Original Article

Effects of Physical Activity and Weight Loss on Skeletal Muscle Mitochondria and Relationship With Glucose Control in Type 2 Diabetes

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OBJECTIVE—Reduced mitochondrial capacity in skeletal muscle occurs in type 2 diabetic patients and in those at increased risk for this disorder, but the extent to which mitochondrial dysfunction in type 2 diabetic patients is remediable by physical activity and weight loss intervention is uncertain. We sought to address whether an intervention of daily moderate-intensity exercise combined with moderate weight loss can increase skeletal muscle mitochondrial content in type 2 diabetic patients and to address the relationship with amelioration of insulin resistance and hyperglycemia.

RESEARCH DESIGN AND METHODS—Muscle biopsies were obtained before and after a 4-month intervention to assess mitochondrial morphology, mitochondrial DNA content, and mitochondrial enzyme activities. Glucose control, body composition, aerobic fitness, and insulin sensitivity were measured.

RESULTS—In response to a weight loss of 7.1 ± 0.8% and a 12 ± 1.6% improvement in $V_{O_{2max}}$ ($P < 0.05$), insulin sensitivity improved by 59 ± 21% ($P < 0.05$). There were significant increases in skeletal muscle mitochondrial density (by 67 ± 17%, $P < 0.01$), cardiolipin content (55 ± 17%, $P < 0.01$), and mitochondrial oxidation enzymes. Energy expenditure during physical activity correlated with the degree of improvement in insulin sensitivity ($r = 0.84$, $P < 0.01$), and, in turn, improvement in mitochondrial content was a strong correlate of intervention-induced improvement in A1C and fasting plasma glucose.

CONCLUSIONS—Intensive short-term lifestyle modifications can restore mitochondrial content and functional capacity in skeletal muscle in type 2 diabetic patients. The improvement in the oxidative capacity of skeletal muscle may be a key component mediating salutary effects of lifestyle interventions on hyperglycemia and insulin resistance. ***Diabetes*** **56:2142–2147, 2007**

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mtDNA, mitochondrial DNA.

The prevalence of type 2 diabetes continues to increase, and a sedentary lifestyle and obesity are recognized as key risk factors (1,2). Obesity and sedentary habits exacerbate insulin resistance in type 2 diabetic patients (3) and pose a risk for mortality (4,5). In type 2 diabetic patients, skeletal muscle mitochondria are reduced in size, and there is reduced activity of the electron transport chain (6–8). A diminished stimulation of oxidative phosphorylation by insulin has also been reported (9). In skeletal muscle of nondiabetic individuals with a family history of type 2 diabetes, decreased expression of nuclear genes encoding proteins of oxidative phosphorylation has been reported (10,11), along with reduced in vivo oxidative phosphorylation (12,13). These findings suggest that mitochondrial impairments in type 2 diabetes may have a heritable component. However, it is also well established that mitochondria are rather adaptable organelles and that skeletal muscle of healthy individuals can manifest considerable plasticity of mitochondrial content, adapting to match energy demands of physical activity (14,15). Such plasticity appears to be preserved in insulin resistance associated with obesity and aging (7,16,17) but has not yet been demonstrated in type 2 diabetes.

Moderate-intensity physical activity, exemplified by a nearly daily habit of brisk walking, and a 5–10% weight loss remain cornerstones for the management of type 2 diabetes. This is effective in the prevention of type 2 diabetes (18,19) and can improve hyperglycemia and other cardiovascular risk factors in patients with type 2 diabetes (20). However, it is uncertain whether this type of intervention can rectify the deficit in muscle mitochondrial capacity in subjects with type 2 diabetes, in whom more marked metabolic disturbances coexist. To address this issue, we implemented a 4-month intervention of weight loss and physical activity in patients with type 2 diabetes and examined muscle mitochondria by four interrelated parameters using biopsy samples of vastus lateralis. Our findings indicate that there is a clear potential for remediation of mitochondrial dysfunction in type 2 diabetic patients and that the changes induced play an important role in mediating the positive metabolic effects on hyperglycemia.

