Mitochondrial Capacity in Skeletal Muscle Is Not Stimulated by Weight Loss Despite Increases in Insulin Action and Decreases in Intramyocellular Lipid Content

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OBJECTIVE—In obesity and type 2 diabetes, exercise combined with weight loss increases skeletal muscle mitochondrial capacity. It remains unclear whether mitochondrial capacity increases because of weight loss, improvements in insulin resistance, or physical training. In this study, we examined the effects of an intervention of weight loss induced by diet and compared these with those of a similar intervention of weight loss by diet with exercise. Both are known to improve insulin resistance, and we tested the hypothesis that physical activity, rather than improved insulin resistance, is required to increase mitochondrial capacity of muscle.

RESEARCH DESIGN AND METHODS—Sixteen sedentary overweight/obese volunteers were randomized to a 16-week intervention of diet ($n = 7$) or diet plus exercise ($n = 9$). Insulin sensitivity was measured using euglycemic clamps. Mitochondria were examined in muscle biopsies before and after intervention. We measured mitochondrial content and size by electron microscopy, electron transport chain (ETC) activity, cardiolipin content, and mitochondrial DNA content. Intramyocellular content of lipid (IMCL) and fiber-type distribution were determined by histology.

RESULTS—The diet-only and diet plus exercise groups achieved similar weight loss (10.8 and 9.2%, respectively); only the diet plus exercise group improved aerobic capacity. Insulin sensitivity improved similarly in both groups. Mitochondrial content and ETC activity increased following the diet plus exercise intervention but remained unchanged following the diet-only intervention, and mitochondrial size decreased with weight loss despite improvement in insulin resistance. IMCL decreased in the diet-only but not in the diet plus exercise intervention.

CONCLUSIONS—Despite similar effects to improve insulin resistance, these interventions had differential effects on mitochondria. Clinically significant weight loss in the absence of increased physical activity ameliorates insulin resistance and IMCL but does not increase muscle mitochondrial capacity in obesity. ***Diabetes*** **57:987–994, 2008**

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ETC, electron transport chain; IMCL, intramyocellular content of lipid; mtDNA, mitochondrial DNA.

Mitochondrial oxidative capacity is decreased in skeletal muscle of obese individuals and is correlated with insulin resistance (1). It remains unclear, however, whether reduced mitochondrial capacity in skeletal muscle is causative for insulin resistance or, instead, arises as a consequence of insulin resistance (2). Recent clinical investigations from our group have demonstrated that the reduced muscle mitochondrial content and functional capacity in obesity and type 2 diabetes are reversible with moderate weight loss combined with moderate-intensity regular physical activity (3–5), a lifestyle intervention modeled on contemporary clinical recommendations (6). It was also observed that increases in mitochondrial parameters correlated with improved insulin sensitivity (4) and, in type 2 diabetes, with glucose lowering (3). One supposition is that physical activity may be the most important factor for improving mitochondrial capacity in insulin resistance, since exercise training promotes mitochondrial biogenesis in skeletal muscle of healthy individuals (7–9). Nonetheless, other explanations should be considered. Calorie restriction evokes a panoply of changes such as reduced visceral adiposity and intramyocellular lipid (IMCL) content and increased insulin sensitivity. The role of improved insulin resistance, in particular, merits consideration because insulin signaling has been postulated to affect mitochondrial capacity in muscle (2); therefore, it is possible that it is the improvement in insulin resistance that induces improvements in mitochondrial content and function in obesity and type 2 diabetes.

It is well established that insulin resistance improves in response to weight loss. The addition of exercise to a weight loss intervention may not improve insulin resistance more than weight loss alone (10). Accordingly, weight loss alone may be more pivotal than physical activity for amelioration of insulin resistance in obesity. Though the relative contribution of exercise and weight loss to improving insulin resistance has received much attention, there are few reports that specifically address the effects of weight loss on muscle mitochondrial biogenesis, which is in contrast to an abundant literature describing the effects of physical activity. This issue of the comparative effects of weight loss and physical activity on muscle mitochondrial has potential clinical importance in view of the association between mitochondrial dysfunction and insulin resistance. Kern et al. (11) observed an increase in skeletal muscle oxidative capacity in obese women following moderate weight loss, but the study did not include a specific metric of aerobic fitness. Simoneau et al. (12), in an intervention study in which moderate

weight loss was induced without changes in physical activity or aerobic fitness, observed no improvement in muscle oxidative enzyme activity (12). More recently, this issue was studied in the CALERIE study, though these participants were not obese (13). Calorie restriction was associated with increased expression of oxidative phosphorylation genes, but the related enzyme activities were unchanged (13).

