Effects of weight loss and physical activity on skeletal muscle mitochondrial function in obesity

Elizabeth V. Menshikova, Vladimir B. Ritov, Frederico G. S. Toledo, Robert E. Ferrell, Bret H. Goodpaster, and David E. Kelley

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Menshikova, Elizabeth V., Vladimir B. Ritov, Frederico G. S. Toledo, Robert E. Ferrell, Bret H. Goodpaster, and David E. Kelley. Effects of weight loss and physical activity on skeletal muscle mitochondrial function in obesity. Am J Physiol Endocrinol Metab 288: E818–E825, 2005. First published December 7, 2004; doi: 10.1152/ajpendo.00322.2004.—The current study was undertaken to address responsiveness of skeletal muscle mitochondrial electron transport chain (ETC) activity to weight loss (WL) and exercise in overweight or obese, sedentary volunteers. Fourteen middle-aged participants (7 male/7 female) had assessments of mitochondrial ETC activity and mitochondrial (mt)DNA in vastus lateralis muscle, obtained by percutaneous biopsy, before and after a 16-wk intervention. Mean WL was 9.7 (1.5%) and the mean increase in VO2 max was [means (SD)] 21.7 (3.7%). Total ETC activity increased significantly, from 0.13 (0.02) to 0.19 (0.03) U/mU creatine kinase (CK; P < 0.001). ETC activity was also assessed in mitochondria isolated into subsarcolemmal (SSM) and intermyofibrillar (IMF-M) fractions. In response to intervention, there was a robust increase of ETC activity in SSM (0.028 (0.007) to 0.046 (0.011) U/mU CK, P < 0.001), and in IMF-M (0.101 (0.015) to 0.148 (0.018) U/mU CK, P < 0.005). At baseline, the percentage of ETC activity contained in the SSM fraction was low and remained unchanged following intervention [19 (3) vs. 22 (2) %], despite the increase in ETC activity. Also, muscle mtDNA content did not change significantly [1665 (213) vs. 1874 (214) mtDNA/nuclear DNA], denoting functional improvement rather than proliferation of mitochondria as the principal mechanism of enhanced ETC activity. Increases in ETC activity were correlated with energy expenditure during exercise sessions, and ETC activity in SSM correlated with insulin sensitivity after adjustment for VO2 max. In summary, skeletal muscle ETC activity is increased by WL and exercise in previously sedentary obese men and women. We conclude that improved skeletal muscle ETC activity following moderate WL and improved aerobic capacity contributes to associated alleviation of insulin resistance.

A CLASSICAL PHYSIOLOGICAL ADAPTATION in response to physical activity is increased oxidative capacity within skeletal muscle (31). Although this response is well described in normal-weight young and in older lean adults, relatively few studies have examined whether this adaptation of skeletal muscle occurs in a similar manner in obesity. Reduced oxidative enzyme activity in muscle, including smaller mitochondria and reduced electron transport chain (ETC) activity, has been found in obese men and women (2, 17, 20, 33). This biochemical characteristic has also been found to correlate with severity of insulin resistance (3, 20, 34). A postulated mechanism is accumulation of intramyocellular lipid. Although weight loss in the absence of improvements of aerobic fitness improves insulin sensitivity of skeletal muscle in obesity (16) and can decrease lipid content (11), weight loss without change in aerobic fitness was not found to improve oxidative enzyme activity in muscle (18, 34). However, Kern et al. (19) did observe improved oxidative enzyme capacity following weight loss in obesity, although physical activity during the intervention was not clearly quantified and might have increased. Because of the findings of impaired mitochondrial function in muscle in obesity, it is important to determine whether improvements can be achieved with an intervention of physical activity combined with weight loss. Failure of such a response might conceivably be a risk for weight regain or might limit improvements in insulin sensitivity. Additionally, little is known in obesity as to whether improvement in oxidative capacity in muscle that might be induced by activity and weight loss is due primarily to an increase of mitochondrial content or to an increased functional capacity of mitochondria.