RESEARCH DESIGN AND METHODS

Inclusion criteria for participation were as follows: *1*) a confirmed diagnosis of type 2 diabetes, *2*) nonpharmacological management alone or with met-

formin and/or sulfonylurea, *3*) stable weight (<3 kg change in the preceding 2 months) with a BMI in the range of 28–38 kg/m^2, *4*) aged between 30 and 55 years, and *5*) sedentary habits. A screening examination, including a graded treadmill exercise test, was done to exclude those with contraindications to moderate-intensity exercise or to weight loss. Laboratory criteria for eligibility were as follows: normal urine sediment, hematocrit, serum creatinine, thyrotropin, alkaline phosphatase, and aspartate and alanine aminotransferase <2.5 × the upper limit of reference range. All research participants gave written informed consent. The protocol was approved by the University of Pittsburgh institutional review board.

Weight loss and physical activity intervention. The behavioral weight loss program and moderate-intensity physical activity intervention lasted 16–20 weeks; the protocol was similar to that in our previous studies (16,21). Medications were not changed or adjusted. The goals were to achieve ≥7% weight loss and exercise on most days of the week at moderate intensity (60–70% of maximal heart rate). 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 behavioral change to reduce calorie intake by ~25%. 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 intervention, participants underwent evaluation of insulin sensitivity, body composition, and fitness and had a muscle biopsy. All procedures were repeated at completion of the intervention, and participants were asked not to exercise for 2 days preceding the metabolic assessments. After an overnight fast, the glycemic response to a 75-g oral glucose challenge was determined. A modified Bruce treadmill protocol was used to measure maximal aerobic capacity. Fat mass and fat-free mass were assessed using dual-energy X-ray absorptiometry. Cross-sectional computed tomography images were obtained, centered at L_{3-4}, T_{12}, and the midthigh, respectively, to examine abdominal adipose tissue distribution and to estimate fat content within liver and in skeletal muscle (22). In liver and skeletal muscle, the mean computed tomography attenuation value was used as a noninvasive index of fat content (23,24), and, for the liver, a value for the ratio of liver to spleen computed tomography attenuation <1.0 was used to indicate hepatic steatosis (22).

For assessment of insulin sensitivity and biopsies, participants 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 were fasted overnight. The next morning, a euglycemic-hyperinsulinemic clamp was performed. A primed (200 mg/m^2), continuous (2 mg/min per m^2) infusion of [6,6-$^2H_2$]glucose was started 3 h before initiating the clamp to measure rates of glucose utilization and endogenous glucose production. These were calculated using non–steady state equations (25) based on plasma [6,6-$^2H_2$]glucose enrichment determined by gas chromatography–mass spectrometry (22). The euglycemic clamp was started with an insulin infusion (40 mU/m^2 per min) and euglycemia maintained for 4 h with a variable dextrose infusion (26). Plasma and serum were sampled before and during the clamp for determinations of free fatty acid, glucose, and insulin concentrations.

Resting metabolic rate, respiratory quotient, glucose, and fat oxidation rates were determined with open-circuit indirect calorimetry, taking into account measured urinary nitrogen excretion (27). These were determined during a 30-min period preceding the clamp and repeated during the last 30 min of the clamp, after which subcutaneous lidocaine was applied and a percutaneous vastus lateralis needle biopsy was performed.

Light microscopy. Muscle samples were dissected free of adipose and connective tissue, 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). Images were acquired with an optical microscope (Microphot-FXL; Nikon, Tokyo, Japan) connected to a digital video camera (Sony, Tokyo, Japan) and analyzed using digital image software (Microscope Vision and Image Analyst, Monaca, PA).

Transmission electron microscopy. A portion of the muscle sample was used for determination of mitochondrial content as previously described (7). Images of 10 transverse sections of muscle fibers were obtained at 36,000× (JEM-1210; JEOL, Tokyo, Japan). Mitochondrial cross-sectional area (size) and mitochondrial volume density (the fraction of cell volume occupied by mitochondria) were measured by digital imaging morphometry and stereological principles of point sampling, in a blind fashion, as previously described (7).