The current study was undertaken to examine how mitochondrial capacity responds to diet-induced weight loss in insulin resistance and concomitantly compare the results with those of an intervention of weight loss plus moderate exercise. We hypothesized that both interventions would reduce systemic and central adiposity and improve insulin resistance but that mitochondrial capacity would increase only with the addition of exercise. A goal of this study was to achieve similar weight loss in the two intervention arms and thereby strengthen comparative examination of changes in insulin sensitivity and of skeletal muscle mitochondria. Muscle biopsies were obtained for determination of mitochondrial size and volume density, electron transport chain (ETC) activity, and the content of cardiolipin and mitochondrial DNA (mtDNA)—a panel of metrics to characterize the effect of intervention on mitochondrial biogenesis.

RESEARCH DESIGN AND METHODS

The protocol was approved by the University of Pittsburgh institutional review board. All participants had a screening medical history, physical examination, and screening laboratory tests. We sought to select a study population with a high likelihood of having insulin resistance: eligibility criteria included a BMI >28 kg/m^2, waist circumference >94 cm (men) or >80 cm (women) (14), weight stability (<3 kg change in the prior 2 months), a sedentary lifestyle (<20 min of exercise activity/week), and age >30 years. Enrolled participants were all Caucasian or African American, reflecting the demographics of Pittsburgh. Participants were excluded if they had diabetes; anemia; cardiopulmonary, neuromuscular, or renal disease; abnormal urine sediment; abnormal thyrotropin; alkaline phosphatase; or serum transaminases greater than 2.5 times the upper limit of the reference range.

Lifestyle intervention. After screening and baseline metabolic assessments, participants were randomized to a diet-only or a diet plus exercise intervention ($n = 7$ and $n = 9$, respectively). The goals were to achieve ≥7% weight loss in both groups within 16–20 weeks of intervention, and for those in the diet plus exercise group to exercise 3–5 days/week at moderate intensity (60–70% of maximal heart rate). The mean ± SE duration of intervention was 19.2 ± 0.4 weeks in the diet plus exercise group and 18.6 ± 0.7 weeks in the diet-only group ($P = 0.42$). To achieve weight loss, a research dietitian met weekly with participants to give instructions to reduce portion size, lower consumption of fat, maintain daily food records, and undertake related behavior change to reduce calorie intake by ~25%. In the diet plus exercise group, most participants chose walking, on a treadmill or otherwise, for exercise and were instructed to begin with 30-min sessions for the first month and increase to 40 min for the next month, at which time a submaximal treadmill stress test was performed to adjust the exercise prescription. At least one session weekly was supervised by an exercise physiologist, heart rate was recorded at each session, and participants were requested to maintain an exercise log and record heart rate during unsupervised exercise. Exercise intensity was quantified by the average heart rate recorded by a wireless monitor (Polar, Kempele, Finland) for each exercise session in the participant's personal exercise log. This provided an estimate of energy expenditure during each exercise bout based on the regression of heart rate and V_{O_2} determined at baseline.

Metabolic assessments. Before and after interventions, all participants underwent metabolic and physical fitness evaluations, which included a 75-g oral glucose tolerance test after an overnight fasting, a glucose clamp to measure insulin sensitivity, body composition studies, exercise tests, and a muscle biopsy. Participants were asked not to exercise for 2 days preceding the metabolic assessments. A modified Bruce treadmill protocol was used to measure maximal aerobic capacity. Fat mass and fat-free mass were assessed by dual-energy X-ray absorptiometry. Cross-sectional computed tomography images were obtained and centered at L_{4-5} and the midthigh to examine abdominal and thigh adipose tissue distribution.

For metabolic studies and biopsies, volunteers were admitted to the University of Pittsburgh General Clinical Research Center. Following a standardized dinner (7 kcal/kg; 50% carbohydrates, 20% protein, and 30% fat), they fasted for at least 12 h overnight. The next morning, a primed (200 mg/m^2), continuous (2 mg/min per m^2) infusion of [6,6-$^2H_2$]-glucose was started 150 min before initiating the euglycemic-hyperinsulinemic clamp to measure rates of glucose utilization (Rd) and endogenous glucose production. These were calculated using non–steady-state equations based on plasma [6,6-$^2H_2$]-glucose enrichment determined by gas chromatography–mass spectometry. The clamp was started with an insulin infusion (40 mU/m^2 per min) and plasma glucose maintained at 90 ± 5 mg/dl (~5 mmol/l) for 4 h with a variable dextrose infusion. Plasma and serum were sampled before and during the steady-state phase of the clamp for determinations of free fatty acid, glucose, and insulin concentrations.

Muscle biopsies. Samples of vastus lateralis skeletal muscle were obtained by percutaneous needle biopsy after local lidocaine anesthesia. Approximately 50–70 mg muscle tissue was obtained each time and immediately dissected of any adipose and connective tissue under low-magnification microscopy. A portion was saved for light and electron microscopy experiments and the remainder immediately stored in liquid nitrogen for biochemical determinations. Histological and biochemical assays were then conducted in parallel in paired samples when available.