These questions can also be asked with respect to mitochondrial distribution within muscle fibers. Subsarcolemmal mitochondria (SSM) are located near the cell surface, whereas intermyofibrillar mitochondria (IMF-M) are located near the Z-line of muscle fibers (7). It is generally considered that IMF-M supply ATP to contracting myofibrils, whereas SSM chiefly provide energy for membrane-related events of cell signaling, substrate transport and ion exchange and in support of adjacent nuclei (14). SSM generally comprise one-third of skeletal muscle mitochondria. We have recently reported that, in addition to the absolute decrease in ETC activity in muscle in type 2 diabetes mellitus (DM) and obesity, there is additionally a disproportionate decrease in ETC activity within the SSM fraction (29). Interestingly, it is this subpopulation of mitochondria that appears to be most responsive to physical activity in rat muscle (4, 21), as well as in human skeletal muscle (5, 6, 8). Taken together, these observations raise the question of whether the deficit in SSM noted in obesity and type 2 DM is simply a manifestation of sedentary behavior and,
accordingly, might be restored by physical activity. The present study was therefore undertaken to examine the effect of increased physical activity combined with moderate weight loss (WL) on muscle mitochondrial (mt)DNA content and ETC activity within SSM and IMF-M fractions in previously sedentary, overweight and obese men and women. Physiological data from this intervention have recently been reported (9), as have information on changes in muscle lipid content (13); these changes included improvements in insulin sensitivity, increased reliance on lipid oxidation during fasting conditions, and unchanged muscle lipid content, albeit with a redistribution to smaller lipid droplets in myocytes.

METHODS

Research volunteers. The research participants in the current investigation were part of a larger cohort of research volunteers previously described (9), who were recruited from the community and had a medical examination before participation. Participants in the current study had pre- and postintervention muscle biopsy samples. Inclusion criteria were that volunteers were overweight or obese (body mass index of ≥27 kg/m²), weight stable, sedentary, and nondiabetic. The protocol was approved by the University of Pittsburgh Institutional Review Board, and all volunteers gave written informed consent.

Body composition and metabolic assessments. Measurements of body composition, aerobic fitness, fasting and insulin-stimulated respiratory quotient (RQ), and insulin sensitivity were performed as previously described in detail (9), and will be summarized briefly. Systemic fat mass (FM) and fat free mass (FFM) were assessed by dual energy X-ray absorptiometry (model DPX-L; Lunar, Madison, WI), and abdominal adipose tissue was measured by cross-sectional computed tomography imaging. Maximal oxygen consumption (VO₂max) was measured using an incremental protocol on an electronically braked cycle ergometer; these data are expressed relative to FFM. Insulin sensitivity was estimated as the rate of glucose disposal during the last 30 min of a 4-h hyperinsulinemic (40 mU/m²/H11002 min) euglycemic clamp. Metabolic flexibility in the response of systemic RQ was calculated as the difference between insulin-stimulated and fasting values for RQ.

Intervention. The 16-wk program of WL and exercise training was also previously described in detail (9) and therefore will be summarized briefly. Systemic fat mass (FM) and fat free mass (FFM) were assessed by dual energy X-ray absorptiometry (model DPX-L; Lunar, Madison, WI), and abdominal adipose tissue was measured by cross-sectional computed tomography imaging. Maximal oxygen consumption (VO₂max) was measured using an incremental protocol on an electronically braked cycle ergometer; these data are expressed relative to FFM. Insulin sensitivity was estimated as the rate of glucose disposal during the last 30 min of a 4-h hyperinsulinemic (40 mU/m²/H11002 min) euglycemic clamp. Metabolic flexibility in the response of systemic RQ was calculated as the difference between insulin-stimulated and fasting values for RQ.

The 16-wk program of WL and exercise training was also previously described in detail (9) and therefore will be summarized briefly. Participants were asked to participate in a minimum of four and a maximum of six exercise sessions weekly, with at least one supervised walking. The goal of the weight loss intervention was to produce a weight loss of 10% body weight via a reduction in daily calorie intake of 500–1,000 kcal. A nutritionist met with participants weekly.

