Restoration of Muscle Mitochondrial Function and Metabolic Flexibility in Type 2 Diabetes by Exercise Training Is Paralleled by Increased Myocellular Fat Storage and Improved Insulin Sensitivity

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OBJECTIVE—Mitochondrial dysfunction and fat accumulation in skeletal muscle (increased intramyocellular lipid [IMCL]) have been linked to development of type 2 diabetes. We examined whether exercise training could restore mitochondrial function and insulin sensitivity in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS—Eighteen male type 2 diabetic and 20 healthy male control subjects of comparable body weight, BMI, age, and $V_{O_{2max}}$ participated in a 12-week combined progressive training program (three times per week and 45 min per session). In vivo mitochondrial function (assessed via magnetic resonance spectroscopy), insulin sensitivity (clamp), metabolic flexibility (indirect calorimetry), and IMCL content (histochemically) were measured before and after training.

RESULTS—Mitochondrial function was lower in type 2 diabetic compared with control subjects ($P = 0.03$), improved by training in control subjects (28% increase; $P = 0.02$), and restored to control values in type 2 diabetic subjects (48% increase; $P < 0.01$). Insulin sensitivity tended to improve in control subjects (delta Rd 8% increase; $P = 0.08$) and improved significantly in type 2 diabetic subjects (delta Rd 63% increase; $P < 0.01$). Suppression of insulin-stimulated endogenous glucose production improved in both groups (−64%; $P < 0.01$ in control subjects and −52% in diabetic subjects; $P < 0.01$). After training, metabolic flexibility in type 2 diabetic subjects was restored (delta respiratory exchange ratio 63% increase; $P = 0.01$) but was unchanged in control subjects (delta respiratory exchange ratio 7% increase; $P = 0.22$). Starting with comparable pretraining IMCL levels, training tended to increase IMCL content in type 2 diabetic subjects (27% increase; $P = 0.10$), especially in type 2 muscle fibers.

CONCLUSIONS—Exercise training restored in vivo mitochondrial function in type 2 diabetic subjects. Insulin-mediated glucose disposal and metabolic flexibility improved in type 2 diabetic subjects in the face of near–significantly increased IMCL content. This indicates that increased capacity to store IMCL and restoration of improved mitochondrial function contribute to improved muscle insulin sensitivity. ***Diabetes*** **59:572–579, 2010**

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Skeletal muscle insulin resistance is one of the earliest hallmarks of the development of type 2 diabetes. The combination of increased intramyocellular lipid (IMCL) and a low oxidative capacity are key features in the development of muscular insulin resistance (1–3). Thus, mitochondrial dysfunction has been suggested to be involved in accretion of IMCL.

In type 2 diabetes, smaller and damaged mitochondria have been reported (4). In line with this, gene expression of a key transcriptional cofactor in mitochondrial biogenesis (PGC1α), and its target genes encoding key enzymes in oxidative mitochondrial metabolism, was lower in (pre-) diabetic subjects (5,6). We confirmed lower expression of PGC1α in type 2 diabetic patients and a restoration toward control values upon treatment with rosiglitazone (7), indicating that PGC1α-mediated defects in mitochondria are reversible.

Importantly, these defects can translate into a lower in vivo ATP synthesis rate in first-degree relatives of type 2 diabetic patients, as determined using ^{31}P–magnetic resonance spectroscopy (MRS) (8). Using an alternative ^{31}P-MRS method, we recently reported that type 2 diabetic patients are also characterized by reduced in vivo mitochondrial function, as reflected by a prolonged postexercise phosphocreatine resynthesis rate (9). More recently, we extended this observation with ex vivo data indicating intrinsic mitochondrial defects in patients with type 2 diabetes (10). Under all of these conditions, compromised mitochondrial function was observed in overweight-to-obese, BMI-matched populations with comparable IMCL content. Together, these data support the hypothesis that a low oxidative capacity may contribute to the development of insulin resistance in the presence of high IMCL content (11).

Current American Diabetes Association/American Heart Association–based guidelines in the prevention and treatment of type 2 diabetes target a diet-induced weight loss of 5–10% body weight and at least 150 min of moderate activity per week (12). Interestingly, although both are

insulin-sensitizing interventions, diet-induced weight loss and physical exercise training differentially affect IMCL content. While a diet-induced reduction in body mass results in declined IMCL content (13), exercise training leads to IMCL accretion (11,14–16). The net effect of combined dietary and exercise interventions may thus be similar IMCL levels pre- and postintervention. Indeed, in patients with type 2 diabetes, it has been shown that a combined exercise-dietary intervention improved insulin sensitivity without changes in IMCL but with an improvement in mitochondrial function (13). On the other hand, diet-induced weight loss alone reduces IMCL content without affecting mitochondrial capacity, suggesting that to improve muscular insulin resistance the balance between IMCL content and oxidative capacity is critical (13).