Light microscopy. Samples were mounted in Cryomatrix (Shandon, Pittsburgh, PA) and then frozen directly in isopenthane cooled to its freezing point with liquid nitrogen. From each tissue block, serial transverse sections (8 μm) were cut using a cryostat at −20°C and then mounted on Superfrost/Plus slides (Fisher Scientific, Pittsburgh, PA). Immunohistochemistry with an anti-myosin monoclonal antibody was performed to identify fiber types (I, IIa, and IIb). Lipid content was determined with oil red O staining as in previous studies (15). Using digital imaging software, regions of interest were outlined in the cytoplasm of each muscle fiber and average staining density determined. All values were subtracted from background density. Images were acquired with an optical microscope (Nikon Microphot-FXL, Tokyo, Japan) connected to a digital video camera (Sony, Tokyo, Japan) and analyzed using digital image software (MVIA, Monaca, PA).

Transmission electron microscopy. Muscle (10 mg) was cut into small pieces (1 × 1 × 2 mm), fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated, and embedded in Epon for transmission electron microscope. After tissue fixation, randomly sampled transverse sections of muscle fibers were obtained followed by 10–12 random micrographs acquired with an electron microscope (JEM-1210; JEOL, Tokyo, Japan) on a final magnification of 36,000×. Mitochondrial volume density, i.e., the fraction of the cell occupied by mitochondria, was determined in images of the intermyofibrillar compartment, as in our previous studies (3,4), using a digitally overlapped grid of 144 intersection points and stereological analysis methodology. Mitochondrial cross-sectional area was determined by digital imaging software (Metamorph 6.3; Molecular Devices, Sunnyvale, CA). Measurements were conducted in a blinded fashion.

Mitochondrial biochemistry. The remainder 20–30 mg of muscle available was homogenized as in our previous studies (16–18). To prepare soluble and particulate fractions, homogenates were centrifuged at 45,000g × 20 min (17). The soluble fraction was mixed (1:1) with buffer containing 50% glycerol (17) and saved for analysis of creatine kinase. The pellet (particulate fraction) containing mitochondria was suspended in storage medium containing 0.1 mg/ml BSA, 20 μmol/l leupeptin, and 25% glycerol (17). To release mitochondria trapped by the myofibrillar matrix, the particulate fraction was treated with the KCl/pyrophosphate method (16,18,19). Samples were stored at −80°C before analyses.

NADH-oxidase activity and cardiolipin content in the particulate fraction were measured by high-performance liquid chromatography (1,2) and expressed relative to creatine kinase activity to normalize for minor variations in muscle content (17). As in our previous studies, creatine kinase activity (units/g wet weight tissue) was not affected by the interventions (diet plus exercise, 5,951 ± 411 vs. 6,352 ± 576; diet only, 5,644 ± 511 vs. 5,520 ± 439; means not statistically different).

mtDNA. mtDNA copy number was measured by quantitative PCR (TaqMan, Applied Biosystems) and expressed relative to nuclear DNA copy number (5). DNA was extracted from biopsy samples (QIAamp DNA Mini Kit; Qiagen, Chatsworth, CA); 20 ng was used as a template against cytochrome b for mtDNA genome and against β-globin for nuclear DNA.

Statistics. Data are presented as means ± SEM unless otherwise indicated. Statistical significance was accepted with P values ≤0.05. Baseline characteristics in Table 1 were statistically examined by two-tailed unpaired t tests after confirming that variables followed a normal distribution. Sex distribution was examined with Fisher's test. To examine the factors of group and intervention, we employed two-way ANOVA with repeated measures. For body weight, fat mass, and insulin sensitivity, we further examined whether the mean percent-

TABLE 1
Basic characteristics of subjects

	Diet and exercise group	Diet-only group
n	9	7
Sex (male/female)	3/6	3/4
Age (years)	42.4 ± 2.7	46.1 ± 2.0
Men	43.3 ± 1.9	43.3 ± 2.9
Women	42.0 ± 4.0	48.2 ± 2.6
BMI (kg/m^2)	34.8 ± 1.1	33.4 ± 1.2
Men	34.6 ± 2.1	33.6 ± 2.0
Women	34.9 ± 1.4	33.3 ± 1.7
Waist (cm)	107.2 ± 3.8	111.8 ± 5.3
Men	114.8 ± 0.25	114.0 ± 9.0
Women	104.7 ± 4.7	110.1 ± 7.4
A1C (%)	5.5 ± 0.1	5.5 ± 0.1
Men	5.4 ± 0.1	5.2 ± 0.3
Women	5.5 ± 0.15	5.6 ± 0.1
Fasting glucose (mmol/l)	5.35 ± 0.15	5.48 ± 0.10
Men	5.69 ± 0.36	5.46 ± 0.24
Women	5.18 ± 0.10	5.49 ± 0.08
2-h postchallenge glucose	7.83 ± 0.50	9.05 ± 0.44
Men	7.42 ± 1.18	9.46 ± 1.02
Women	8.04 ± 0.54	8.75 ± 0.35

Data are means ± SE unless otherwise indicated. There were no statistically significant differences between groups at baseline.

age of change with intervention was similar between groups using two-tailed *t* tests.