Analysis of mitochondrial ETC activity. Muscle biopsy samples of vastus lateralis (~10–15 mg wet wt) were homogenized using a Polytron homogenizer according to the procedures described by Krieger et al. (21). Soluble and particulate fractions were prepared, as previously described (28), by centrifugation (45,000 g for 20 min) to pellet a particulate fraction containing >95% of tissue mitochondria. SSM and IMF-M fractions were isolated as described previously (30). Mitochondrial preparations were suspended in medium containing 0.5 mM EGTA, 0.1 mg/ml BSA, 25 mM KH₂PO₄, and pH 7.0 at 21°C and kept at ~80°C until assay. Activity of rotenone-sensitive NADH:O₂ oxidoreductase was determined in the particulate fractions by HPLC-based assay, as described previously (30). Activity of citrate synthase (CS) was determined in the soluble and particulate fractions by HPLC monitoring of the generation of CoASH after conversion to a fluorescent adduct in a reaction with ThioGlo-1, as previously described (30). A calibration curve was obtained by plotting the peak area of CoA-S-ThioGlo-1 fluorescent adduct in arbitrary units against known amounts of CoA-SH. Activity of creatine kinase (CK) was measured as an index of muscle fiber content in biopsy samples by monitoring of the generation of NADPH in a coupled enzymatic reaction (hexokinase/glucose-6-phosphate dehydrogenase), as previously described (17, 28). Activity of succinate oxidase (succinate:O₂ oxidoreductase) was measured by estimation of fumarate accumulation. The fumarase assay was based on using fumarase and malic dehydrogenase reactions to oxidize fumarate and reduce NAD to NADH (32). Activity of succinate oxidase was measured at 30°C in a reaction medium containing 0.5 mM EGTA, 0.1 mg/ml BSA, 40 μg/ml alamethicin, 4 mM succinate, and 25 mM KH₂PO₄, pH 7.0 at 21°C, as described previously (30). After termination of the reaction by addition of H₂SO₄, the assay pH was adjusted to ~9.0 and then fumarase and malic dehydrogenase were added to oxidize fumarate and reduce NAD to NADH. HPLC with fluorescence detection was used to measure NADH (30).

mtDNA determinations. DNA (mitochondrial and nuclear) was extracted from tissue samples using a QIAamp DNA minikit (Qiagen, Chatsworth, CA). The concentration of each sample was determined using a GeneQuant spectrophotometer (Pharmacia Biotech). mtDNA content was measured using the real-time PCR as described earlier (36). Detection of a 69-bp fragment of mtDNA (nucleotides 14918–14986) and a 77-bp fragment of β-globin, both based on markers published by Miller et al. (24), were used to determine relative copy number of mtDNA per diploid nuclear genome. Primers and FAM-labeled Taqman TAMRA probes (no. 450025; Applied Biosystems, Foster City, CA) were designed using Primer Express software, version 1.5 (Applied Biosystems) and are listed in Table 1. PCR for the detection of mtDNA and β-globin was performed as two separate reactions but within the same run for each sample. All samples were run in duplicate for each gene. Reactions were carried out in the presence of 1× Taqman Universal PCR Master Mix (no. 4304437,

Table 1. Sequences of amplification primers and probes

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>mtDNA forward</td>
<td>5’-GAC GCC TGA ACC GCC TT-3’</td>
</tr>
<tr>
<td>mtDNA reverse</td>
<td>5’-GCG GAT GAT TCA GCC ATA ATT TA-3’</td>
</tr>
<tr>
<td>β-Globin forward</td>
<td>5’-TGA AGG CTC ATG CCA AGA AA-3’</td>
</tr>
<tr>
<td>β-Globin reverse</td>
<td>5’-AAA GGT GCC CTT GAG GTC TGC-3’</td>
</tr>
<tr>
<td>Probe</td>
<td></td>
</tr>
<tr>
<td>mtDNA probe</td>
<td>5’-6FAM-CATCAA TCG CCC ACA TCA CTC GAG AC-TAMRA-3’</td>
</tr>
<tr>
<td>β-Globin probe</td>
<td>5’-6FAM-CQA GCG CAT CAC TAA AGG CAC CGA-TAMRA-3’</td>
</tr>
</tbody>
</table>

mt, Mitochondrial.
EXERCISE INCREASES MUSCLE ETC ACTIVITY IN OBESITY

Applied Biosystems), 1 μM each of forward and reverse primer, 0.25 μM (FAM) of labeled Taqman/TAMRA probe, and 20 ng of sample DNA to a final volume of 25 μl. Amplification reactions were performed in an ABI Prism 7700 spectrophuorometric thermal cycler (Applied Biosystems) with the following cycle conditions: 50°C for 2 min uracil-N-glycosylase incubation, 95°C denaturation and enzyme activation step for 10 min followed by 40 cycles of 95°C denaturation for 15 s, and 60°C annealing and elongation for 60 s. The Sequence Detection System software (SDS v. 1.7) of the ABI-Prism 7700 was used to generate the FAM fluorescence. The threshold cycle number (Ct) was calculated using SDS software v. 1.7 (Applied Biosystems) and an automatic setting of the baseline. The baseline value was the average fluorescence value of PCR cycles 3–15 plus 10 times its standard deviation. These values were used for the relative copy number (Rc) calculations by expressing Ct differences of the β-globin and mtDNA PCR as described earlier (36): Rc = 2^{-ΔCt}, where ΔCt = Ct β-globin − Ct mtDNA.

Transmission electron microscopy. To complement the assessment of mitochondrial distributions based on subcellular separation methods, as described above, transmission electron microscopy (TEM) was performed to examine the thickness of the layer of SSM (37). Muscle samples used for TEM were prepared as previously described (17).