At present, the effect of exercise without dietary restrictions and without targeted weight loss on IMCL content, mitochondrial function, and insulin sensitivity is unknown. In addition, it is not known whether the response to exercise training in type 2 diabetes—with respect to insulin sensitivity, mitochondrial content and function, and IMCL—differs from the response in BMI-matched normoglycemic control subjects. Therefore, we aimed to investigate the effect of a well-controlled 12-week training program in type 2 diabetic patients and carefully matched obese healthy control subjects on insulin sensitivity, in vivo mitochondrial function and content, and IMCL content.

RESEARCH DESIGN AND METHODS

Eighteen male type 2 diabetic subjects and 20 healthy male control subjects matched for body weight, BMI, and age were included. Exclusion criteria were cardiac disease, impaired liver or renal function, BMI >35 kg/m^2, diabetes complications, exogenous insulin therapy, and prior participation in training studies. For control subjects, a family history of type 2 diabetes was added to the exclusion criteria. Glucose tolerance was examined by an oral glucose tolerance test (17). Diabetic patients were diagnosed with type 2 diabetes for at least 1 year before the start of the study, had well-controlled diabetes (A1C ±7.2%), and were using oral antidiabetes agents (metformin only or in combination with sulfonurea derivatives). Medication use did not change during the study. None of the subjects were on a diet, and all had a sedentary lifestyle. The institutional medical ethics committee approved the study. Body composition was measured through hydrostatic weighing (18), and maximal work load and oxygen uptake were assessed during a graded cycling test until exhaustion (19).

Exercise training protocol. Subjects enrolled in a tightly controlled exercise program for 12 weeks. Aerobic exercise was carried out on a cycling ergometer twice a week for 30 min at 55% of a previously determined maximal work load (W_{max}). Resistance exercise was performed once a week and comprised one series of eight repetitions at 55% of subjects' previously determined maximal voluntary contraction (MVC) and two series of eight repetitions at 75% MVC and focused on large muscle groups (Chest press, leg extension, lat pull down, leg press, triceps curls, biceps curls, abdominal crunches, and horizontal row). The MVC test was preceded by a familiarization trial. Warming-up and cooling-down sessions of 5 min were performed on a stationary bike at 45% W_{max}. Every 4 weeks, MVC was reassessed; maximal aerobic capacity was reassessed after 6 weeks, and training loads were readjusted accordingly. Supervised training sessions were performed with four subjects at a time.

Hyperinsulinemic-euglycemic clamp. A 6-h hyperinsulinemic-euglycemic insulin clamp (40 mU/m^2 per min) was performed before and after the training period essentially as previously described (7). Dietary habits were stable, and physical exercise was avoided 3 days prior to the clamp. Diabetic subjects discontinued antidiabetes medication 7 days prior to the clamp. Glucose tracer ([6,6-$^2H_2$]glucose) was used to determine rates of glucose appearance (Ra) and disposal (Rd). The first 3 h (t = 0–180 min, where t represents time) were used to determine non–insulin stimulated Ra and Rd. At t = 180, a primed constant infusion of insulin started and glucose infusion rates were adjusted to maintain euglycemia. In the final 30 min of the non–insulin stimulated period (t = 150–180) and under steady clamp conditions (t = 330–360), blood was sampled and indirect calorimetry (ventilated hood) was performed. Muscle biopsies from the m. vastus lateralis were obtained under local anesthesia (2% lidocaine) before and after the clamp.

Tracer calculations. Isotopic enrichment of plasma glucose was determined by electron ionization gas chromatography–mass spectrometry. Steele's single-pool non–steady-state equations (20) were used to calculate glucose Ra and Rd. Volume of distribution was assumed to be 0.160 l/kg for glucose. Insulin-stimulated glucose disposal was computed as the difference between Rd under insulin-stimulated conditions and Rd under basal non–insulin-stimulated conditions (delta Rd). Endogenous glucose production was calculated as Ra − exogenous glucose infusion rate. Nonoxidative glucose disposal was calculated as Rd − carbohydrate oxidation.

Blood sample analysis. Arterialised blood samples were collected from a hand vein. Plasma free fatty acids (FFAs) and glucose were measured spectrophotometrically. Insulin concentration was determined using a radioimmunoassay (Linco Research, St. Charles, MO).

Metabolic flexibility. Fat and carbohydrate oxidation in the basal and insulin-stimulated state was calculated according to the methodology of Frayn (21), with protein oxidation considered negligible. Metabolic flexibility was expressed as the change in respiratory exchange ratio from the fasted state to the insulin-stimulated condition.

^{31}P-MRS–based measurement of mitochondrial function. ^{31}P-MRS measurements were performed in vastus lateralis muscle on a 1.5 T whole-body scanner (Intera; Philips Health Care, Best, the Netherlands) essentially accoring the methodology of Schrauwen-Hinderling et al. (9). Data are expressed as rate constant (ln 2/PCr recovery time in sec). The higher the rate constant, (s^{-1}), the better in vivo mitochondrial function.

IMCL content. IMCL content was assessed histochemically in muscle cross-sections using a modified oil red O staining for fluorescence microscopy (28) and combined with muscle fiber typing.

