Characteristics of skeletal muscle mitochondrial biogenesis induced by moderate-intensity exercise and weight loss in obesity

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Menshikova EV, Ritov VB, Ferrell RE, Azuma K, Goodpaster BH, Kelley DE. Characteristics of skeletal muscle mitochondrial biogenesis induced by moderate-intensity exercise and weight loss in obesity. J Appl Physiol 103: 21–27, 2007. First published March 1, 2007; doi:10.1152/japplphysiol.01228.2006.—There are fewer mitochondria and a reduced oxidative capacity in skeletal muscle in obesity. Moderate-intensity physical activity combined with weight loss increases oxidative enzyme activity in obese sedentary adults; however, this adaptation occurs without a significant increase in mitochondrial DNA (mtDNA), which is unlike the classic pattern of mitochondrial biogenesis induced by vigorous activity. The objective of this study was to examine the hypothesis that the mitochondrial adaptation to moderate-intensity exercise and weight loss in obesity induces increased mitochondrial cristae despite a lack of mtDNA proliferation. Content of cardiolipin and mtDNA and enzymatic activities of the electron transport chain (ETC) and tricarboxylic acid cycle were measured in biopsy samples of vastus lateralis muscle obtained from sedentary obese men and women before and following a 4-mo walking intervention combined with weight loss. Cardiolipin increased by 60% from 47 ± 4 to 74 ± 8 μg/mU CK (P < 0.01), but skeletal muscle mtDNA content did not change significantly (1,901 ± 363 to 2,169 ± 317 Rc, where Rc is relative copy number of mtDNA per diploid nuclear genome). Enzyme activity of the ETC increased (P < 0.01); that for rotenone-sensitive NADH-oxidase (96 ± 1%) increased more than for ubiquinol-oxidase (48 ± 6%). Activities for citrate synthase and succinate dehydrogenase increased by 29 ± 9% and 40 ± 6%, respectively. In conclusion, moderate-intensity physical activity combined with weight loss induces skeletal muscle mitochondrial biogenesis in previously sedentary obese men and women, but this response occurs without mtDNA proliferation and may be characterized by an increase in mitochondrial cristae.

mitochondria; cardiolipin

SKELETAL MUSCLE adapts its oxidative capacity to the prevailing pattern of bioenergetic demand, which in turn is largely determined by habits of physical activity or the counterpart of sedentary behavior. In obesity, skeletal muscle has a reduced oxidative capacity (2, 24–26, 43), which is a metabolic characteristic associated with risk for weight gain and insulin resistance (32, 33, 44, 56). In clinical investigations, we have observed that an intervention of moderate-intensity exercise, mostly briskly paced walking, combined with moderate weight loss induced increases of oxidative capacity of skeletal muscle in obese adults. This increase of oxidative capacity of muscle, although remaining substantially below the levels found in younger, lean adults who habitually perform high-intensity exercise (36), was associated with improved insulin sensitivity and an increased capacity for fat oxidation (10, 30). Morphonologically, the walking intervention induced increased size in skeletal muscle mitochondria (47), a response typical for adaptation to physical activity (14). Yet, these responses to moderate-intensity exercise combined with weight loss occurred absent a change in muscle content of mitochondrial DNA (mtDNA) (30). This response is unlike the proliferation of mtDNA that characteristically occurs as a key component of the adaptation to vigorous activity.

The adaptive response to vigorous aerobic exercise has been extensively examined and is characterized by increases of mtDNA as well as increased oxidative capacity (16–19). Proportionality between mtDNA content in skeletal muscle and its oxidative capacity has been commonly observed, prompting the “gene dosage” theory that postulates mtDNA replication is an integral mechanism for exercise-induced mitochondrial biogenesis (51–53). In animal studies, electrical stimulation of skeletal muscle induces proportional increases of citrate synthase (CS) activity and mtDNA content (13, 20, 52). In clinical investigations as well, in healthy, lean individuals the activity of CS correlates with mtDNA content in vastus lateralis muscle (8, 49). Endurance-trained athletes generally have a higher mitochondrial content in muscle than do sedentary individuals, and mtDNA content is proportional to mitochondrial volume density (34). Consistent with these concepts of a “gene dosage” theory, our laboratory recently reported that a moderate-intensity walking intervention in elderly men and women induced amplification of muscle mtDNA that was proportional to increases in oxidative capacity and that substantially rectified the low baseline mtDNA content of muscle (29).