Mitochondrial biochemistry. Total activities of rotenone-sensitive NADH oxidase, citrate synthase, creatine kinase, and cardiolipin content were determined in 20–30 mg muscle (28–30). Muscle was homogenized and centrifuged to prepare a particulate fraction containing >95% tissue mitochondria. Rotenone-sensitive NADH oxidase activity and cardiolipin content were determined in this fraction. Citrate synthase activity was determined as the sum of activities in soluble and particulate fractions. Cardiolipin, citrate synthase, and NADH oxidase activities were expressed relative to creatine kinase activity, measured in each homogenate to account for minor variations in muscle fiber content, as earlier described (29). Creatine kinase activity did not change with the intervention (3,724 ± 198 vs. 4,049 ± 263 units/g wet wt tissue for pre- and postintervention, respectively; P = NS).

Mitochondrial DNA. Mitrochondrial DNA (mtDNA) copy number was determined by quantitative PCR (TaqMan; Applied Biosystems) and expressed relative to nuclear DNA (16). DNA was extracted from biopsy samples (QIAamp DNA Mini Kit; Qiagen, Chatsworth, CA). Twenty nanograms were used as a template against cytochrome *b* for mtDNA genome and against β-globin for nuclear DNA. The threshold cycle number was calculated using SDS software (version 1.7; Applied Biosystems).

Statistics. Data are presented as means ± SEM unless otherwise indicated. Statistical significance was accepted at a P value ≤0.05. The effect of the intervention was examined with two-tailed paired t tests. Pearson analysis was used to determine correlations between variables.

RESULTS

Body composition, aerobic fitness, and glycemic control. Participants achieved a mean weight loss of 7.1 ± 0.9% ($P < 0.001$) (Table 1). Mostly, this was due to decreased fat mass, notably including a decrease in visceral adiposity. There was considerable reduction in hepatic fat content, noted by increased liver computed tomography attenuation (Hounsfield units), and there was a lesser but significant increase in computed tomography attenuation values for skeletal muscle, denoting a modest decrease in muscle lipid content. The percentage of decrease in abdominal adiposity, for both the visceral and subcutaneous depots, was greater than that for lower extremity subcutaneous adiposity ($P < 0.01$), and the decrease in visceral abdominal tissue was strongly correlated with the concomitant reduction in hepatic steatosis ($r = 0.93$, $P < 0.001$).

Physical fitness was particularly improved by the intervention, noted by a mean 12 ± 1.6% increase in maximum aerobic capacity ($V_{O_{2max}}$), a mean 40 ± 8% increase in the maximal MET output attained during exercise testing, and a decrease in resting heart rate from 76 ± 4 to 68 ± 4 bpm ($P < 0.01$). An average of 1.6 ± 0.3 exercise sessions each week, representing one-third of weekly sessions, were conducted under direct supervision, and the average energy expenditure per session was 355 ± 40 kcal. Estimates of energy expenditure during unsupervised sessions, also based on recorded heart rate responses, were similar (311 ± 55 kcal) and correlated with measurements obtained during supervised sessions ($r = 0.92$, $P < 0.001$).

The intervention led to substantial improvements in hyperglycemia. A1C decreased from 7.9 ± 0.5 to 6.5 ± 0.3% ($P < 0.01$), and there were significant improvements in the fasting and 2-h postchallenge plasma glucose values, as these decreased to near diagnostic thresholds for diabetes.

