Subsarcolemmal and intermyofibrillar mitochondria play distinct roles in regulating skeletal muscle fatty acid metabolism

Timothy R. Koves,1,3,5 Robert C. Noland,1 Andrew L. Bates,2 Sarah T. Henes,2 Deborah M. Muoio,3,4,5 and Ronald N. Cortright1,2

1Department of Physiology, Brody School of Medicine, and 2Department of Exercise and Sport Science, College of Health and Human Performance, East Carolina University, Greenville; and 3Department of Medicine, 4Department of Pharmacology and Cancer Biology, and 5Sarah W. Stedman Nutrition and Metabolism Center, Duke University, Durham, North Carolina

Submitted 9 August 2004; accepted in final form 4 January 2005

Koves, Timothy R., Robert C. Noland, Andrew L. Bates, Sarah T. Henes, Deborah M. Muoio, and Ronald N. Cortright. Subsarcolemmal and intermyofibrillar mitochondria play distinct roles in regulating skeletal muscle fatty acid metabolism. Am J Physiol Cell Physiol 288: C1074–C1082, 2005. First published January 12, 2005; doi:10.1152/ajpcell.00391.2004.—Skeletal muscle contains two populations of mitochondria that appear to be differentially affected by disease and exercise training. It remains unclear how these mitochondrial subpopulations contribute to fiber type-related and/or training-induced changes in fatty acid oxidation and regulation of carnitine palmitoyltransferase-1β (CPT1β), the enzyme that controls mitochondrial fatty acid uptake in skeletal muscle. To this end, we found that fatty acid oxidation rates were 8.9-fold higher in subsarcolemmal mitochondria (SS) and 5.3-fold higher in intermyofibrillar mitochondria (IMF) that were isolated from red gastrocnemius (RG) compared with white gastrocnemius (WG) muscle, respectively. Malonyl-CoA (10 μM), a potent inhibitor of CPT1β, completely abolished fatty acid oxidation in SS and IMF mitochondria from RG, whereas oxidation rates in the corresponding fractions from RG were inhibited only 89% and 60%, respectively. Endurance training also elicited mitochondrial adaptations that resulted in enhanced fatty acid oxidation capacity. Ten weeks of treadmill running differentially increased palmitate oxidation rates 100% and 46% in SS and IMF mitochondria, respectively. In SS mitochondria, elevated fatty acid oxidation rates were accompanied by a 48% increase in citrate synthase activity but no change in CPT1 activity. Nonlinear regression analyses of mitochondrial fatty acid oxidation rates in the presence of 0–100 μM malonyl-CoA indicated that IC50 values were neither dependent on mitochondrial subpopulation nor affected by exercise training. However, in IMF mitochondria, training reduced the Hill coefficient (P < 0.05), suggesting altered CPT1β kinetics. These results demonstrate that endurance exercise provokes subpopulation-specific changes in mitochondrial function that are characterized by enhanced fatty acid oxidation and modified CPT1β-malonyl-CoA dynamics.

Endurance exercise training increases mitochondrial size, number (14, 20), and phosphorylation activity (9), and thereby enhances the muscle’s capacity to oxidize both glycolytic (2, 18) and lipid (2, 40) substrates. These adaptations, which are thought to play a major role in mediating contraction-induced fiber type alterations, not only facilitate enhanced athletic performance but also contribute to improved whole body metabolic fitness. In contrast, deficiencies in mitochondrial oxidative capacity have been associated with the pathophysiology of aging (43) and metabolic diseases (26, 28).

Studies on mitochondrial function in skeletal or cardiac muscle present the added dimension of two distinct mitochondrial subpopulations that might exist as a continuous reticulum (25, 30) but differ according to their subcellular localization, morphology and biochemical properties (8, 33, 42). Subsarcolemmal (SS) mitochondria are located just underneath the sarcolemma and have a large, lamellar shape. In contrast, the intermyofibrillar (IMF) mitochondria are smaller, more compact, and located between the contractile filaments. Several studies have shown that SS and IMF mitochondria possess inherent differences in their capacity to adapt to changes in contractile activity (5, 21, 31). Compared with IMF mitochondria, the SS mitochondria display greater exercise-induced increases in volume, state III respiration, and enzyme activities (5, 31). The SS mitochondria also show more pronounced decrements in response to muscle disuse (31).