Therefore, our recent observation that moderate-intensity exercise combined with weight loss does not increase muscle mtDNA yet does improve muscle oxidative capacity in previously sedentary obese men and women (30) may suggest a different pattern of mitochondrial adaptation from that observed with high-intensity exercise. The modification might be related to the influence of baseline obesity, to an effect on muscle of the weight loss intervention, or to an effect of moderate-intensity as opposed to vigorous exercise. Mitochondrial biogenesis has been identified in yeast that is characterized by cell elongation and mitochondrial expansion yet does not require mtDNA replication (39). Instead, the key adaptation is an increased mitochondrial cristae density (39). The present studies were undertaken to test the hypothesis that moderate-intensity physical activity induces an analogous pat-
tern of mitochondrial biogenesis in skeletal muscle of previously sedentary obese adults. To test this hypothesis, mtDNA content and that of cardiolipin were determined. Cardiolipin is a phospholipid that is unique to the inner mitochondrial membrane, a structural component present in a ratio of 1–2:4:4 with phosphatidylcholine and phosphatidylethanolamine, respectively (7). Therefore cardiolipin is an excellent quantitative marker for the amount of the inner mitochondrial membrane (23). Furthermore, cardiolipin contributes to the functional integrity of the electron transport chain (ETC) (28, 40, 55).

**METHODS AND MATERIALS**

*Research volunteers.* Vastus lateralis muscle samples were obtained by percutaneous biopsy from seven obese, nondiabetic volunteers (4 men and 3 women) before a physical activity and weight loss intervention were started, and biopsies were repeated 4 mo later. The clinical characteristics are shown in Table 1 and have been previously reported along with a detailed description of the intervention and metabolic evaluations (30). Research participants had baseline determinations of body composition, aerobic fitness, insulin sensitivity, and resting energy expenditure and substrate oxidation, together with muscle biopsy procedures before beginning a 4-mo program of moderate-intensity exercise training combined with nutritional intervention.

For comparison with these participants, muscle biopsies of the vastus lateralis muscle were obtained from highly trained endurance athletes participating in competitive cycling. These data were ascertained to represent an "upper boundary" of training-induced effects in skeletal muscle. The clinical characteristics of these individuals (n = 4) are also presented in Table 1. The endurance-trained individuals were participating in a minimum of 5 days/wk of aerobic exercise training, and all were involved in competitive cycling or triathlons. Although their maximal O2 uptake (VO2 max) was not determined in this study, another group of similar athletes studied in our laboratory had a relatively high aerobic capacity as evidenced by their VO2 max of 61.0 ± 5.4 ml·kg⁻¹·min⁻¹ (9). The protocol was approved by the University of Pittsburgh Institutional Review Board, and all volunteers gave written informed consent.

*Intervention.* A 16-wk program of exercise training was conducted after completion of baseline metabolic and body composition assessments, as previously described (10, 30). Participants were instructed to perform a minimum of four and a maximum of six exercise sessions (weeks 5–8, exercise sessions were increased to 40 min at the same intensity. During weeks 9–16, exercise sessions were continued at 40 min, but the intensity increased to 75% of maximal heart rate. For the independent (unsupervised) exercise sessions, participants were instructed to use a stationary cycle or treadmill at home if this equipment was available or to walk or bicycle outdoors for a similar duration.