Western blotting. Uncoupling protein 3 (UCP3) content was determined by Western blotting, using a rabbit polyclonal antibody. Five different structural components of the electron transport chain were measured at the protein level as a reflection of mitochondrial density. The components include ND6 subunit of complex I, the 30 kDa Ip subunit of complex II, the 47 kDa core protein 2 of complex III, subunit II of cytochrome C oxidase (COXII), and the α subunit of the F1F0 ATP synthase (complex V) and were measured using monoclonal antibodies (MitoSciences, OR, USA) (29). Gels were loaded with equal amounts of protein of pre- and posttraining lysates of two control and two type 2 diabetic subjects per gel to allow valid comparison between pre- and posttraining samples. To adjust for inter-gel variation, and hence variation in the mean of the control group and the type 2 diabetic group, the optical density of the band of interest per subject was normalized to the mean optical density of the complete gel. Protein content was expressed as arbitrary units (AU).

Statistics. Data are presented as mean ± SE. In four control and three type 2 diabetic subjects no pre- or post data for MRS analyses could be obtained due to claustrophobia or metal parts in their body. Statistical analyses were performed two-sided using SPSS for Windows 15.0 software (SPSS, Chicago, IL). Statistical significance was set at $P < 0.05$. A two-way ANOVA model for repeated measures was applied using control and type 2 diabetic subjects as between-subject variables and pre- and posttraining data as repeated within-subject variables. Using this model we did not find significant interaction effects.

RESULTS

Subject characteristics. Control subjects and type 2 diabetic patients were included and matched for body weight (94.7 ± 2.7 and 93.8 ± 2.9 kg), BMI (29.7 ± 0.8 and 30.0 ± 0.8 kg/m^2), and age (59.0 ± 0.8 and 59.4 ± 1.1 years). Training induced a modest but near-significant decline in body weight and BMI in control subjects (94.7 ± 2.7 to 93.6 ± 2.7 kg and 29.7 ± 0.8 to 29.4 ± 0.8 kg/m^2, respectively, both P = 0.06), but did not result in significant changes in type 2 diabetic subjects (Table 1). Fat mass tended to decline in control subjects (30.0 ± 1.8 to 29.2 ± 2.0 kg after training; P = 0.09) and declined modestly but significantly after training in type 2 diabetic subjects (29.4 ± 1.9 to 28.0 ± 1.8 kg; P = 0.04). Fat free mass was similar between both groups and did not change after training (Table 1).

Exercise capacity. Before training, maximal oxygen uptake was comparable in both groups and increased upon training (+6.4 ± 2.6%, P = 0.04 in control subjects and

TABLE 1
Subject characteristics

	Control		Type 2 diabetes	
	Pretraining	Posttraining	Pretraining	Posttraining
Age (years)	59.0 ± 0.8	—	59.4 ± 1.1	—
Years since diagnosis	—	—	3.9 ± 0.9	—
Weight (kg)	94.7 ± 2.7	93.6 ± 2.7	93.8 ± 2.9	92.8 ± 3.1
Height (cm)	178.5 ± 1.3	—	176.7 ± 1.3	—
BMI (kg/m^2)	29.7 ± 0.8	29.4 ± 0.8	30.0 ± 0.8	29.8 ± 0.9
Body fat (%)	31.5 ± 1.4	30.6 ± 1.6	31.1 ± 1.4	29.9 ± 1.3*
Fat mass (kg)	30.0 ± 1.8	29.2 ± 2.0	29.4 ± 1.9	28.0 ± 1.8*
Fat-free mass (kg)	64.6 ± 2.0	65.4 ± 2.0	64.3 ± 1.7	64.8 ± 1.8
Vo_{2max} (ml · min^{-1} · kg^{-1})	28.8 ± 1.0	30.2 ± 1.2*	27.5 ± 1.2	31.1 ± 1.2*
Wmax (Watt)	207 ± 10	236 ± 9*	202 ± 9	233 ± 9*
Average strength (kg)	85.8 ± 3.2	104.0 ± 3.5*	83.7 ± 3.5	102.4 ± 4.2*
Fasting glucose (mmol/l)	5.9 ± 0.1	5.5 ± 0.1*	9.0 ± 0.4†	9.0 ± 0.4†
A1C (%)	5.8 ± 0.1	5.7 ± 0.1*	7.2 ± 0.2†	7.2 ± 0.2†
Triacylglycerol (mmol/l)	1.52 ± 0.13	1.49 ± 0.15	1.77 ± 0.16	1.68 ± 0.14

Data are expressed as means ± SE. *Posttraining significantly different from pretraining. †Type 2 diabetic group data significantly different from control group data.

+11.3 ± 2.2%, $P < 0.01$ in type 2 diabetic subjects). Maximal oxygen uptake and Wmax increased similarly in control and type 2 diabetic subjects (+15.0 ± 2.4% and +16.9 ± 3.0% in control and type 2 diabetic subjects, respectively, $P < 0.01$) and persisted after correction for body mass. Resistance training profoundly improved muscle strength (+22.2 ± 2.0% and +23.9 ± 1.9% in control and type 2 diabetic subjects, respectively, $P < 0.01$, Table 1).