The present study sought to determine whether these two subpopulations play discrete roles in mediating training-induced increases in skeletal muscle fatty acid oxidation, and/or in conferring the distinct metabolic properties of red (type I) compared with white (type II) fiber types. In a previous report we provided evidence of fiber type-dependent differences in the kinetic properties of the muscle isoform of carnitine palmitoyltransferase-1β (CPT1β) (29), the enzyme that controls mitochondrial uptake of fatty acid and functions as the rate-determining step in β-oxidation. CPT1β localizes to the outer mitochondrial membrane and is largely regulated by cytosolic concentrations of its biological inhibitor malonyl-CoA. However, we identified a malonyl-CoA-insensitive CPT1β fraction that is predominately active in mitochondria from red skeletal muscle (29). In the present study, we investigated whether this malonyl-CoA-resistant CPT1β subfraction might be differentially active in SS compared with IMF mitochondria. Furthermore, because endurance training is known to increase mito-
chondrial oxidative capacity (i.e., metabolic shift toward enhanced lipid utilization), we also tested whether this expansion in capacity is generalized or specific to a given mitochondrial subpopulation. Our results indicate that the oxidative properties of muscle mitochondria depend on fiber type and are enhanced by endurance training. Moreover, we show that SS and IMF mitochondria play distinct metabolic roles in mediating both fiber type-specific and exercise-induced regulation of CPT1β and fatty acid oxidation. These findings support the emergent view that skeletal muscle possesses distinct mitochondrial subpopulations that are inherently unique in different muscle fiber types and that display distinct adaptations in response to energy stress.

**MATERIALS AND METHODS**

**Animal Care.** Male Sprague-Dawley rats (Harlan, Indianapolis, IN) were subjects for all studies. The animals were housed in a temperature-controlled environment with a 12:12-h light-dark cycle and provided ad libitum access to rat chow and water. On the day of the experiments, food was removed 4 h before anesthesia was administered. All procedures conformed to East Carolina University Animal Care and Use Committee standards, and the committee approved the study.

**Exercise protocol.** Animals weighing 200–225 g were randomly assigned to either a training (n = 7) or control (n = 7) group. Earlier, Molé and Holloszy (39) demonstrated the absence of a difference in palmitate oxidation between ad libitum-fed controls and control pairs fed to match body weights of the trained group. Therefore, both trained and sedentary animals were allowed ad libitum access to food and water. The rats in the training group were habituated to the treadmill over a 2-wk period to final conditions of 35 m/min and 8% grade. Rats were run 1 h/day, 5 days/wk, for a period of 10–12 wk. Similar training protocols have been shown to produce increases in the capacity of skeletal muscle to oxidize fatty acids (2, 40). Animals were euthanized 48 h after their last training bout.

**Muscle sample preparation.** Animals were administered a mixture containing 90 mg/ml ketamine and 10 mg/ml xylazine at 0.1 ml/kg body wt−1 and muscles were quickly excised, cleaned, rinsed, blotted, weighed, and placed into ice-cold modified Chappel-Perry solution 1 consisting of (in mM) 100 KCl, 10 Tris-HCl, 10 Tris-Base, 5 MgCl2, 1 EDTA, and 1 ATP, pH 7.5.

**Mitochondrial preparations.** Mitochondrial suspensions were prepared according to the methods of Bizeau et al. (5) and Palmer et al. (42) with modifications for the preparation of IMF mitochondria. To prepare the SS mitochondria, gastrocnemius samples were placed in fresh solution 1 (see above) so that they were twofold diluted (wt/vol), minced, suspended 10-fold (wt/vol) in solution 1, and homogenized at 40% power (~9.500 rpm) in an Ultra Turrax homogenizer (model T-25; IKA, Wilmington, NC) for 10 s. The homogenate was centrifuged at 800 g for 10 min at 4°C. The supernatant was filtered through double-layered gauze and centrifuged at 800 g for 10 min at 4°C. The supernatant was filtered through double-layered gauze, and mitochondria were pelleted, washed, and resuspended as described for SS mitochondria.