The target for weight loss was at least 7%, as described previously (10, 30). To achieve this magnitude of weight loss, a reduction in calories (a reduction of 500–1,000 kcal/day on the basis of recent food records/history) and a low-fat (<30% of calories from fat) diet were used as part of the weight intervention. The weight loss program was administered individually to participants to allow them to enter the weight control intervention immediately after their baseline physiological assessments. A nutritionist/behaviorist met with each participant weekly. Participants were weighed weekly, and their weight was recorded. All participants kept detailed 7-day food records for the entire 16-wk exercise training period.

*Reagents and equipment.* Tetraoleoyl cardiolipin (internal standard) was purchased from Avanti Polar lipids (Alabaster, AL). HPLC-grade chloroform, stabilized by 0.7% ethanol, was obtained from Fisher Scientific (Pittsburgh, PA). Other HPLC-grade solvents and reagents were purchased from Sigma Chemicals (St Louis, MO). 1-Pyrenyldiazomethane (PDAM) was obtained from Molecular Probes (Eugene, OR). A Shimadzu high-performance liquid chromatograph (model LC-10AT vp) equipped with an autosampler (model SIL-10AD), a tray cooler, and a Shimadzu fluorescence detector (model RF-10AxI) were used for these studies. The analog signal of the detector was processed and stored in digital form with Shimadzu Class-VP software (Shimadzu Scientific Instruments, Columbia, MD).

*Lipid extraction.* Lipid extraction of muscle biopsy specimens to assay cardiolipin was performed using the particulate fraction prepared from tissue homogenate, as previously described (35, 38). An aliquot of the particulate fraction, corresponding to 1–3 mg of tissue (wet weight) and containing more than 95% of tissue mitochondria (24), was resuspended in 500 μl of washing medium containing 10 mM EDTA and 0.1 mg/ml BSA (pH 8.3 at 21°C) and centrifuged for 20 min at 4°C at 22,000 RPM (45,000 g). Supernatant was discarded, and 10 μl of 10% dodecyl maltoside in H2O, 10 μl of H2O, 10 μg BHT (1 μl, 10 mg/ml in methanol), and 200–400 ng of tetraoleoyl cardiolipin as an internal standard (2–4 μl, 100 μg/ml in ethanol) were added to the remaining pellet, and final additions of 300 μl of hexane and 200 μl of isopropanol. The mixture was then sonicated, under nitrogen, for 2–3 min at 4°C using a horn probe connected to a Torbeo 36810 (Cole-Parmer) generator set to maximum power (20 dB). The sonicated mixture was kept on ice under nitrogen for 120 min to extract lipids, after which the mixture was centrifuged at 4°C at 10,000 RPM (12,000 g) for 10 min. The supernatant was transferred to clean glass tubes and dried in a Speed Vac vacuum concentrator. The residual lipid film was redissolved in 330 μl of a 2:1 chloroform:methanol mixture and kept at −80°C under nitrogen until assay for cardiolipin.

*Cardiolipin assay.* The cardiolipin was measured using a recently published method (37) that is based on fluorescent derivatization of cardiolipin by 1-pyrenyldiazomethane. Briefly, a 150-μl aliquot of the lipid solution containing 100–200 ng tetraoleoyl cardiolipin (as an internal standard) was mixed with 6 μl of 50 mM H2SO4, incubated on ice for 5 min, and then mixed with 100 μl H2O. Following

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**Table 1. Clinical characteristics of the research volunteers**

<table>
<thead>
<tr>
<th></th>
<th>Obese (4M/3F)</th>
<th>Lean (2M/2F) + Endurance Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preintervention</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>40.6±0.8</td>
<td>42.0±3.8</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>102.3±3.5</td>
<td>93.5±4.9*</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>34.1±1.4</td>
<td>31.1±1.4*</td>
</tr>
<tr>
<td>VO2max, ml·kg⁻¹·min⁻¹</td>
<td>36.5±1.7</td>
<td>44.7±2.1*</td>
</tr>
<tr>
<td>Fasting RQ</td>
<td>0.81±0.01</td>
<td>0.78±0.01*</td>
</tr>
<tr>
<td>Clamp RQ</td>
<td>0.89±0.01</td>
<td>0.92±0.01</td>
</tr>
<tr>
<td>Rd. mg·min⁻¹·kg·FFM⁻¹</td>
<td>6.48±0.51</td>
<td>9.92±0.98*</td>
</tr>
</tbody>
</table>