Whole-body insulin-stimulated glucose disposal. Insulin-stimulated glucose disposal (delta Rd) was considerably higher in control than in type 2 diabetic subjects (Table 2). Training induced a near-significant increase in delta Rd in control (17.1 ± 2.4 to 18.4 ± 2.1 μmol · kg^{-1} · min^{-1}, $P = 0.08$) and a profound significant increase in type 2 diabetic subjects (from 6.8 ± 1.4 to 11.1 ± 1.4 μmol · kg^{-1} · min^{-1}, $P < 0.01$). The significant increase observed in patients with type 2 diabetes originates from a reduction

TABLE 2
Substrate kinetics pre- and posttraining

	Control		Type 2 diabetes	
	Pretraining	Posttraining	Pretraining	Posttraining
Plasma insulin (mU/l)				
Basal	18.1 ± 2.4	16.1 ± 2.1*	16.4 ± 1.2	14.6 ± 0.8*
Clamp	112.5 ± 5.4	112.1 ± 5.5	107.6 ± 4.8	103.1 ± 2.7
Plasma FFA (μmol/l)				
Basal	479.0 ± 22.9	454.9 ± 28.3	519.4 ± 25.3	500.1 ± 34.1
Clamp	84.7 ± 7.2	67.5 ± 6.9*	107.1 ± 8.7	87.6 ± 8.7*
Rd glucose (μmol · kg^{-1} · min^{-1})				
Basal	8.7 ± 0.7	8.3 ± 0.6	11.6 ± 0.7†	9.9 ± 0.6*
Clamp	25.8 ± 2.3	26.7 ± 2.3	18.4 ± 1.4†	21.0 ± 1.4*†
Delta	17.1 ± 2.4	18.4 ± 2.1	6.8 ± 1.4†	11.1 ± 1.4*†
Endogenous glucose production (μmol · kg^{-1} · min^{-1})				
Basal	8.7 ± 0.6	8.7 ± 0.6	10.3 ± 0.6	9.1 ± 0.7
Clamp	2.8 ± 0.8	1.0 ± 1.0*	2.9 ± 0.5	1.4 ± 0.3*
Delta	−5.7 ± 1.1	−7.2 ± 1.2	−7.9 ± 0.6	−7.7 ± 0.8
CHO oxidation (μmol · kg^{-1} · min^{-1})				
Basal	6.5 ± 0.5	7.1 ± 0.5	8.1 ± 0.6	7.3 ± 0.4
Clamp	12.5 ± 0.8	13.0 ± 0.7	11.7 ± 0.8	13.2 ± 0.8
Delta	5.9 ± 0.7	5.9 ± 0.6	3.6 ± 0.8†	5.9 ± 0.7*
Nonoxidative glucose disposal (μmol · kg^{-1} · min^{-1})				
Basal	2.3 ± 0.7	1.1 ± 0.6	3.5 ± 0.9	2.6 ± 0.8
Clamp	13.5 ± 1.7	13.7 ± 2.3	6.7 ± 1.2†	8.0 ± 1.2†
Delta	11.3 ± 1.9	12.6 ± 1.9	3.2 ± 1.4†	5.3 ± 1.2†
Lipid oxidation (μmol · kg^{-1} · min^{-1})				
Basal	1.08 ± 0.05	1.03 ± 0.05	1.08 ± 0.05	1.09 ± 0.05
Clamp	0.63 ± 0.04	0.55 ± 0.04	0.75 ± 0.04†	0.59 ± 0.05*
Delta	−0.46 ± 0.05	−0.48 ± 0.06	−0.32 ± 0.06	−0.49 ± 0.06*

Data are expressed as means ± SE. *Posttraining significantly different from pretraining. †Type 2 diabetic group data significantly different from control group data.