Insulin sensitivity and calorimetry. Insulin sensitivity, as determined by insulin-stimulated glucose utilization during euglycemic clamps, was improved after interven-

TABLE 1
Baseline and postintervention characteristics of research participants with type 2 diabetes

	Baseline	Postintervention	*P*
n	10	10	—
Age (years)	44 ± 3	—	—
BMI (kg/m^2)	34.0 ± 0.7	31.9 ± 0.7	<0.001
Weight (kg)	99.5 ± 3.8	92.4 ± 3.4	<0.001
Fat mass (kg)	36.6 ± 2.1	31.1 ± 2.1	<0.001
Lean body mass (kg)	60.0 ± 4.1	59.1 ± 3.7	—
Visceral fat (cm^2)	242.0 ± 28.0	183.0 ± 17.0	<0.01
Subcutaneous abdominal fat (cm^2)	382.0 ± 31.0	360.0 ± 30.0	—
Subcutaneous midthigh fat (cm^2)	108.0 ± 19.0	101.0 ± 21.0	—
Intermyofascial midthigh fat (cm^2)	16.0 ± 3.0	12.0 ± 1.0	—
Thigh muscle CT attenuation (HU)	48.2 ± 1.0	50.3 ± 0.9	<0.01
Liver-to-spleen CT attenuation ratio	0.83 ± 0.09	1.11 ± 0.06	<0.01
$V\mathrm{O}_{2max}$ (ml $O_2 \cdot \mathrm{min}^{-1} \cdot$ kg lbm^{-1})	43.5 ± 1.6	48.6 ± 1.6	<0.001
Maximal METs	8.7 ± 0.5	12.0 ± 0.5	<0.001
A1C (%)	7.85 ± 0.52	6.47 ± 0.25	<0.01
Oral glucose tolerance test (75g)			
Glucose, 0 min (mg/dl)	164.0 ± 15.0	126.0 ± 6.0	<0.05
Glucose, 120 min (mg/dl)	260.0 ± 16.0	205.0 ± 15.0	<0.01

Data are means ± SEM unless otherwise indicated. CT, computed tomography; lbm, lean body mass.

tion with a mean increase of 59 ± 21% (Table 2). There was also greater stimulation of glucose oxidation during insulin infusion and more complete suppression of lipid oxidation and plasma free fatty acids. Fasting endogenous glucose production declined just slightly but did suppress more fully with insulin, indicating improved hepatic insulin sensitivity. Rates of resting energy expenditure were unchanged, as were glucose and fat oxidation rates in fasting conditions.

Skeletal muscle mitochondrial content and enzyme activity. Skeletal muscle mitochondrial density was determined by transmission electron microscopy (Table 3). Mitochondrial density increased on average by 67 ± 17% and was accompanied by increased mean mitochondrial size. Representative micrographs are shown in Fig. 1. Cardiolipin content, a phospholipid specific to the inner mitochondrial membrane, increased on average by 55 ± 17% and was correlated with mitochondrial density (r = 0.67, $P < 0.01$). Intervention had only a modest effect on mtDNA content, which increased by 20% ($P = 0.05$), but there was an increase in citrate synthase activity, which also correlated with mitochondrial density ($r = 0.61$, $P < 0.01$) and cardiolipin content ($r = 0.76$, $P < 0.001$). NADH oxidase activity also increased and was highly correlated with activity of citrate synthase and cardiolipin content ($r = 0.82$, $P < 0.001$). The distribution of fiber types within vastus lateralis muscle tissue was unchanged following intervention.

Correlates of intervention effects on insulin sensitivity and mitochondria. The relationship between changes in insulin sensitivity with changes in aerobic fitness ($V\mathrm{O}_{2max}$ and maximum MET) did not achieve statistical significance, nor did the correlations with weight loss and regional adiposity. A caveat is the relatively tight uniformity of weight loss within the 5–7% range and narrow range of variation in the improvement of $V\mathrm{O}_{2max}$ values. However, as shown in Fig. 2*A*, the improvement in insulin sensitivity was strongly associated with the mean caloric expenditure during supervised exercise sessions ($r = 0.75$, $P = 0.01$), a correlation that was reproduced for nonsupervised sessions ($r = 0.66$, $P = 0.03$).