Values are means ± SE. M, men; F, women; BMI, body mass index; VO2max, maximal oxygen uptake; FFM, fat-free mass; RQ, respiratory quotient measured by indirect calorimetry; Rd, rate of insulin-stimulated glucose utilization. *P ≤ 0.05.
centrifugation to separate the two phases, a chloroform solution of PDAM (50 μl; 1.0 mg/ml) was added to phase on the bottom of the tube and placed on ice for 30 min, under nitrogen, to complete derivatization. Two microliters of concentrated acetic acid was added at the end of incubation, followed by 100 μl of chloroform, and 500 μl of 50% methanol in water, and tubes were vortexed and then centrifuged for 2 min. After phase separation, the chloroform phase was transferred to glass tubes, solvent was evaporated to vacuum, and the solid residue was redissolved in 80 μl of tetrahydrofuran. Before injection on HPLC, samples were diluted by 20 μl of H2O, and a 10-μl aliquot was injected onto a C18 reverse-phase column (Zorbax XDB-C18, 4.6 × 150 mm; Agilent Technologies). The column was protected by a guard cartridge (Eclipse XDB-C18; Agilent Technologies) and was connected to the fluorescence detector. The column was eluted (1 ml/min) by a mobile phase composed of HPLC-grade ethanol and 0.5 mM H3PO4. In the eluate, fluorescence of 1-pyrenyl-methyl methyl ester derivatives of cardiolipin was monitored at an emission of 395 nm, after excitation at 340 nm. All separations were performed at a stable ambient temperature (21°C).

Enzyme assays. Creatine kinase (CK), NADH-oxidase, and CS all were measured using HPLC-based assays as previously described (38) with the modifications in sample preparation described below. To provide better access of substrates to mitochondria, aliquots of the mitochondria fraction were treated by nitrogen cavitation. These samples were saturated by nitrogen at 1,000 psi for 10 min using a cell disruption minibomb (model 4639, Parr Instrument), concluding with samples were saturated by nitrogen at 1,000 psi for 10 min using a cell disruption minibomb (model 4639, Parr Instrument), concluding with disruption of mitochondria from the elements of cytoskeleton (1). Incubation mixture used for mitochondrial assays contained the combination of mitochondrial matrix, as has been previously described in detail (11, 24). To measure activity of mitochondrial ubiquinol:O2 oxidoreductase (complex III/IV), a pyridine nucleotide HPLC-based enzymatic assay was developed that utilized the enzyme diaphorase to regenerate ubiquinol by oxidizing an NADH. A substrate for ubiquinol (1:1) and can be monitored using the procedure we previously developed for the mitochondrial assay (38). The difference between the amount of NAD generated in the presence and absence of “uncaging” mitochondria from the elements of cytoskeleton (1). Incubation mixture used for mitochondrial assays contained the channel-forming antibiotic alamethicin to provide free access of substrates to the mitochondrial matrix, as has been previously described in detail (11, 24). To measure activity of mitochondrial ubiquinol:O2 oxidoreductase (complex III/IV), a pyridine nucleotide HPLC-based enzymatic assay was developed that utilized the enzyme diaphorase to regenerate ubiquinol by oxidizing an NADH. A substrate for ubiquinol:O2 oxidoreductase was 2,3-dimethoxy-5-methyl-6-geranyl-1,4-benzoquinone (CoQ2), an analog of coenzyme Q. The oxidation of NADH in diaphorase reaction is stoichiometric to the oxidation of ubiquinol (1:1) and can be monitored using the procedure we previously developed for the NADH-oxidase assay (38). The difference between the amount of NAD generated in the presence and absence of a specific blocker of complex III, myxothiazol (46), was used to calculate the activity of ubiquinol-oxidase.