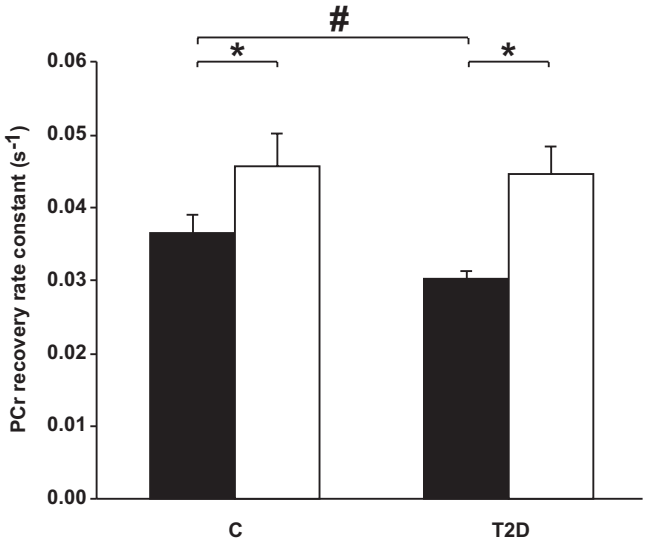

FIG. 1. In vivo mitochondrial function measured in vastus lateralis muscle expressed as the rate constant (s^{-1}) before (black bars) and after (white bars) training. A high rate constant reflects high in vivo mitochondrial function. Data are expressed as means ± SE. Pre- and posttraining leg-extension exercise was performed at 0.5 Hz to an acoustic cue on an magnetic resonance–compatible ergometer and a weight corresponding to 60% of the predetermined maximum. Spectra were fitted in the time domain with the AMARES algorithm (22) in the jMRUI software (23). Five peaks were fitted with Gaussian curves (Pi, PCr, and three ATP peaks). The time course of the PCr amplitude [PCr(t)] during the last 20 sec of exercise (steady state) and during the recovery period was fitted as previously described (9), asssuming a monoexponential PCr recovery. Postexercise PCr resynthesis is driven almost purely oxidatively (24), and the resynthesis rate reflects in vivo mitochondrial function in health (25) and disease (rev. in 26,27). #Data for type 2 diabetic (T2D) subjects significantly different from that of the control (C) group. *Posttraining significantly different from pretraining.

in basal glucose disposal rate as well as from improved disposal rate under insulin-stimulated conditions (Table 2). Nevertheless, insulin-stimulated glucose disposal remained higher in control after training compared to type 2 diabetic subjects. Basal endogenous glucose production before training tended ($P = 0.07$) to be lower in control than in type 2 diabetic subjects. Insulin sensitivity of the liver in control and type 2 diabetic subjects improved significantly (endogenous glucose production drops from 2.8 ± 0.8 pretraining to 1.0 ± 1.0 in control, $P = 0.01$ and from 2.9 ± 0.5 pretraining to 1.4 ± 0.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in type 2 diabetic subjects, $P < 0.01$). Insulin-mediated nonoxidative glucose disposal was significantly higher in control than in type 2 diabetic subjects and was not significantly affected by exercise training ($P = 0.14$ and 0.13 in control and type 2 diabetic subjects, respectively, Table 2). In contrast, delta glucose oxidation in type 2 diabetic subjects restored to control values (Table 2).

In vivo mitochondrial function by ^{31}P-MRS. We confirmed compromised mitochondrial function in type 2 diabetic compared to BMI-matched control subjects (rate constant: 0.036 ± 0.002^{-1} and 0.030 ± $0.001s^{-1}$ in control and type 2 diabetic subjects, respectively, $P = 0.03$). Mitochondrial function improved after training in control and type 2 diabetic subjects (+28% and +48%, respectively), resulting in similar in vivo mitochondrial function after training ($P = 0.84$) (Fig. 1). There was no difference in end-exercise pH values between control and type 2 diabetic subjects and no difference between pre- and posttraining values (7.05 ± 0.02 and 7.02 ± 0.02 for control pre- and posttraining and 7.01 ± 0.02 and 6.99 ± 0.03 for type 2 diabetic subjects pre- and posttraining, respectively).

Metabolic flexibility. Prior to training, metabolic flexibility was significantly higher in control subjects compared to type 2 diabetic subjects (Fig. 3), due to a more profound increase in insulin-stimulated glucose oxidation (5.9 ± 0.7 and 3.6 ± 0.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in control and type 2 diabetic subjects, respectively, $P = 0.04$), with a concomitant tendency ($P = 0.10$) to more profound insulin-mediated suppression of fat oxidation (−0.46 ± 0.05 and −0.32 ± 0.06 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in control and type 2 diabetic subjects, respectively) (Table 2). Exercise training did not affect metabolic flexibility in control but fully restored flexibility in type 2 diabetic subjects (control versus type 2 diabetic subjects; $P = 0.84$, Fig. 3), due to improved insulin-stimulated increases in glucose oxidation (from 3.61 ± 0.78 to 5.94 ± 0.72 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; $P = 0.02$) and suppression of fat oxidation (from −0.32 ± 0.06 to −0.49 ± 0.06 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; $P = 0.01$) (Table 2). Remarkably, insulin-stimulated substrate oxidation