$V\mathrm{O}_{2max}$ values correlated with mitochondrial density

TABLE 2
Effect of the weight loss and physical activity intervention on glucose metabolism and insulin sensitivity

	Baseline	Postintervention	*P*
Insulin-stimulated conditions			
Plasma glucose (mg/dl)	95.0 ± 3.0	95.0 ± 2.0	—
Plasma insulin (μU/ml)	80 ± 4.0	88.0 ± 3.0	—
Plasma free fatty acids (mmol/l)	0.16 ± 0.02	0.09 ± 0.01	<0.01
Glucose disposal (mg $\cdot$ min^{-1} kg lbm^{-1})	4.06 ± 0.61	6.27 ± 0.86	<0.05
EGP (mg $\cdot$ min^{-1} kg lbm^{-1})	1.31 ± 0.28	0.61 ± 0.20	<0.05
Inhibition of EGP (%)	64.0 ± 8.0	83.0 ± 6.0	<0.05
Resting metabolic rate (kJ/day)	8,044.0 ± 554.0	7,809.0 ± 444.0	—
Glucose oxidation (mg $\cdot$ min^{-1} kg lbm^{-1})	2.63 ± 0.23	3.56 ± 0.17	<0.001
Fat oxidation (mg $\cdot$ min^{-1} kg lbm^{-1})	0.92 ± 0.10	0.56 ± 0.10	<0.001
Fasting conditions			
EGP (mg $\cdot$ min^{-1} kg lbm^{-1})	3.62 ± 0.12	3.38 ± 0.16	—
Resting metabolic rate (kJ/day)	8,146.0 ± 594.0	7,624.0 ± 418.0	—
Glucose oxidation (mg $\cdot$ min^{-1} kg lbm^{-1})	1.70 ± 0.16	1.76 ± 0.18	—
Fat oxidation (mg $\cdot$ min^{-1} kg lbm^{-1})	1.33 ± 0.10	1.19 ± 0.09	—

Data are means ± SEM unless otherwise indicated. EGP, endogenous glucose production; lbm, lean body mass.

TABLE 3
Effects of the weight loss and physical activity intervention on skeletal muscle mitochondrial content and functional capacity

	Baseline	Postintervention	*P*
Electron microscopy			
Mitochondrial density (%)	3.69 ± 0.40	5.74 ± 0.51	<0.001
Mean mitochondrial size (μm^2)	0.062 ± 0.004	0.084 ± 0.008	<0.05
Biochemistry			
Cardiolipin (μg/mU CK)	58.8 ± 5.4	87.3 ± 8.8	<0.01
mtDNA copy number (mtDNA/nDNA)	2,114.0 ± 156.0	2,591.0 ± 320.0	0.05
Citrate synthase (unit/mU CK)	3.28 ± 0.49	5.12 ± 0.90	<0.05
NADH oxidase (unit/mU CK)	0.16 ± 0.03	0.27 ± 0.06	<0.05
Muscle fiber type (%)			
Type I	47.0 ± 2.0	50.0 ± 1.0	—
Type IIa	42.0 ± 1.0	37.0 ± 1.0	—
Type IIb	11.0 ± 1.0	13.0 ± 1.0	—

Data are means ± SEM unless otherwise indicated. CK, creatine kinase.

($r = 0.54$, $P < 0.05$), mitochondrial size ($r = 0.46$, $P < 0.05$), cardiolipin ($r = 0.71$, $P < 0.01$), citrate synthase ($r = 0.56$, $P < 0.05$), and NADH oxidase activity ($r = 0.66$, $P < 0.01$). Similar correlations were found when maximum MET was used instead of $V_{O_{2max}}$ (patterns of association affirming the influence of physical activity status on mitochondria). There were not, however, significant correlations between mitochondrial parameters with weight loss or regional adiposity.

We next examined the relationship between improvements in hyperglycemia and mitochondria. The relative change in fasting plasma glucose correlated strongly with the change in cardiolipin ($r = -0.75$, $P = 0.01$) and citrate synthase ($r = -0.68$, $P < 0.05$). Likewise, the change in A1C also correlated with the change in cardiolipin (shown in Fig. 2*B*) and citrate synthase ($r = -0.73$, $P < 0.05$). Taken together, these patterns suggest that improvements in skeletal muscle mitochondrial content are close markers of improvement in hyperglycemia in diabetes.