mtDNA Quantification. DNA (mitochondrial and nuclear) was extracted from muscle biopsy samples using a QIAamp DNA minikit (QIAGEN, Chatworth, CA), and the concentration of each sample was determined using a GeneQuant spectrophotometer (Pharmacia Biotech). The relative copy number of mtDNA per diploid nuclear genome was measured by real-time PCR on the basis of the methods of Szuhai et al. (31). Primers and FAM-labeled Taqman TAMRA probes (Applied Biosystems, 450025) were designed using Primer Express software, version 1.5 (Applied Biosystems), as described previously (30). This approach to the estimation of mtDNA is valid if the efficiency of mtDNA amplification and nuclear DNA amplification are approximately equal. This was tested; mean amplification efficiency measured in triplicate was 1.995 ± 0.027 (99.8%) for mtDNA and 2.018 ± 0.022 (100%) for nuclear DNA, a sufficient similarity in the efficiency of parallel amplification to meet recommendations for this methodology (Applied Biosystems).

RESULTS

Effects of intervention on cardiolipin and mtDNA. During the 4-mo physical activity and nutrition intervention, the average number of exercise sessions weekly was 4.2 ± 0.3 days. At completion of the intervention, the mean weight loss was 8.8 ± 1.6 kg and the mean change in VO2 max was an increase of 22.5 ± 2.6%, as shown in Table 1. Total cardiolipin content in vastus lateralis muscle increased from 46 ± 4 to 74 ± 8 μg/mU CK (P < 0.01) following intervention, a 59 ± 15% increase. The activity of CK did not change significantly (5,902 ± 838 vs. 5,434 ± 405 U/g wet weight, pre- and postintervention, respectively; not significant). Cardiolipin content in skeletal muscle was 172 ± 14 μg/mU CK in vastus lateralis muscle obtained from the highly trained, lean volunteers, or ~1.0 mg/g wet weight, which likely represents an upper physiological limit for skeletal muscle. This is fourfold higher than the cardiolipin content in the preintervention samples from obese volunteers and remained twofold greater than levels reached at the conclusion of the 4-mo intervention (see Table 3). The predominant species was tetrailinoleoyl-cardiolipin, which accounted for 79% of cardiolipin in sedentary (baseline) obese samples and a slightly but significantly higher percentage in samples from the trained, lean volunteers. The distribution of molecular forms of cardiolipin remained unchanged following intervention (Table 2).

Real-time PCR was used to determine relative copy number of mtDNA per diploid nuclear genome in skeletal muscle biopsies. mtDNA content in skeletal muscle determinations was measured in paired baseline and postintervention samples obtained from 19 obese, previously sedentary individuals who completed the 4-mo moderate-intensity activity intervention. There was not a significant change in mtDNA (2,100 ± 203 vs. 2,248 ± 177 mtDNA/nuclear DNA, baseline and postintervention, respectively; P = 0.6). Baseline mtDNA correlated well with postintervention values (r = 0.66; P < 0.01). In the cohort of seven volunteers for whom in-depth analyses of muscle were performed for the present studies, muscle mtDNA increased by 20 ± 13% following intervention, from 1,901 ± 363 to 2,169 ± 317 mtDNA/nuclear DNA, but this was not statistically significant (P = 0.4).

Activity of ETC and tricarboxylic acid cycle (Table 3, Fig. 1). There was a significant increase in cardiolipin content normalized to mtDNA content (3.1 ± 0.9 vs. 3.8 ± 0.3, baseline vs. postintervention; P < 0.05), consistent with an increased surface area of the inner mitochondrial membrane. An increase in the surface area of the inner mitochondrial membrane would allow for additional complexes of the ETC and would increase the lipid phase for coenzyme Q; both changes might contribute to increased ETC activity. Activity of rotenone-sensitive NADH-oxidase, which reflects activity of the ETC from complex I to IV, increased by 96 ± 21%, from 0.15 ± 0.01 to 0.29 ± 0.03 U/mU CK (P < 0.002). This was