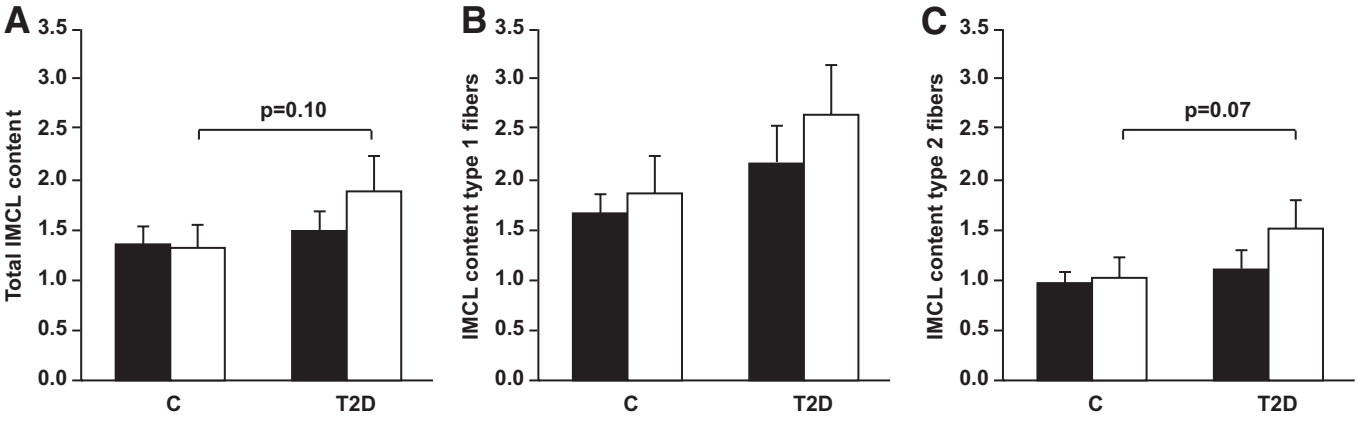

FIG. 2. IMCL content before (black bars) and after (white bars) training in all muscle fibers (*A*), in type 1 muscle fibers (*B*), and in type 2 muscle fibers (*C*). Data are expressed as means ± SE. Muscle fiber typing was performed using a monoclonal primary antibody against the slow isoform of myosin heavy chain 1 (MHC1), which was visualized using a secondary fluroscein isothiocyanate (FITC)-conjugated secondary antibody. Thus, MHC1-positive cells were considered type 1 muscle fibers, whereas MHC1-negative cells were considered type 2 muscle fibers. Immunolabeling of the basement membrane protein laminin was performed to identify the cellular border. Thus, we were able to identify the typology of individual muscle cells. Tresholding the Oil red O signal allowed us to compute the relative fraction of cell area containing lipid droplets per individual muscle fiber of either type. C, control subjects; T2D, type 2 diabetic subjects.

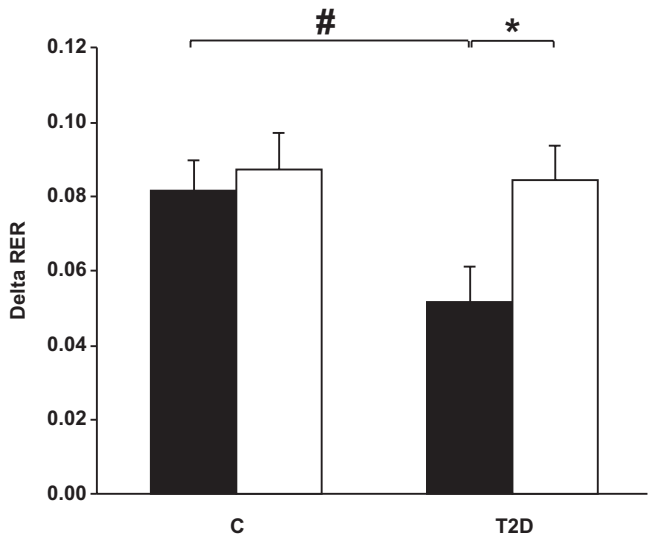

FIG. 3. Metabolic flexibility, measured as the change in respiratory quotient (respiratory exchange ratio [RER]) from the fasted state to the insulin-stimulated state before (black bars) and after (white bars) training. Data are expressed as means ± SE. #Type 2 diabetic (T2D) group significantly different from control (C) group. *Posttraining significantly different from pretraining.

rates were comparable between type 2 diabetic and control subjects after training, indicating that the lower insulin-stimulated glucose uptake after training in type 2 diabetic subjects was completely accounted for by a lower insulin-stimulated nonoxidative glucose uptake.

IMCL. IMCL content pretraining was comparable in both groups and did not change after training in the control group (Fig. 2). In the group with type 2 diabetes, however, training tended to increased IMCL content (from 1.5 ± 0.2 to 1.9 ± 0.3 AU; $P = 0.10$), which was predominantly accounted for by a near-significant increase in IMCL in type 2 muscle fibers (from 1.1 ± 0.2 to 1.5 ± 0.3 AU; $P = 0.07$ [Fig. 2]).

Blood sample analysis. Fasting glucose levels and A1C were significantly lower in control than in type 2 diabetic subjects prior to the training program. Twelve weeks of exercise training induced a slight but significant decrease in A1C and in fasting glucose levels in the control group. There was no change in A1C or fasting glucose levels in the group with type 2 diabetes (Table 1). Fasting plasma insulin levels were comparable pretraining and decreased significantly in both groups after training (Table 2). Fasting plasma FFA levels were similar across groups and did not change after training. Under insulin stimulation, however, FFA levels tended to be lower in the control group compared with those in the group with type 2 diabetes and decreased significantly after training in both groups (Table 2), suggesting improved insulin-mediated suppression of adipose tissue lipolysis posttraining in both groups.

Markers of mitochondrial density. Mitochondrial density was evaluated by measuring the protein content of five structural subunits of the distinct complexes of the electron-transport chain. Neither the individual complexes (Table 3) nor the averaging of the protein content of these complexes revealed difference in mitochondrial density between the control and type 2 diabetic groups pretraining (0.66 ± 0.11 and 0.59 ± 0.08 AU, respectively; NS). Training resulted in increased mitochondrial density in both groups (Table 3). The increase in mitochondrial density tended to be more pronounced in type 2 diabetic subjects compared with that in control subjects ($P = 0.07$).