RESULTS

Baseline and postintervention clinical characteristics. As shown in Table 1, the baseline (preintervention) characteristics of volunteers in both groups were similar, and insulin resistance was apparent in both groups. In both groups, mean glycemia after an oral glucose tolerance test was greater than the diagnostic threshold of impaired glucose tolerance.

Significant weight loss was achieved in both the diet-only and the diet plus exercise interventions (Table 2): mean individual weight loss was −10.8 ± 1.6% in the diet-only group and −9.2 ± 1.2% in the diet plus exercise group, values that were not statistically different from each other. There was a nearly identical decrease in fat mass in both groups (19 ± 3 and 18 ± 3% in the diet-only and diet plus exercise groups, respectively). Comparable reductions in adiposity within various regional depots occurred in both groups.

Though loss of fat mass was similar in both intervention groups, there was a difference in effect on aerobic fitness (Table 2). At baseline, maximal aerobic capacity ($V\mathrm{O}_{2\mathrm{max}}$; normalized to lean body mass) was similar in both groups. Participants in the diet-only group were instructed not to alter previous patterns of physical activity, and at the end of intervention $V\mathrm{O}_{2\mathrm{max}}$ of these participants had not significantly changed (mean change 1.6 ± 4.0% from baseline). In contrast, there was a significant increase in $V\mathrm{O}_{2\mathrm{max}}$ in the diet plus exercise group (10.7 ± 3.9% from baseline). Intervention logs indicated that diet plus exercise participants participated in the prescribed four sessions weekly: 1.5 ± 0.2 sessions weekly under supervision at the research facility and 2.5 ± 0.4 sessions weekly without supervision. Thus, the intent of the study was attained, which was to have both groups achieve similar weight loss but differ in the change in fitness and with little change in the diet-only intervention, allowing us to examine the effects of exercise beyond those attributable to weight loss alone.

Effects on insulin sensitivity. As shown in Table 3, both groups had elevated fasting insulin concentrations, which decreased following intervention. Systemic insulin sensitivity was quantified using euglycemic clamps. Pre- and postintervention steady-state plasma glucose concentrations were similar between groups. Steady-state plasma insulin during clamp studies was slightly lower after interventions, consistent with an increase in insulin clearance after weight loss. As shown in Fig. 1, insulin-stimulated glucose disposal (*M*) increased following intervention in both groups. The mean percentage of increase in response to intervention was 38 ± 9% in the diet plus exercise group and 29 ± 7% in the diet-only group. These increases were statistically similar and remained similar when *M* was normalized for insulin concentrations during steady-state conditions (*M/I*). Therefore, the addition of moderate-intensity exercise to the weight loss intervention, despite attainment of improved aerobic fitness, did not further improve insulin resistance.

Effects on muscle fiber type, IMCL, and mitochondria. Skeletal muscle fiber-type distribution was not altered by either intervention, as shown in Fig. 2. However, differing responses upon IMCL were observed, as shown in Fig. 3. IMCL did not change from baseline in response to

TABLE 2
Effect of each intervention on aerobic capacity and adiposity

	Diet and exercise group			Diet-only group		
	Before	After	*P*	Before	After	*P*
Whole-body adiposity (kg)						
Weight	94.8 ± 4.4	86.3 ± 4.6	<0.01	95.0 ± 4.3	84.4 ± 2.7	<0.01
Fat mass	40.2 ± 1.7	33.1 ± 1.9	<0.01	39.5 ± 2.9	32.0 ± 3.0	<0.01
Lean mass	52.6 ± 3.8	51.3 ± 3.5	NS	53.0 ± 4.5	50.9 ± 4.1	<0.05
Regional adiposity (cm^2)						
Midthigh SAT	147.8 ± 13.3	119.5 ± 12.9	<0.01	136.0 ± 22.9	111.4 ± 21.7	<0.05
Abdominal SAT	468.3 ± 29.8	388.3 ± 31.4	<0.01	430.4 ± 53.7	340.6 ± 44.8	<0.01
VAT	202.5 ± 27.8	150.9 ± 23.1	<0.01	207.9 ± 24.7	172.1 ± 32.0	<0.05
Maximal aerobic capacity (ml $O_2 \cdot \mathrm{min}^{-1} \cdot$ kg LBM^{-1})	47.6 ± 2.5	52.4 ± 2.2	<0.05	44.5 ± 2.5	45.2 ± 2.9	NS

Data are means ± SE unless otherwise indicated. There were no statistically significant differences between groups. LBM, lean body mass; NS, not significant; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