Statistics. Data are presented as means (SD, unless otherwise indicated). ANOVA was used to compare men and women. A paired t-test was used to compare within-subject pre- and postintervention values. Linear regression was used to examine for correlation between physiological variables (e.g., insulin sensitivity) and parameters of mitochondrial function.

RESULTS

Effects of intervention on weight loss, aerobic capacity, and insulin sensitivity. Baseline clinical characteristics of the research volunteers and after 16 wk of intervention are shown in Table 2. Men and women were of similar age [means (SD)] [39.2 (4.7) yr]. Men had higher absolute values for V̇O2 max but expressed relative to FFM, which was greater in men, baseline V̇O2 max was similar in men and women. Women had a significantly higher percentage of FM, whereas men had greater visceral adipose tissue and FFM; these are expected sex-related differences. In response to intervention, the mean WL was 9.7 (5.7)% and V̇O2 max increased by 22 (14)% with both changes being highly significant with respect to baseline (P < 0.001). WL and increases in V̇O2 max were similar in men and women. On average, men exerted at a modestly higher intensity, as determined by monitored heart rate responses [339 (121) vs. 207 (85) kcal/session, P < 0.05].

As a result of the intervention, there was a significant improvement in insulin sensitivity, estimated on the basis of the steady-state rate of exogenous glucose infusion during a glucose clamp. During steady-state conditions of insulin infusion, arterial glucose was identical [90.3 (3.0) vs. 90.2 (2.8) mg/dl, pre- and postintervention, respectively], and arterial insulin was lower at the postintervention clamp [69.7 (4.5) vs. 58.7 (2.7) μU/ml, P < 0.05]. Rates of insulin-stimulated glucose utilization, adjusted for steady-state insulin, increased by a mean of 92 [19%, from 0.097 (0.008) to 0.175 (0.018) mg-min⁻¹·kg⁻¹·FFM⁻¹·μU⁻¹·ml⁻¹, P < 0.001]; these increases were similar in men and women. There was also a significant increase (P < 0.01) in metabolic flexibility in men and women, which is defined as the difference between insulin-stimulated and fasting values for RQ; these data are shown in Table 2 and reflect decreases in fasting RQ as well as increases in insulin-stimulated values. Considering preintervention and postintervention values, there was a strong correlation between insulin sensitivity and metabolic flexibility (r = 0.86, P < 0.001).

Muscle oxidative enzyme activity. Mean values, pre- and postintervention, for skeletal muscle activity of CS, rotenone-sensitive NADH oxidase, and succinate oxidase are shown in Table 3, and individual responses for succinate oxidase are shown in Fig. 1. In response to intervention, activities of CS (P < 0.01), NADH oxidase (P < 0.001), and succinate oxidase (P < 0.001) each increased significantly. There was not a difference in CK activity in preintervention compared with postintervention muscle [6,384 (546) and 6,384 (492) U/g tissue wet wt]. Succinate oxidase activity was also determined in subfractions of SSM and IMF-M. After intervention, there was a highly significant increase of succinate oxidase activity in both the SSM and IMF-M muscle fractions. The percentage increase in activities of NADH oxidase [69 (18)%, p ≤ 0.01] and succinate oxidase [52 (9)%], which reflect ETC activity, was greater than the increase in CS activity [18 (5)%]. There was a difference in mitochondrial matrix enzyme of the TCA cycle.

There were sex differences in skeletal muscle CS and succinate oxidase activities at baseline and after intervention, with higher mean values in men. There was also a sex difference in succinate oxidase activity in SSM and IMF-M fractions. In