UCP3 content. We confirm our previous reports showing a ~50% lower UCP3 content in type 2 diabetic than in control subjects (30,31) (Table 3). Training increased UCP3 content twofold in control subjects and almost fourfold in type 2 diabetic subjects. This resulted in similar UCP3 content in control and type 2 diabetic subjects after training (Table 3).

DISCUSSION

Mitochondrial dysfunction has been reported in type 2 diabetic patients (4,32,33) and in young, lean, insulin-resistant offspring of parents with type 2 diabetes (2), although not all studies support this (34,35). The present study confirms our previous observation of compromised mitochondrial function measured in vivo in patients with type 2 diabetes (9,10). Importantly, exercise training in patients with type 2 diabetes completely restored mitochondrial function toward values observed in control subjects after training. In patients with type 2 diabetes, restoration of mitochondrial function was paralleled by improved (but not restored) insulin-stimulated glucose disposal and by complete restoration of metabolic flexibility and insulin-stimulated substrate oxidation toward control values—both in the face of a near-significant increase in IMCL content. In control subjects, training also improved mitochondrial function, while insulin-stimulated glucose disposal increased only marginally and metabolic flexibility and IMCL content remained unaltered.

The ability of patients with type 2 diabetes to increase mitochondrial function indicates that despite aberrations in the transcriptional control of mitochondrial biogenesis (5,6), a lifestyle intervention comprising physical exercise

TABLE 3
Mitochondrial density and UCP3 protein content (AU)

	Control		Type 2 diabetes	
	Pretraining	Posttraining	Pretraining	Posttraining
Complex I	0.61 ± 0.19	1.11 ± 0.32	0.66 ± 0.18	1.65 ± 0.42
Complex II	0.61 ± 0.18	1.13 ± 0.30	0.60 ± 0.15	1.74 ± 0.37*
Complex III	0.73 ± 0.06	1.18 ± 0.12*	0.60 ± 0.05	1.55 ± 0.13*†
Complex IV	0.66 ± 0.07	1.21 ± 0.09*	0.57 ± 0.07	1.63 ± 0.12*†
Complex V	0.76 ± 0.12	1.06 ± 0.12*	0.62 ± 0.07	1.51 ± 0.14*†
Average of the complexes	0.66 ± 0.11	1.14 ± 0.16*	0.59 ± 0.08	1.62 ± 0.20*
UCP3	0.71 ± 0.12	1.43 ± 0.20*	0.39 ± 0.05†	1.50 ± 0.22*
UCP3 normalized to mitochondrial density	1.22 ± 0.21	1.37 ± 0.16	0.73 ± 0.09†	1.03 ± 0.11*

Data are expressed as means ± SE. *Posttraining significantly different from pretraining. †Type 2 diabetic group data significantly different from control group data.

is potent enough to overcome these apparent defects. Increased mitochondrial content and improved function have previously been observed in type 2 diabetic patients following a combined dietary exercise intervention targeting >7% of body weight loss (13). Here, we show that exercise training, even without substantial loss of body mass, not only improves mitochondrial function but even results in complete restoration toward the control values observed in age- and BMI-matched normoglycemic control subjects. The observation of compromised mitochondrial function in patients with type 2 diabetes compared with that of control subjects, despite comparable mitochondrial density (as indicated by measurement of the protein content of five structural components in the electron-transport chain), supports previous findings of intrinsic defects in mitochondria of patients with type 2 diabetes (10,36). Interestingly, mitochondrial protein content markedly increased after exercise training, suggesting that at least a major part of the restoration of mitochondrial function after training is due to increased mitochondrial biogenesis. Although it remains to be established whether exercise training also improves intrinsic mitochondrial function, it is of interest to note that protein expression of UCP3, a protein with a putative role in ameliorating lipotoxicity and oxidative stress via mild uncoupling (37), was significantly lower in type 2 diabetic patients compared with that in control subjects, confirming previous work (30,31). UCP3 content restored to control values after training in type 2 diabetic subjects even after adjustment for the increase in structural components of the electron-transport chain. This may indicate that exercise training in patients with type 2 diabetes not only improves mitochondrial content but also results in adaptive responses within mitochondria to cope better with the myocellular metabolic stress in the insulin-resistant state.

Part of the metabolic stress in type 2 diabetes may originate from myocellular fat storage. IMCL content correlates negatively with insulin sensitivity in untrained subjects (11,38,39). On the other hand, endurance-trained athletes also have high levels of IMCL (11,40) while being insulin sensitive. It has thus been suggested that low fat oxidative capacity and a concomitant increase in fatty acid metabolites induces insulin resistance rather than IMCL levels per se (11,41). Our present study confirms previous findings of reduced mitochondrial function in type 2 diabetes with IMCL content similar between control subjects and type 2 diabetic patients (9,10). This suggests that high IMCL levels combined with compromised mitochondrial function may contribute to impeded insulin sensitivity. This notion is substantiated by our observation that exercise training improved mitochondrial function and alleviated muscular insulin resistance in patients with type 2 diabetes even though IMCL levels increased posttraining.