TABLE 3
Hyperinsulinemic-euglycemic clamps before and after intervention

	Diet and exercise group			Diet-only group		
	Before	After	*P*	Before	After	*P*
Fasting						
Plasma FFA (mmol/l)	0.54 ± 0.04	0.53 ± 0.04	NS	0.48 ± 0.04	0.54 ± 0.06	NS
Plasma glucose (mmol/l)	5.47 ± 0.15	5.23 ± 0.12	<0.05	5.37 ± 0.17	5.25 ± 0.14	NS
Plasma insulin (μU/ml)	19.2 ± 2.0	12.4 ± 1.8	<0.01	13.0 ± 1.7*	10.2 ± 1.4	<0.05
During hyperinsulinemic clamp						
Plasma glucose (mmol/l)	4.98 ± 0.03	5.06 ± 0.07	NS	5.12 ± 0.01	5.12 ± 0.03	NS
Plasma insulin (μU/ml)	89.7 ± 6.6	80.7 ± 5.9	<0.05	86.0 ± 8.7	77.9 ± 7.2	<0.05

Data are means ± SE unless otherwise indicated. *P* values for the effect of intervention are shown. *$P < 0.05$ between groups before intervention. FFA, free fatty acid, NS, not significant.

diet plus exercise, a finding consistent with prior observations (15,21). In contrast, there was a significant decrease in IMCL following the diet-only intervention. As shown in Fig. 4, diet plus exercise increased mitochondrial density, reflecting an increase in mitochondrial content. The mean increase was 49 ± 16% from baseline. In contrast, mitochondrial density remained unchanged in the diet-only group. Also, distinct patterns of mitochondrial ultrastructural adaptation occurred between the two interventions (Fig. 4): mean mitochondrial size decreased in the diet-only group, on average by −17 ± 4% from baseline ($P < 0.05$), but remained unchanged in the diet and exercise group. Biochemical findings corroborated the microscopy data (Fig. 5). Cardiolipin content, a marker for the amount of the inner mitochondrial membrane, increased in the diet plus exercise group but not in the diet-only group. Likewise, ETC activity increased in response to the diet plus exercise intervention but remained unchanged following the diet-only intervention. mtDNA encodes important components required for mitochondrial respiration, so we examined whether the mtDNA content (expressed as mtDNA copies relative to nuclear DNA copies) was affected by interventions. There were no significant changes in mtDNA following the diet-only intervention (2,297 ± 439 vs. 2,508 ± 414; pre- and postintervention, respectively) or the diet plus exercise intervention (2,049 ± 296 vs. 2,185 ± 338).

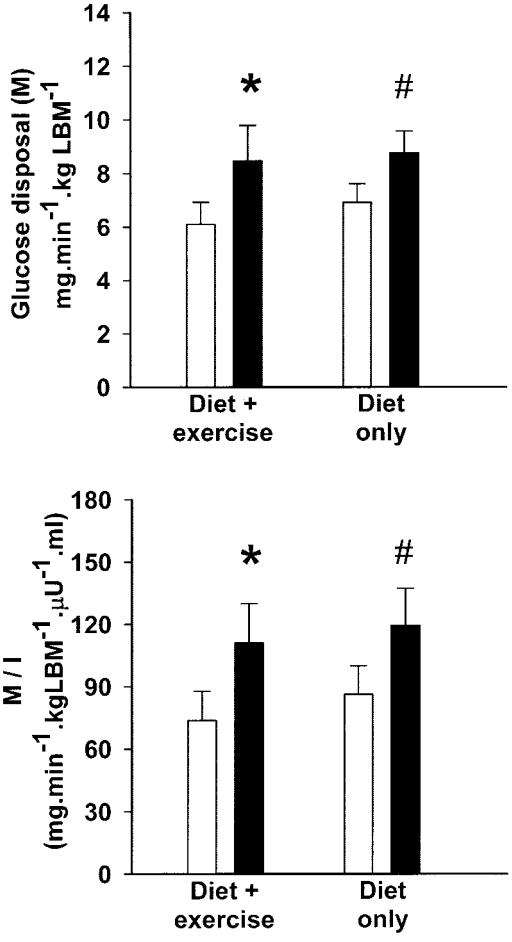

FIG. 1. Insulin sensitivity measured by euglycemic clamps performed before (white bars) and after (black bars) intervention. Glucose disposal (*M*) adjusted for lean body mass (LBM) improved after intervention in both groups. *$P < 0.05$ and #$P < 0.01$ after intervention. *M/I* indicates glucose disposal normalized for insulin concentrations during steady state. There were no differences of statistical significance in insulin sensitivity between groups.