Table 2. Clinical characteristics of research volunteers

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Weight, kg</th>
<th>BMI, kg/m²</th>
<th>Fat mass, %fat mass</th>
<th>FFM, kg</th>
<th>VAT, cm</th>
<th>V̇O2 max, ml·kg⁻¹·FFM⁻¹·min⁻¹</th>
<th>GINF/IRI, mg·kg⁻¹·FFM⁻¹·min⁻¹·IRI⁻¹</th>
<th>Fasting RQ</th>
<th>Clamp RQ</th>
<th>Metabolic flexibility (ΔRQ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-INT</td>
<td>Post-INT</td>
<td>Pre-INT</td>
<td>Post-INT</td>
<td>Pre-INT</td>
<td>Post-INT</td>
<td>Pre-INT</td>
<td>Pre-INT</td>
<td>Pre-INT</td>
<td>Pre-INT</td>
<td>Pre-INT</td>
</tr>
<tr>
<td>Males (n = 7)</td>
<td>39.5 (6.4)</td>
<td>103.7 (11.6)</td>
<td>31.7 (3.3)</td>
<td>28.0 (6.8)</td>
<td>74.5 (7.9)</td>
<td>102 (77)</td>
<td>39.0 (6.9)</td>
<td>8.9 (3.0)</td>
<td>0.07 (0.02)</td>
<td>0.07 (0.02)</td>
</tr>
<tr>
<td>Females (n = 7)</td>
<td>38.8 (2.8)</td>
<td>96.1 (14.7)</td>
<td>29.4 (4.2)</td>
<td>22.6 (9.7)</td>
<td>70.7 (8.0)</td>
<td>126 (51)</td>
<td>48.7 (7.2)</td>
<td>14.9 (4.9)</td>
<td>0.08 (0.03)</td>
<td>0.11 (0.04)</td>
</tr>
</tbody>
</table>

Values are means (SD). INT, intervention; BMI, body mass index; FFM, fat-free mass; VAT, visceral adipose tissue; VO2 max, maximal O2 consumption; GINF, glucose infusion rate; IRI, immunoreactive insulin; RQ, respiratory quotient. *P < 0.01; †P < 0.05.
women, the SSM fraction contained 12 (5)% of succinate oxidase activity and in men the percentage was 27 (9)% significantly higher than in women \( P < 0.001 \). As a point of reference, in previously reported data \( (29) \), we found that, in lean, physically active women and men, respectively, the SSM fraction contained 25 (4) and 34 (7)% of muscle succinate oxidase activity, a larger fraction than that observed in obese sedentary women or men enrolled in the current study. Moreover, absolute activities of succinate oxidase in the SSM fraction were approximately eight- and fourfold higher, respectively, in lean women and men than in the obese, sedentary volunteers of the current study.

Despite the differences in baseline activities of succinate oxidase between men and women, both men and women manifested significant increases following intervention and a similar proportionate increase. Succinate oxidase activity in the SSM fraction increased significantly, in men by 65% and in women by 62%. Individual responses, by sex, for SSM and IMF-M are shown in Fig. 1, and the mean responses by sex are shown in Table 3. Yet despite the increase in absolute activity of succinate oxidase activity in SSM and IMF fractions, the relative deficit of ETC activity in the SSM fraction, when considered as a percentage of overall ETC activity, persisted in both sexes.

TEM was used to measure the thickness of the layer of SSM located near muscle nuclei, as previously described \( (37) \). The thickness of the layer of SSM increased slightly but not significantly following weight loss and exercise intervention \( [0.107 (0.021) \text{ vs. } 0.133 (0.026)] \text{ m, } P = 0.45 \).

**mtDNA content.** The effect of intervention on muscle content of mtDNA, as an index of mitochondrial content, was assessed for 11 of the 14 volunteers, those from whom there remained sufficient pre- and postintervention sample for this determination. The findings are shown in Table 4. RT-PCR was used to determine relative copy number of mtDNA per diploid nuclear genome in skeletal muscle. After intervention, mtDNA content did not increase significantly \( [1,665 (213) \text{ vs. } 1,874 (214)] \text{ copies of mtDNA/diploid nuclear DNA, pre- and postintervention, respectively, } P = 0.21 \). The mean increase in mtDNA was 16 (8%). In contrast, in these 11 volunteers, mean succinate oxidase activity increased significantly \( (P < 0.01) \), by 50 (9)%, as also shown in Table 4. The increase in ETC activity was not significantly correlated with the change in mtDNA \( (r = 0.47, P = 0.14) \). However, the ratio of succinate oxidase activity divided by mtDNA did increase significantly \( (P < 0.001) \), by a mean of 32 (7)%. This indicates that improved ETC activity following intervention was more clearly attributable to an increased functional capacity of mi-

### Table 3. Effect of weight loss and exercise intervention in men and women on citrate synthase, rotenone-sensitive NADH oxidase, and succinate oxidase activities in the mitochondrial fraction of skeletal muscle