DISCUSSION

During the past few years, an emerging body of data has indicated that mitochondrial capacity is reduced in type 2 diabetes (6,9–12,31,32), but the extent to which this reduction is either reversible or a permanent defect had not yet been characterized in type 2 diabetes. Notably, mitochondrial function, content, and gene expression were found reduced in individuals at risk for type 2 diabetes (10–12), thus raising the possibility of an inheritable etiology. In this study, we present relevant findings concerning this issue. We found that both mitochondrial content and functional capacity can be improved by simple lifestyle changes advocated as cornerstones of treatment of type 2 diabetes. In response to moderate weight loss and regular exercise, we observed unambiguous improvements in adiposity, aerobic capacity, hyperglycemia, and insulin sensitivity, thus creating an ideal scenario of metabolic changes to examine the plasticity of skeletal muscle mitochondria.

Four complementary measures examined such plasticity. Transmission electron microscopy demonstrated an

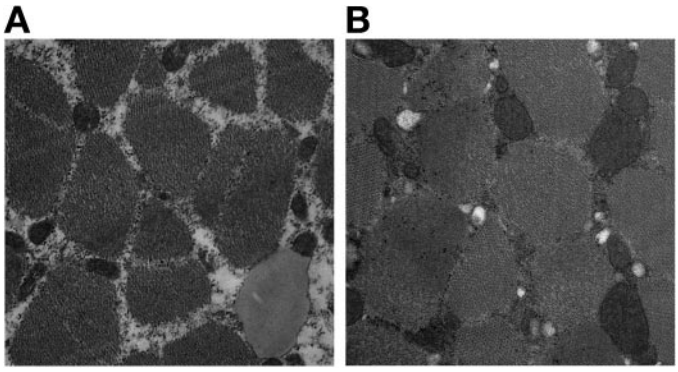

FIG. 1. Representative transmission electron micrographs of skeletal muscle tissue obtained from the same volunteer before (*A*) and after (*B*) intervention. Mitochondrial density and size were increased after intervention.

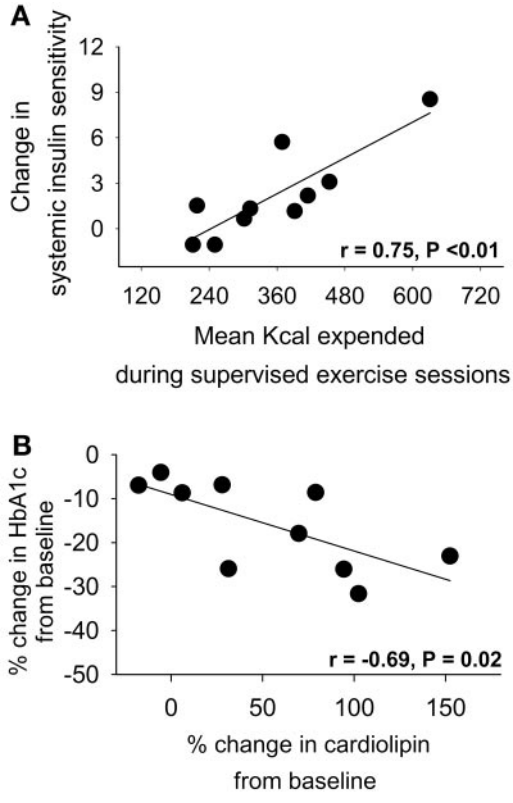

FIG. 2. *A*: Scatter plot showing the correlation between mean energy expended during supervised exercise sessions and the change in insulin sensitivity. *B*: Scatter plot showing the correlation between changes in A1C (%) (relative to baseline) vs. mitochondrial cardiolipin content (%) (relative to baseline). Correlation coefficients were calculated with Spearman analysis.