DISCUSSION

The effects of weight loss in improving obesity-related insulin resistance are well established, but there is less information concerning the impact on muscle mitochondria, organelles that have begun to emerge as an independent target of intervention for improving insulin resistance. In the current study, the diet-only and diet plus exercise interventions achieved ~10% weight loss and nearly identical loss of fat mass. This degree of weight loss is generally regarded as clinically significant, and consistent with this there was improvement in insulin-stimulated glucose disposal, indicating improved insulin sensitivity in muscle tissue. Notably, the improvement in insulin resistance was quite similar between the diet-only and diet plus exercise groups. However, there were differences between these interventions in the effect on muscle lipid content and muscle mitochondria. IMCL was reduced by the diet only intervention, yet this intervention did not improve mitochondrial capacity. In contrast, diet plus exercise led to an unambiguous increase in mitochondrial capacity but did not lower IMCL, the latter finding consistent with the notion that exercise training favors storage of triglyceride in muscle. These observations provide a valuable intervention-based perspective to examine the interactions of insulin resistance, obesity, and skeletal muscle mitochondria.

The etiology of reduced mitochondrial capacity in skeletal muscle in insulin resistance is not yet fully established. Acute insulin administration increases mitochondrial ATP production (2,22), and chronic treatment of diabetic individuals with subcutaneous insulin improves gene transcription for mitochondrial biogenesis (23). These studies

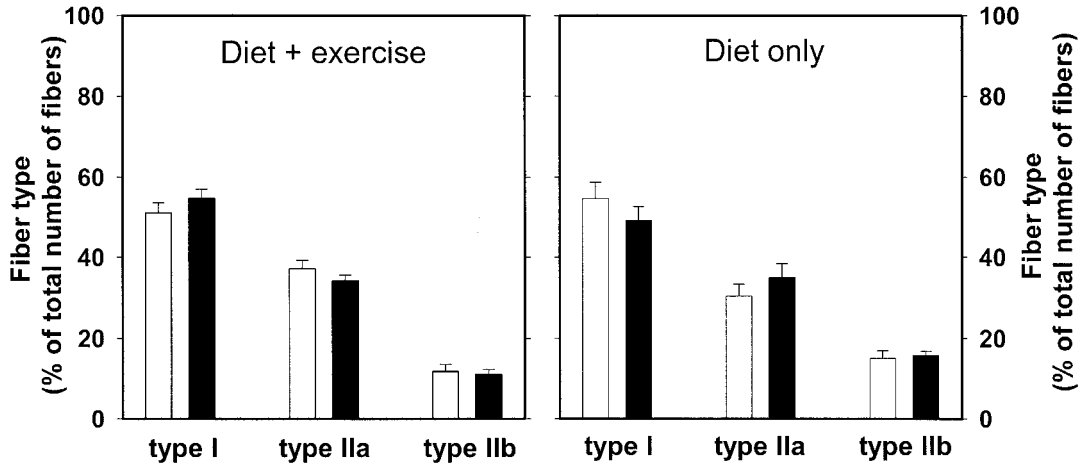

FIG. 2. Proportion of skeletal muscle fiber types before (white bars) and after (black bars) intervention. Fiber type distribution did not change after intervention in both groups.

give indirect support to the hypothesis that decreased mitochondrial capacity in insulin resistance might arise as a consequence of impaired insulin action. The alternative hypothesis is that mitochondrial dysfunction occurs as a primary abnormality and leads to insulin resistance (24). Studies from our laboratory and from other investigations have clearly shown that reduced mitochondrial capacity associated with obesity and type 2 diabetes is not a fixed defect. Rather, mitochondrial capacity can be considerably stimulated by an intervention combining moderate weight loss with moderate-intensity exercise (3,4,25). This intervention evokes a variety of physiological changes including decreased insulin resistance; therefore, the direction of causality between mitochondrial dysfunction and insulin resistance, if one does exist, cannot be determined. Accordingly, the impetus for the current study was to achieve weight loss with and without concomitant exercise and to compare effects on insulin resistance and on muscle mitochondria. In obese individuals with insulin resistance, improvement in insulin resistance occurs quickly upon initiating reduced caloric intake. Therefore, we postulated that if mitochondrial dysfunction in muscle arises as a consequence of insulin resistance, improvement in mitochondrial parameters should follow a period of sustained improvement in insulin resistance induced by weight loss. However, we did not observe this to occur, despite unequivocally improved insulin resistance and reduced IMCL. This result leads us to draw two conclusions: *1*) amelioration of insulin resistance is not contingent on a concomitant increase in mitochondrial capacity in muscle, and *2*) reduced mitochondrial content and functional capacity in obesity are not solely consequences of insulin resistance.

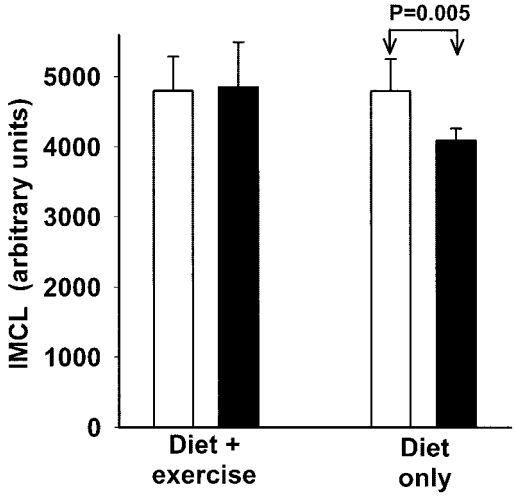

FIG. 3. Content of IMCL before (white bars) and after (black bars) intervention. Only the diet-only intervention induced changes in IMCL content.