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th></th>
<th>Women</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-INT</td>
<td>Post-INT</td>
<td>Pre-INT</td>
<td>Post-INT</td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>3.4 (1.5)</td>
<td>4.2 (1.7)*</td>
<td>2.3 (0.5)</td>
<td>2.6 (0.5)</td>
</tr>
<tr>
<td>NADH oxidase</td>
<td>0.18 (0.12)</td>
<td>0.34 (0.26)*</td>
<td>0.16 (0.07)</td>
<td>0.22 (0.05)*</td>
</tr>
<tr>
<td>Succinate oxidase</td>
<td>0.153 (0.092)</td>
<td>0.233 (0.130)*</td>
<td>0.104 (0.053)</td>
<td>0.155 (0.043)*</td>
</tr>
<tr>
<td>SSM succinate oxidase [%]</td>
<td>0.043 (0.031) [27 (9)]</td>
<td>0.069 (0.048)*[29 (6)]</td>
<td>0.012 (0.074) [12 (5)]</td>
<td>0.023 (0.009)*[15 (5)]</td>
</tr>
<tr>
<td>IMF-M succinate oxidase [%]</td>
<td>0.110 (0.063) [73 (9)]</td>
<td>0.164 (0.087)[71 (6)]</td>
<td>0.092 (0.049 [88 (5)]</td>
<td>0.132 (0.04)*[88 (5)]</td>
</tr>
</tbody>
</table>

*Values are means (SD). SSM, subsarcolemmal mitochondria; IMF-M, intramyofibrillar mitochondria. *\( P < 0.01 \).*

 Fig. 1. A: pre- and postintervention values for (ETC) activity in the subsarcolemmal (SS) mitochondrial fraction of skeletal muscle obtained by biopsy from men (A) and women (C). Activity of succinate oxidase is expressed relative to the content of creatine kinase (CK) activity in the biopsy sample, the latter an index of muscle fiber content. B: activity of succinate oxidase in the intramyofibrillar (IMF) mitochondrial fraction before and after intervention.
BMI was also statistically significant. After inclusion of V˙O2 max, which accounted for 33% of the variance in insulin sensitivity, ETC activity in the SSM fraction was of borderline statistical significance. The ETC/mtDNA ratio in each individual was calculated by dividing the succinate oxidase activity (normalized for CK) by the mtDNA (normalized for nuclear DNA), and multiplying by 1,000.

Table 4. Effect of exercise on mtDNA content and ETC activity in mitochondrial fraction of skeletal muscle

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 5)</th>
<th>Women (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-INT</td>
<td>Post-INT</td>
</tr>
<tr>
<td>mtDNA</td>
<td>1754 (974)</td>
<td>2069 (676)</td>
</tr>
<tr>
<td>Succinate oxidase</td>
<td>0.166 (0.109)</td>
<td>0.243 (0.152)</td>
</tr>
<tr>
<td>ETC/mtDNA ratio</td>
<td>0.118 (0.094)</td>
<td>0.135 (0.103)</td>
</tr>
</tbody>
</table>

Values are means (SD). ETC, electron transport chain. mtDNA is expressed as mtDNA copies/nuclear DNA copies. Succinate oxidase is expressed as U/mU creatine kinase (CK). The ETC/mtDNA ratio in each individual was calculated by dividing the succinate oxidase activity (normalized for CK) by the mtDNA (normalized for nuclear DNA), and multiplying by 1,000.

mitochondria rather than simply an increased number of mitochondria.

Association between muscle ETC activity and physiological variables. As a result of the intervention, there were changes in weight, aerobic capacity, and muscle ETC activity, along with improvement in insulin sensitivity and improvement in metabolic flexibility of RQ in the transitions between fasting and insulin-stimulated conditions. Accordingly, we examined for potential correlations within this set of variables. By use of all pre- and postintervention values, with insulin sensitivity as the dependent variable, in a stepwise regression analysis, V˙O2 max was the strongest simple correlate (F = 12.9, P < 0.001), and BMI was also statistically significant (F = 5.5, P < 0.05). After inclusion of V˙O2 max, which accounted for 33% of the variance in insulin sensitivity, ETC activity in the SSM fraction was the variable of highest statistical significance (F = 6.4, P < 0.05), and total ETC activity was of borderline significance (F = 3.2, P = 0.08). Addition of SSM ETC activity to V˙O2 max accounted for 47% of the variance in insulin sensitivity. With regard to metabolic flexibility as the dependent variable, again, V˙O2 max was the strongest simple correlate (F = 4.2; P < 0.05). After inclusion of V˙O2 max, ETC activity in the SSM was of borderline statistical significance (F = 3.4, P = 0.08). In regard to which aspects of the intervention were associated with muscle ETC activity, we examined V˙O2 max, weight loss (and %weight loss), and average intensity of exercise sessions. Considering pre- and postintervention values, ETC activity in SSM was strongly associated with V˙O2 max (F = 13.6, P < 0.001), as shown in Fig. 2, and with body mass index (BMI; F = 5.4, P < 0.05), although BMI did not add independent significance after inclusion of V˙O2 max. A similar pattern was evident for ETC activity in IMF-M, although the association with V˙O2 max (F = 7.4, P < 0.01) was not as strong as with ETC activity in SSM. In regard to the intervention induced change in ETC activity in SSM, the Kcal/session expended in exercise was strongly correlated (F = 13.7, P < 0.01).