Table 2. Molecular forms of cardiolipin in human skeletal muscle

<table>
<thead>
<tr>
<th>Cardiolipin Molecular Forms, %</th>
<th>Trilinoleoyl</th>
<th>Trilinoleoyl-oleoyl</th>
<th>Dilinoleoyl-dioleoyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trained lean (n = 4)</td>
<td>83.7±0.9</td>
<td>13.0±0.8</td>
<td>3.3±0.1</td>
</tr>
<tr>
<td>Obese</td>
<td>79.1±0.8</td>
<td>15.9±0.5</td>
<td>5.0±0.3</td>
</tr>
<tr>
<td>Preintervention</td>
<td>78.9±0.9</td>
<td>15.8±0.6</td>
<td>5.3±0.3</td>
</tr>
<tr>
<td>Postintervention</td>
<td>80.1±0.8</td>
<td>15.3±0.5</td>
<td>4.5±0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.01, athletes vs. obese.
a larger increase than for cardiolipin, as reflected in a significant change in the ratio of rotenone-sensitive NADH-oxidase activity normalized to cardiolipin content, increasing from 3.2 ± 0.2 to 4.1 ± 0.4 U/mg cardiolipin (P < 0.05).

Activity of myxothiazol-sensitive ubiquinol:O2 oxidoreductase, the fragment of the ETC that includes complexes III and IV, increased from 0.54 ± 0.05 to 0.79 ± 0.06 U/mU CK following intervention (P < 0.02), an increase of 48 ± 6%. This is a substantial increase, yet half that found of rotenone-sensitive NADH-oxidase, as shown in Fig. 1. Activity of ubiquinol:O2 oxidase normalized to cardiolipin content remained stable following intervention (11.9 ± 1.4 vs. 11.2 ± 1.0 U/mg cardiolipin; baseline and postintervention, respectively).

To gain additional perspective on intervention-induced changes in cardiolipin content and ETC activity, activity of two enzymes of the tricarboxylic acid (TCA) cycle were assessed. CS is a soluble enzyme of the mitochondrial matrix, and its activity is generally regarded as reflecting mitochondrial volume density in skeletal muscle (4). Activity of CS increased by 29 ± 9%, from 3.1 ± 0.2 to 3.9 ± 0.3 U/mU CK (P < 0.05). Succinate dehydrogenase (SDH) is also a component of the TCA cycle, yet unlike CS, SDH is embedded in the inner mitochondrial membrane. Activity of SDH increased 40 ± 6%, from 0.19 ± 0.01 to 0.26 ± 0.03 U/mU CK (P < 0.01). These increases in CS and SDH activity were less than occurred for rotenone-sensitive NADH-oxidase activity. The ratio of SDH normalized to cardiolipin content remained stable (4.1 ± 0.4 vs. 3.7 ± 0.3 U/mg cardiolipin; pre- compared with postintervention). The response to intervention for each of the enzyme activities, as well as for mtDNA and cardiolipin content, was similar in men compared with women (data not shown).

**DISCUSSION**

A main finding of the present study is that moderate-intensity physical activity combined with weight loss improves oxidative enzyme activity in skeletal muscle in previously sedentary obese men and women without a significant change in mtDNA content in skeletal muscle. Instead, the main change that does occur is increased cardiolipin, denoting increased surface area of the inner mitochondrial membrane. Along with the expanded surface area of the inner mitochondrial membrane, there were increases in enzymatic activity of the ETC, increases that were greater than increases that occurred for TCA enzyme activity. There was a particularly robust increase in the activity of rotenone-sensitive NADH-oxidase. This pattern may suggest increased formation of mitochondrial super-complexes (of complexes I, III and IV), which are recognized to have enhanced efficiency of electron transfer (27, 40, 41). On the basis of these findings, we conclude that moderate-intensity physical activity combined with weight loss in previously sedentary obese adults elicits a pattern of mitochondrial biogenesis that differs from that delineated for the adaptation to high-intensity aerobic activity in which mtDNA amplification is a prominent component. The relative proportionality of change induced by moderate-intensity physical activity and weight loss as shown in Fig. 1 emphasizes the predominant effect to increase ETC activity and cardiolipin content.