Our data do not negate that insulin may promote effects on skeletal muscle mitochondria, as has been well documented under experimental conditions (23). However, our data do not support the notion that insulin resistance is a prime determinant of skeletal muscle mitochondrial dysfunction in obesity. The obese participants with insulin resistance experienced a robust improvement in insulin action, chronically compounded over 16 weeks. Yet, increases in mitochondrial content and oxidative function did not occur. In contrast, the diet plus exercise intervention resulted in a clear increase in mitochondrial content and oxidative capacity, confirming prior results in other cohorts of overweight/obese and type 2 diabetic subjects (3,4). Such increases in mitochondrial content were observed in the setting of changes in body adiposity and insulin resistance similar to those that occurred with the diet-only intervention but with a differential effect on aerobic fitness. This observation indicates that physical activity is a chief factor controlling mitochondrial capacity in insulin resistance, calling into question whether it is sedentary behavior that is responsible for reduced mitochondrial capacity in obesity-related insulin resistance.

There are sparse prior data concerning the effects of weight loss on muscle oxidative capacity. Kern et al. (11) reported an increase in muscle oxidative capacity among obese women following weight loss, but the contribution of physical activity in that intervention is uncertain, and aerobic capacity was not assessed. Simoneau (12) reported that weight loss in obese insulin resistance adults did not increase oxidative enzyme activities in vastus lateralis despite improvement in insulin sensitivity. In the present study, more detailed mitochondrial assessments were undertaken. In response to the diet-only intervention, as noted above, we did not observed a change from baseline mitochondrial capacity, but we did find subtle

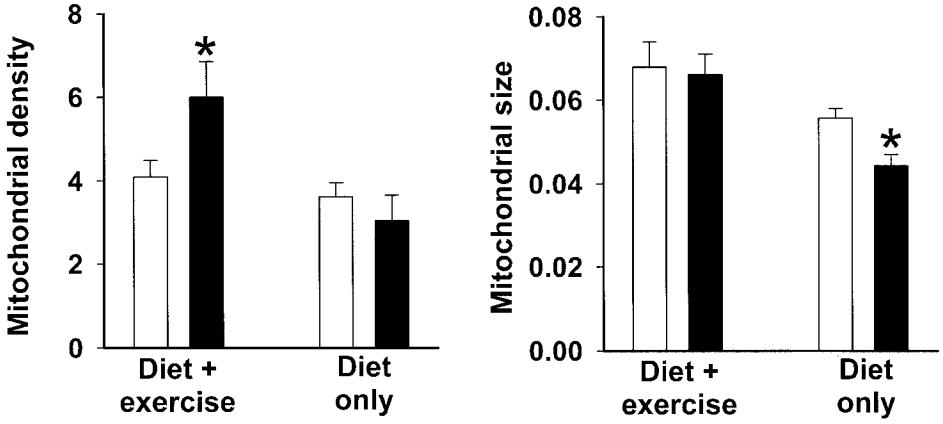

FIG. 4. Mitochondrial density and size assessed by transmission electron microscopy and morphometry before (white bars) and after (black bars) intervention. *Left panel*: mitochondrial density (%). *Right panel*: mean mitochondrial size (μm^2). *$P < 0.005$ after intervention.

ultrastructural mitochondrial changes. Mitochondrial size decreased by ~17%, an observation not previously reported. Interestingly, mitochondrial size has been correlated with insulin sensitivity (1,4). In the present investigation, we show that weight loss induced even smaller mitochondria, thus dissociating this phenotype from insulin resistance. It is potentially conceivable that the decrease in mitochondrial size is related to reduced energy metabolism, as occurs with weight loss (26). Conversely, mitochondrial size has been shown to be increased in exercise-trained humans (27,28) and to increase after exercise training in healthy, young subjects (29). Increased mitochondrial size has been postulated to support the high energy demands of exercise activity (30), reducing the diffusion distances to energy-demanding compartments like the sarcoplasmic reticulum and contractile apparatus (30). We have previously shown that some degree of mitochondrial enlargement can occur in obese nondiabetic and diabetic subjects following an intervention of moderate-intensity exercise with weight loss (3,4). However, in the present study, no mitochondrial enlargement was observed in the diet plus exercise group. The reason behind this incongruity among studies is unclear. A novel finding of our study is that weight loss may decrease mitochondrial size; thus, one conceivable possibility is that the opposing effects of exercise training and weight loss on mitochondrial size offset each other. Consistent with this notion, the magnitude of weight loss in the diet plus exercise group (9.2 ± 1.2%) was more marked than in our former studies, in which we employed a similarly structured diet plus exercise intervention in sedentary obese individuals (8.4 ± 2.0%) (4) and sedentary individuals with type 2 diabetes (7.1 ± 0.9%) (3). However, other explanations should be considered. It remains uncertain whether differences in sex distribution among studies might have played a role as well, since the present study had more women ($n = 6$) than men ($n = 3$), while our former studies had a balanced male/female distribution. Despite these uncertainties, it is unlikely that the age of subjects was an influencing factor because the mean age in the present study (42 years) is quite similar to that of our former studies with nondiabetic (39 years) and diabetic (44 years) subjects (3,4).