DISCUSSION

Skeletal muscle oxidative capacity is reduced in sedentary obese compared with lean, more physically active individuals (2, 22, 33) and is associated with a disproportionate reduction of ETC activity in SSM (29). This reduction in oxidative capacity may contribute to the accumulation of lipid within muscle fibers in obesity and to insulin resistance. Lower oxidative capacity has also been related to risk for weight gain (35). Reduced muscle oxidative capacity in obesity may simply be a consequence of sedentary habits. Weight loss interventions without changes in aerobic capacity do not appear to enhance muscle oxidative capacity in obesity (18, 34). The current study was undertaken, therefore, to assess the impact of increases in moderate-intensity physical activity, when added to a weight loss intervention, on muscle mitochondria in obese, previously sedentary men and women. The metabolic effects of this intervention in a larger cohort of research volunteers, including those of the current study, have previously been reported (9). Pertinent to this examination of muscle mitochondria, the intervention increased insulin sensitivity and was associated with increased reliance on fat oxidation during fasting conditions (9). Muscle lipid content remained unchanged after this intervention, but the mean size of lipid droplets within myocytes decreased, a change that was correlated with the improvement in insulin sensitivity (12) and that occurred without a change in the distribution of muscle fiber types, two findings pertinent to the current report of changes in ETC activity.

The current study focused on whether this intervention would increase ETC activity in skeletal muscle and, if so, whether this was due to mitochondrial biogenesis and would change subcellular distribution of mitochondria within muscle fibers. More specifically, we wanted to address whether there would be an increase in the proportion of muscle ETC activity in SSM. Pre- and postintervention muscle biopsy samples were obtained from 14 participants and examined for ETC activity, for distribution of ETC activity in SSM and IMF fractions, and for content of mtDNA. The main finding of the current study was that the combination of moderate weight loss and increased physical activity, sufficient to improve V˙O2 max by ~20%, increased ETC activity in muscle substantially, by ~60%.

Fig. 2. Values for succinate oxidase activity in the SS mitochondrial (SSM) fraction are plotted against corresponding values for maximal O2 consumption (V˙O2 max) before intervention (○) and after weight loss and physical activity intervention (●). There was a significant correlation (r = 0.59, P < 0.01).
There were several important collateral findings that give insight into the nature of the mitochondrial response. First, the increase in ETC activity was substantially greater than the corresponding rise in mtDNA. In fact, the postintervention change in mtDNA was not statistically significant. Though mitochondrial proliferation was not induced, the ratio of ETC activity normalized to mtDNA content increased significantly. These findings indicate enhanced functional capacity rather than proliferation of mitochondria as the principal mechanism for improved ETC activity. A second finding in this regard was that there was an ~20% increase in activity of CS, a matrix enzyme of the TCA cycle, which, although statistically significant, was less than that of ETC activity, which increased by ~60%. This indicates a particular and specific enhancement of the ETC activity. Third, increased ETC activity occurred in both SSM and IMF-M fractions and by the same proportion. This indicates that improved functional capacity of mitochondria occurred across the mitochondrial reticulum and was not confined to a specific subpopulation. A corollary of this is that we did not observe that the intervention rectified the disproportionate reduction of ETC in SSM, which was present before intervention and that we have also observed in type 2 DM (29).

The strongest correlate of ETC activity was physical activity, as both $\text{VO}_2^{\text{max}}$ and mean energy expenditure of exercise sessions correlated more clearly than did weight loss per se, and this is consistent with prior studies (34). It has been estimated that 6 wk of physical activity is sufficient to evoke a new steady state of mitochondrial biogenesis in humans (14), a process generally associated with a 50–100% increase in mitochondrial content in skeletal muscle. In a recently completed physical activity intervention in normal-weight, elderly men and women, our laboratory did observe a 60% increase in mtDNA in vastus lateralis and that this increases in mtDNA was tightly correlated with a similar amplitude of increase in muscle ETC activity (10). Clearly, then, the response that we observed in the current study, which involved obese, previously sedentary, middle-aged men and women, is somewhat different from that noted in lean young or elderly volunteers, since, as noted above, there was a much larger increase in ETC activity than in mtDNA content. There is a suggestion of potential sex differences with regard to ETC activity in SSM. At baseline and after intervention, men had higher ETC activity and a higher proportion of overall ETC activity within SSM than did women. Both men and women had similar percentage increases in ETC activity in SSM, but the sex differences noted at baseline persisted after intervention. An important caveat is that these comparisons involve only seven men and seven women and will need to be examined more extensively in future studies.