One of the intriguing aspects of skeletal muscle physiology is its considerable metabolic plasticity. This is manifest in a capacity to dramatically increase blood flow, oxygen consumption, and rates of substrate transport and oxidation to accommodate the increased demand for energy production during physical activity. These adaptations to physical activity extend to the molecular physiology of skeletal muscle. The increase in oxidative enzyme capacity that occurs as an adaptation to high-intensity exercise has been of longstanding scientific interest and is associated with an increase in mitochondrial volume density, together with increased size of mitochondria and an amplification of mtDNA content (6, 21, 22, 50). Skeletal muscle also adapts to sedentary conditions by reducing oxidative enzyme activity (5). In obesity, skeletal muscle manifests a lower oxidative capacity, together with increased intramyocellular lipid content (12), characteristics that are associated with insulin resistance and that constitute risk for weight gain and resistance to weight loss (15).

Exercise is strongly advocated as an intervention for the prevention and treatment of obesity, and our laboratory is among those that have examined the manner in which skeletal muscle in obesity adapts to physical activity and weight loss (12). The form of physical activity employed for intervention in previously sedentary, middle-aged to elderly obese individ-

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**Table 3. Effect of intervention on the parameters of mitochondrial content and functional capacity in skeletal muscle**

<table>
<thead>
<tr>
<th></th>
<th>Preintervention</th>
<th>Postintervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>mtDNA, Rc</td>
<td>1.901±363</td>
<td>2.169±317</td>
</tr>
<tr>
<td>Cardiolipin, μg/mU CK</td>
<td>46.9±3.6</td>
<td>73.7±7.9</td>
</tr>
<tr>
<td>NADH-oxidase, U/mU CK</td>
<td>0.15±0.01</td>
<td>0.29±0.03†</td>
</tr>
<tr>
<td>Ubiquinol-oxidase, U/mU CK</td>
<td>0.54±0.05</td>
<td>0.79±0.06*</td>
</tr>
<tr>
<td>Succinate dehydrogenase, U/mU CK</td>
<td>0.19±0.01</td>
<td>0.26±0.03*</td>
</tr>
<tr>
<td>Citrate synthase, U/mU CK</td>
<td>3.06±0.19</td>
<td>3.91±0.31*</td>
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</table>

Values are means ± SE. mtDNA, mitochondrial DNA; Rc, relative copy number of mtDNA per diploid nuclear genome; CK, creatine kinase. *P ≤ 0.05; †P ≤ 0.01.

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**Fig. 1.** Postintervention percentage change in parameters of mitochondrial content and functional capacity are shown: mitochondrial DNA (mtDNA), citrate synthase (CS), succinate dehydrogenase (SDH), ubiquinol-oxidase; cardiolipin (CL), and rotenone-sensitive NADH-oxidase.
mtDNA proliferation, although this has not been established to also reflect an obesity-related impairment in capacity for response to moderate-intensity physical activity in obesity may differ from the classic patterns of mitochondrial biogenesis that have been described for adaptation to vigorous activity. Notably, we observed that the adaptation to moderate-intensity exercise in obesity occurs without an increase in muscle mtDNA content (30). The reason(s) for this difference could be related to as yet unknown baseline characteristics of skeletal muscle in insulin-resistant obese adults that curtail amplification of mtDNA in response to physical activity, to a counter-balancing effect of weight loss on muscle bioenergetic capacity (42), or to differences between adaptation to moderate-intensity as opposed to high-intensity exercise. Certainly, these factors may be interactive, and further research is needed to distinguish among these possibilities, by comparing, for instance, the effects of weight loss with or without physical activity, or to examine moderate- vs. high-intensity intervention in lean individuals.