In the present study, there was no significant change in skeletal muscle mtDNA content after weight loss with or without the addition of exercise. Other studies have also found that mitochondrial biogenesis can be induced by an intervention of weight loss and moderate intensity exercise in the absence of increased mtDNA (3,5). Recently, however, the CALERIE (Comprehensive Assessment of Long-Term Effects of Reducing Intakes of Energy) study showed that calorie restriction in healthy, nonobese individuals increases skeletal muscle mtDNA (13). Because of the clear differences in characteristics of the research volunteers in the two studies, we believe that our data are not necessarily incongruent with the latter observation. Subjects in the CALERIE study were lean or overweight and may not have had insulin resistance. Additionally,

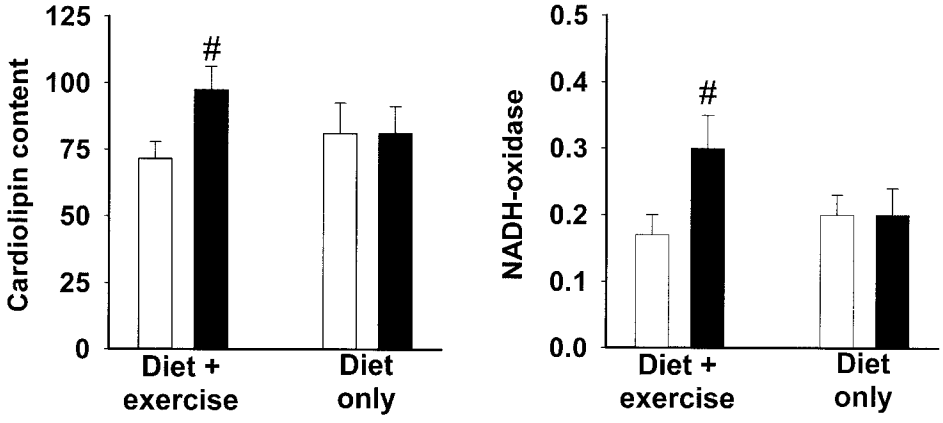

FIG. 5. Mitochondrial capacity assessed by biochemistry: mitochondrial cardiolipin content (μg/creatine kinase) and mitochondrial NADH-oxidase activity (U/mU creatine kinase) were measured before (white bars) and after (black bars) intervention. #$P < 0.01$ after intervention.

mtDNA content may not be the most accurate marker of mitochondrial mass in skeletal muscle because of the existence of a reticulated network of mitochondria in skeletal muscle (31).

Acute lipid loading of skeletal muscle achieved by infusion of a lipid emulsion is associated with decreased expression of peroxisome proliferator–activated receptor-γ coactivator-1 and nuclear encoded mitochondrial genes (32), an observation suggesting that lipotoxicity may contribute to the pathogenesis of decreased oxidative enzyme activity in insulin resistance. On the other hand, recent studies in rodents indicate that a high-fat diet is accompanied by increased oxidative capacity in skeletal muscle (33). These observations implicate a possible role of intramyocelullar lipid content on mitochondria. In the current study, weight loss was associated with decreased IMCL content, but this was not accompanied by changes in mitochondrial capacity. The most straightforward interpretation is that the chief determinant of oxidative capacity in skeletal muscle is physical activity or, more specifically, the energy demand created during frequent bouts of physical activity rather than the level of IMCL or insulin resistance per se.

A limitation of our study is that it only examined vastus lateralis mitochondria. Therefore, it is uncertain whether our findings can be generalized to mitochondria of other muscles. Although this is a limitation, it allows for a direct comparison with findings from other human studies on insulin resistance and muscle mitochondria, since the majority of these published studies have also employed vastus lateralis muscle biopsies (1–5,13,22,23,25,34).

In summary, moderate weight loss, even when accompanied by reductions in IMCL and a significant improvement in insulin resistance, does not augment mitochondrial content or function in obese adults with insulin resistance. These findings indicate that the reduced mitochondrial content of insulin resistance is unlikely a primary consequence of insulin resistance and that improvement in skeletal muscle insulin resistance can occur independently of changes in mitochondrial capacity.

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