obese and type 2 diabetes mellitus patients NOGD is disturbed; glucose Rd and NOGD during hyperinsulinemia are much lower compared with control subjects (18, 19). Thus, our finding of a very low NOGD during hyperinsulinemia at baseline reflects the severely insulin-resistant state of our subjects with a core defect in glucose storage as glycogen. This was improved after the VLCD±E intervention.

A low capacity to oxidize lipids (in combination with FFA oversupply) leads to accumulation of lipids in the skeletal muscle. IMCL content is elevated in the skeletal muscle in obese type 2 diabetes mellitus patients and is associated with insulin resistance in this group. Not IMCL *per se*, but IMCL derivatives, such as diacylglycerol 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. Serine-phosphorylated insulin receptor substrate-1 is unable to activate PI3K and leads to disruption in the PI3K-PKB/AKT insulin signaling cascade (20). 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, but it is also high in endurance-trained athletes; here IMCL represent a physiological role as readily available energy source. This is referred to as the athlete's paradox (21). In literature, exercise in obese nondiabetic and obese diabetic subjects increased (22), decreased (14, 23), or led to unchanged (24, 25) 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 (26). Type 2 diabetes mellitus patients are characterized by low basal fat oxidation and increased lipogenesis rates (27). The current study showed that only VLCD+E increased the reliance on lipid oxidation and lipolysis during fasting after the intervention. The fact that fatty acid oxidation is impaired in insulin resistant states led to the speculation that mitochondrial dysfunction is the cause of IMCL accumulation and the ensuing insulin resistance. Indeed, a decreased mitochondrial density and/or function has been reported in insulin-resistant offspring of type 2 diabetes mellitus patients (28–30) and type 2 diabetes mellitus patients (31–34). However, three of these studies reported decreased mitochondrial function at normal IMCL levels (32–34), suggesting that impaired mitochondrial function is not a prerequisite for IMCL accumulation. Rather, it might be that mitochondrial dysfunction is the consequence of the increased amount of fatty acid metabolites, for example, via the formation of lipid peroxides (35). In that case it might be that the lipid-induced mitochondrial dysfunction induces progressive deterioration of oxidative capacity and further accumulation of lipid intermediates in the skeletal muscle cell.

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 (15, 24). This suggests that sedentary behavior might be responsible for the reduction in mitochondrial capacity in obese type 2 diabetes mellitus patients. Indeed, here we showed that only combining VLCD+E increases mitochondrial copy number. However, the increase in mitochondrial copy number in the VLCD+E group was not associated with a further decrease in IMCL and greater increase in glucose disposal rate compared with 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 type 2 diabetes mellitus, although a causal relationship has not been established. To the best of our knowledge, this is the first study that shows a significant increase in type 1 oxidative muscle fibers in type 2 diabetes mellitus 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 because both groups lost similar but excessive amounts of weight after the intervention.

Even moderately intense regular exercise leads to improved fitness in obese type 2 diabetes mellitus patients as shown in the present and previous studies (23, 24, 36), whereas aerobic capacity did not improve in the group receiving dietary advice without exercise program.

Patients in the VLCD+E group lost about 3.5 kg more body weight than the VLCD-only group (P = NS within the groups). We did not use a dual-energy x-ray absorptiometry scan, so we can give only estimates of fat mass lost by subtracting LBM as measured by bioelectrical impedance analysis from total body weight. With that equation, the VLCD+E group lost 5 kg more fat mass ($P < 0.05$) than the VLCD-only group. Given the fact that the amount of exercise performed would have led to 2500 kcal/wk extra (40,000 kcal per 16 wk) and that 1 kg fat approximates 8000 kcal, this would precisely have led to 5 kg fat loss extra (the difference between of 5 kg in fat mass and 3.5 kg in total body weight being an increase in muscle mass). Unfortunately, we cannot be sure about the amount of fat lost. It might very well have been possible that the exercise group was more sedentary during the rest of the day and that the difference in total body weight therefore was not significant.

The limitation of this study was the lack of follow-up, with a hyperinsulinemic euglycemic clamp after 1 yr. Because exercise induced significantly more fat mass loss, further improvement in fasting lipid oxidation, and mitochondria copy number, it could well be that insulin sensitivity did further improve after a follow-up period in the VLCD+E group compared with the VLCD-only group.

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 LBM, 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 type 2 diabetes mellitus.

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