Mitochondrial biogenesis entails integrated responses of both nuclear and mitochondrial genomes, with the majority of constituents of the ETC encoded by the nuclear genome. Nuclear transcription factors such as peroxisome proliferator-activated receptor-γ coactivator-1α, and nuclear respiratory factors 1 and 2, coordinate expression of nuclear encoded proteins targeted to mitochondria (15). As well, nuclear encoded mitochondrial transcription factor A regulates expression and replication of mtDNA (14). Mitochondrial biogenesis, as well as remodeling of mitochondria, entails importation of protein and lipids, leading to changes in mitochondrial protein content, mitochondrial volume, and phospholipid content. One possibility suggested by the current findings is that the predominant factor was enhanced protein and phospholipid synthesis leading to improved functional capacity of the ETC or alterations in the balance between mitochondrial fusion and fission (23), rather than mitochondrial proliferation as such. Perhaps this is related to characteristics of the intervention. Although the 16-wk duration of the exercise intervention in the current study is certainly sufficient to evoke a new steady state of mitochondrial biogenesis and did have a robust effect of substantially improving $\text{VO}_2^{\text{max}}$, it should be considered that this represented moderate- rather than high-intensity exercise. Mitochondrial proliferation is governed in part by a signaling pathway that depends on intracellular Ca$^{2+}$ and AMP oscillations (15). Changes in intracellular Ca$^{2+}$ flux can be triggered by muscle contraction, but it is not clear that reductions in ATP and increases of AMP are evoked by activity levels such as treadmill walking and stationary cycling, as was done by the research volunteers in the current study. Also, the collateral effect of weight loss on the pattern of mitochondrial biogenesis evoked by physical activity is largely unknown. Prior studies in humans generally indicate that oxidative enzyme activity does not increase following weight loss intervention without physical activity (18, 34). Short-term nutritional challenges, such as fasting or alteration of carbohydrate and fat intake, also acutely modulate metabolic gene expression in human muscle (1, 27), and caloric restriction generally leads to lower ETC activity in muscle and other tissues.

Prior studies, mostly done in animal models and with higher-intensity exercise, indicate that the SSM fraction can be especially responsive to the effects of exercise (4–6). Our findings are not inconsistent with this, as the absolute increase in ETC in the SSM fraction was robust and was significantly correlated with parameters of exercise, namely changes in $\text{VO}_2^{\text{max}}$ and the average intensity per exercise. Also, although the thickness of the layer of SSM did not change, the functional capacity for ETC activity certainly did, a finding consonant with the relatively stable level of mtDNA. Nonetheless, increased ETC activity was noted in IMF fractions as well, and a particular or selective enhancement of ETC in SSM was not noted. Future studies, involving either more intense physical activity or a longer intervention duration may be required to more fully address whether a relative deficiency of SSM can be restored in obesity.

In cross-sectional studies, a correlation between mitochondrial dysfunction and insulin resistance has been observed (17, 25, 26), and the current study provided an opportunity to examine this association in the context of an intervention. We did not observe a significant simple correlation between pre- and postintervention ETC activity and corresponding values for insulin sensitivity or metabolic flexibility. Instead, insulin sensitivity or metabolic flexibility correlated significantly with aerobic fitness and adiposity. Nonetheless, after adjusting for these effects, we did observe a significant correlation with ETC activity, most particularly with ETC activity in the SSM fraction, which supports the hypothesis that mitochondrial function contributes to skeletal muscle insulin sensitivity. Similar findings pertained to the association with metabolic flexibility, denoting improved capacity for transitions between glucose and lipid oxidation in response to insulin-stimulated and fasting conditions, respectively.
In summary, ETC activity in skeletal muscle in previously sedentary obese men and women was substantially improved by an intervention of moderate weight loss and moderate-intensity physical activity. These biochemical changes in skeletal muscle mitochondria occurred in conjunction with improvement in substrate metabolism and insulin sensitivity in muscle. Interestingly, the increase in ETC activity in skeletal muscle in these previously sedentary obese men and women was greater than that of mtDNA content, signifying a more pronounced effect on functional capacity of mitochondria than on proliferation. We conclude that ETC activity is an important component of the improved metabolic capacity of skeletal muscle that occurs following physical activity interventions in obesity.

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