increase in both mitochondrial density and size. Cardiolipin content, an indirect estimate of the total mass of inner mitochondrial membrane, was correspondingly increased. Likewise, markers of oxidative capacity were also improved (citrate synthase and NADH oxidase). The overall pattern is suggestive of mitochondrial biogenesis (14,33). There was, however, just a modest increase in mtDNA content, indicating that a substantial expansion of mitochondrial content did not require an equivalent proportional increase in mtDNA copy number. A likely explanation for this finding is that except for conditions of marked deficiency of mtDNA content, control of mitochondrial biogenesis appears mainly to be in the expression of nuclear genes encoding mitochondrial proteins. Taken together, our study indicates that in type 2 diabetic patients, considerable potential for remediation of mitochondrial capacity still exists—at least among those we examined, who are relatively early in the progression of this disorder. This remediation occurred by nonpharmacological means broadly advocated for patients with this disorder. This is an important finding because exercise intensity and duration are important determinants of physiological responses of skeletal muscle, and our volunteers did not require intense exercise; rather, they performed moderate, weekly exercise, consistent with a healthy lifestyle.

The robust improvement in systemic insulin sensitivity seen in our study provides a physiological context to examine the changes in mitochondria. However, this improvement in insulin sensitivity occurred in response to a multifaceted intervention; thus, it is difficult to determine the respective contribution of each component of the intervention or physiological parameter that changed with intervention. Obesity, especially visceral adiposity, and hepatic steatosis are aspects of body composition that are recognized in cross-sectional studies to correlate with the severity of insulin resistance (22,34). However, in the current study, a 7% mean reduction in weight, a 24% decrease in visceral adiposity, and a substantial improvement in hepatic steatosis did not significantly account for interindividual variation in improvement of insulin sensitivity by regression analysis. Quite likely, these associations were relatively weak because of the homogeneity of weight loss; nearly all subjects lost at least 5% of weight and manifested large decreases of visceral abdominal tissue and hepatic steatosis. The low variance in the weight loss parameters likely made it more feasible to discern the impact of other factors that contributed to variance for improvement of insulin sensitivity. On the other hand, the amount of energy expended during exercise sessions was a robust predictor of improvement in insulin sensitivity and accounted for nearly three-quarters of the variance in improvement, reinforcing the concept that physical activity is an important determinant of insulin sensitivity of skeletal muscle in type 2 diabetes.

An interesting correlate of metabolic improvement that emerged was mitochondrial capacity. Even more robust than the relation of improved insulin sensitivity with improvement in A1C was the association of hyperglycemia with skeletal muscle mitochondria. We previously reported that improvements in mitochondrial density and size correlate with improvements in systemic insulin sensitivity in nondiabetic obese adults (7). Our findings in type 2 diabetic subjects extend this notion. Although such correlations may not necessarily reflect causality, these correlations are noteworthy because there are precedents for a cause-effect relationship. In a recent report, Fritz et al. (35) reported that skeletal muscle changes of increased oxidative gene expression induced by low-intensity exercise were a correlate of systemic metabolic improvements in type 2 diabetes, including insulin sensitivity. Moreover, rats selectively bred for low oxidative capacity in skeletal muscle develop the panoply of cardiovascular risk factors that typify the metabolic syndrome (36).

Mitochondrial content of skeletal muscle can be construed as a cell biology marker of the integrated effect of repeated sessions of physical activity. It is well recognized that exercise modulates mitochondrial content in healthy, lean, normal individuals (14,33). Thus, it is conceivable that the exercise training component of the intervention was the main inducer of improvements in mitochondrial capacity. A limitation of our study is that it was not designed to be mechanistic, and therefore we do not know which molecular mechanisms were most important for mitochondrial biogenesis. It is conceivable that exercise-induced activation of peroxisome proliferator–activated coactivator-α, as well as AMP kinase signaling pathways, might have played a role, and this warrants exploration in future studies.

It has been postulated that mitochondrial dysfunction in type 2 diabetic patients arises as a consequence rather than a cause of insulin resistance (9) and may arise from heritable factors (10–13). Although we have given emphasis to exercise in influencing mitochondria in type 2 diabetic patients, our study does not necessarily exclude those former postulates. The important point in our findings is that skeletal muscle mitochondria can be substantially ameliorated in type 2 diabetes by short-term lifestyle modifications. In this context, improved oxidative capacity of muscle is closely accompanied by the improvement in hyperglycemia.

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