The absence of an increase in muscle mtDNA content in response to moderate-intensity physical activity in obesity may also reflect an obesity-related impairment in capacity for mtDNA proliferation, although this has not been established to occur. There is a baseline reduction of −25% in muscle mtDNA content in obesity (36), yet an even greater reduction in mtDNA content in muscle among elderly, nearly 50% lower than that of lean, young trained individuals (29). In nonobese, elderly men and women, a moderate-intensity physical activity intervention induced a 60% increase in muscle mtDNA content, an increase that was exactly proportionate to the increase in oxidative capacity (29). In general, there is a relative excess of mtDNA transcripts compared with transcripts for mitochondrial proteins encoded by nuclear genes. It is the availability of transcripts for mitochondrial proteins encoded by nuclear genes rather than mtDNA transcripts that is regarded as limiting assembly of the ETC (34, 48). From this, it can be postulated that either there is at baseline sufficient mtDNA (and its transcripts) in muscle of obese individuals to adapt to exercise or that the molecular physiology of muscle adaptation to moderate-intensity exercise may not require amplification of mtDNA.

Our observations support the concept that mitochondrial biogenesis induced by moderate-intensity exercise and weight loss in obesity centers on increasing surface area of the inner mitochondrial membrane. This conclusion is based on the substantial increase in cardiolipin content. Recently, our laboratory developed a one-step derivatization procedure with HPLC detection for quantitative measurement of cardiolipin, with sufficient sensitivity to be used in the relatively small tissue samples obtained by percutaneous muscle biopsy in clinical research studies (37). Cardiolipin is a mitochondria-specific phospholipid that, along with two other common phospholipids, phosphatidylcholine and phosphatidylethanolamine, are the major structural components of the inner mitochondrial membrane. Cardiolipin is a marker of inner mitochondrial membrane, and this makes cardiolipin a useful metric for gauging the content of mitochondria and the amount of the mitochondrial inner membrane in particular (23). However, because there has not been a simple procedure for measuring cardiolipin in small biological samples, analysis of cardiolipin has not been widely used in clinical investigations. In the present study, the content of cardiolipin increased by ∼50% above baseline values, denoting an equivalent increase in the surface area of the inner mitochondrial membrane. Moreover, the ratio of cardiolipin to mtDNA increased.

An increase in the surface area of the inner mitochondrial membrane provides infrastructure for more constituents of the ETC. Consonant with this, there was an increase in ubiquinol-oxidase activity, which is the activity of complexes III and IV of the ETC. Ubiquinol-oxidase activity normalized to cardiolipin content remained stable, as did that for SDH activity, findings that indicate that the increase in formation of the inner mitochondrial membrane provided infrastructure for more proteins of the ETC. It is generally considered that the inner mitochondrial membrane has a higher than usual ratio of protein to lipid content compared with other membranes and that it maintains a relatively narrow range for this ratio (7). Nonetheless, the specific activity of rotenone-sensitive NADH-oxidase normalized to cardiolipin increased significantly following moderate-intensity exercise. The increase in activity of rotenone-sensitive NADH-oxidase was twice that for ubiquinol-oxidase activity. From this finding, we speculate that another important aspect of the molecular physiology of muscle adaptation to moderate-intensity physical activity may include an increased formation of ETC supercomplexes, or so-called “respirasomes” (3, 40, 41, 54), a hypothesis that will require further research to test.

In conclusion, the adaptation of skeletal muscle to moderate-intensity exercise and weight loss in previously sedentary obese men and women yields mitochondria with a greater inner membrane surface area and an increased enzymatic capacity for oxidative phosphorylation. This occurs without amplification of mtDNA content in muscle. Further research is warranted to delineate the mechanisms that control this pattern of mitochondrial biogenesis, whether it does entail increased formation of supercomplexes, and how these adaptations mediate improved efficiency in oxidation of fatty acids.

ACKNOWLEDGMENTS

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GRANTS

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