Training-induced increases in IMCL content may originate from improved partitioning of fatty acids in IMCL due to exercise-induced increases in diacylglycerol-acyl transferase (DGAT1) (42,43), the rate-limiting enzyme in IMCL synthesis. Indeed, enhancing IMCL storage capacity by overexpression of DGAT1 improved insulin sensitivity (42). These findings support the idea that the capacity to effectively store fatty acids as IMCL along with appropriate mitochondrial function are major determinants of myocellular insulin sensitivity. We observed increased IMCL content in type 2 diabetic patients after combined endurance and resistance training in glycolytic type 2 muscle fibers, which in human possess lower IMCL levels than the more oxidative type 1 fibers. It could therefore be suggested that, due to the resistance exercise, previously inactive type 2 fibers were now recruited and increased their storage capacity for fatty acids as IMCL, thereby contributing to the insulin-sensitizing effect of training. This implies that it might be of added value for insulin-sensitizing training interventions to also include exercise at an intensity that requires recruitment of type 2 muscle fibers.

Metabolic inflexibility is another characteristic of insulin-resistant muscles (44), possibly reflecting a reduced ability of mitochondria to shift fuel selection. Metabolic inflexibility in insulin resistance may reflect reduced insulin-stimulated glucose uptake, thereby reducing the availability of glucose for oxidation, rather than a mitochondrial defect in substrate selection (45). The present study partly supports this notion. Impaired metabolic flexibility in type 2 diabetes before training was indeed accompanied by a reduced insulin-stimulated rate of glucose disappearance. Moreover, upon training, insulin-stimulated glucose disposal improved in the type 2 diabetic subjects in conjunction with improved metabolic flexibility. Although the improvement in insulin-stimulated glucose disposal completely matched the restoration of metabolic flexibility, restoration of mitochondrial function may be needed to facilitate this. In control subjects, training did not alter metabolic flexibility and also only marginally improved insulin-stimulated glucose disposal. It thus seems that after training, insulin-stimulated glucose oxidation was working at its maximal capacity in both control and type 2 diabetic subjects. Very interestingly, despite a restoration of metabolic flexibility, mitochondrial function, and insulin-stimulated glucose oxidation, insulin-stimulated glucose disposal was still lower in type 2 diabetic than in control subjects. This was completely accounted for by a lower nonoxidative glucose disposal. Thus, upon exercise training the oxidative component of insulin-stimulated glucose disposal is fully restored—in contrast to nonoxidative glucose disposal. Compromised nonoxidative glucose disposal in type 2 diabetes has previousl been reported (46), and treating insulin-resistant first-degree relatives of type 2 diabetic patients with metformin normalizes nonoxidative glucose disposal (47), supporting the notion that restoring nonoxidative glucose disposal may be crucial for normalizing insulin sensitivity and possibly plasma glucose in type 2 diabetes.

In a model of one-legged exercise training, nonoxidative glucose disposal improved along with increased fractional velocity of glycogen synthase (48). The different training regimes applied (one vs. two-legged exercise six times per week vs. three times per week and aerobic exercise solely vs. a combination of aerobic and resistance exercise) in the one-legged exercise study vs. the present study are likely to explain the differences. It should be noted that also in the present study nonoxidative glucose disposal improved in ~30% of patients with type 2 diabetes (albeit nonsignificantly) but was still lower than that of the control group. More recently, restoration of nonoxidative glucose disposal upon exercise training in type 2 diabetic patients has been reported (49). In that study, however, nonoxidative glucose disposal was measured as the residual of glucose disposal rate minus oxidative glucose disposal and may therefore be biased by hepatic glucose production; thus, the results are hard to compare with the data from the present study. Future studies are needed to identify the mechanism(s) underlying the defective nonoxi-

dative glucose disposal in patients with type 2 diabetes and how to reinstate these defects before full restoration of insulin-stimulated glucose disposal can occur.

While skeletal muscle insulin resistance is a hallmark of type 2 diabetes, insulin resistance of liver and adipose tissue also contributes to the pathogenesis of type 2 diabetes. In this respect, it is relevant to note that exercise training also resulted in beneficial adaptations beyond those reported for muscle. Likewise, we observed that under hyperinsulinemic clamp conditions plasma FFA levels were significantly lower post- than pretraining, possibly reflecting improved antilipolytic activity of insulin in adipose tissue. In addition, exercise training in type 2 diabetic patients improved the ability of insulin to inhibit hepatic glucose output. At present, the routes or mechanisms responsible for these beneficial training-mediated multiple organ adaptations are unknown and warrant further study.

In conclusion, restoration of mitochondrial dysfunction in type 2 diabetes by physical exercise improves insulin-mediated glucose disposal in the presence of increased IMCL storage. Restoration of mitochondrial function and metabolic flexibility in type 2 diabetes by exercise is at least partly accounted for by increased mitochondrial content and possibly by intrinsic mitochondrial adaptations. The insulin-sensitizing effect of exercise training occurs in the absence of major changes in body mass and is not restricted to improved muscle insulin sensitivity but extends to improved hepatic and adipose tissue insulin sensitivity.

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