**Oxidation studies.** Oxidation studies on muscle mitochondria were performed according to methods originally described by Veerkamp et al. (51) and modified by Kim et al. (28). In brief, 40 μl of the final mitochondrial suspensions were incubated in the presence of 160 μl of an oxidation buffer containing (in mM) 0.150 palmitate ([1-14C]palmitate at 0.5 μCi/ml−1; PerkinElmer Life Sciences), 100 μm, 10 Tris-HCl, 10 K2HPO4, 100 KCl, 1 MgCl2, 1 t-carnitine, 0.1 malate, 2 ATP, 0.05 CoA, 1 DTT, and 0.3% BSA and either 0 or 0.01–100 μM malonyl-CoA (Sigma-Aldrich, St. Louis, MO) for 30 min at 37°C. Specific activity was maintained between 7,000 and 8,000 dpm/nmol palmitate−1 for all experiments. The reaction was terminated by the addition of 100 μl of 70% perchloric acid. 14CO2 was trapped in 200 μl of 1 N NaOH. Radioactive 14CO2 was assessed by counting 150 μl from NaOH traps by liquid scintillation counting in 4 ml of Unisint BD (National Diagnostics, Atlanta, GA).

For these experiments, palmitate oxidation and the subsequent inhibition by malonyl-CoA was used as a surrogate for CPT1β activity. This strategy was based on the observation that CPT activity can be measured in both microsomal and mitochondrial fractions as well as in mitochondrial fragments, in which malonyl-CoA insensitive CPT2 might be expressed (36). Traditionally, CPT1 specific activity is calculated by subtracting the residual activity (presumably attributed to CPT2) that is measured in the presence of a maximally inhibiting dose of malonyl-CoA. In contrast, we quantified [14C]-labeled CO2 produced only from intact mitochondria, as an index of palmitate oxidation.

**Enzyme activity assays.** Muscle enzyme activities were determined from 10 μl (~10 μg protein) of the mitochondrial suspensions or whole tissue homogenates. Protein concentrations were determined by bicinchoninic acid protein assay (Pierce, Rockford, IL). CPT1β activity was determined using modifications of the methods of McGarry et al. (37) at 37°C for 6 min, a time at which we observed that the formation of [1-14C]palmitoyl-carnitine remained linear. [1-14C]Palmitoyl-carnitine formed was extracted with 350 μl of water-saturated butanol and quantified by liquid-scintillation counting. Citrate synthase (CS) activity was assessed in a 10-fold diluted mitochondrial suspension using the spectrophotometric method of Srere (49).

**Analyses.** All data are reported as means ± SE. Differences between mitochondrial subpopulations due to training were analyzed using one-way ANOVA. Fiber type and subpopulation differences were analyzed using two-way ANOVA. When significant differences were observed, comparisons between mean values were made using a Student–Newman–Keuls post hoc test. Significance levels were set at a priori at P < 0.05 for all comparisons. In the malonyl-CoA inhibition studies, IC50 values and Hill coefficients were calculated using Prism software (GraphPad, San Diego, CA) with parameters set for best-fit nonlinear regression equations and variable slope constraints. Differences between IC50 values and Hill coefficients were calculated using unpaired t-tests and Welch’s corrections if variances were unequal. All other statistical analyses were also performed using GraphPad Prism.

**RESULTS**

**Isolation procedures.** Because CPT1β is an outer mitochondrial membrane protein that is susceptible to protease degradation, our first objective was to assess mitochondrial mem-
brane integrity in SS and IMF mitochondria fractions using a mechanical method described earlier in the literature (6). CPT1β integrity was assessed by evaluating maintenance of malonyl-CoA inhibitable palmitate oxidation (24). When incubated in the presence of 150 μM [1-14C]palmitate, a 10 μM dose of malonyl-CoA inhibited palmitate oxidation 84% in SS mitochondria. This inhibition was similar (93%) in IMF mitochondria (Table 1). In comparison, when IMF mitochondria were isolated using Nagarse digestion, rates of palmitate oxidation were similar to the mechanical method, but malonyl-CoA sensitivity was lost (Table 1).

**Characteristics of palmitate oxidation in mitochondrial subpopulations.** Assay parameters were optimized using the quadriceps because this muscle provided enough mitochondria to test a sufficient range of conditions. In the presence of 150 μM palmitate and variable concentrations of mitochondrial protein (15–70 μg), rates of 14CO2 production were linear up to 45 min at 37°C (data not shown). Under most conditions, rates of palmitate oxidation in IMF and SS mitochondria were similar, although mean values tended to be higher in the IMF fraction. Based on these results, subsequent experiments were performed at mitochondrial protein concentrations between 40 and 50 μg per well for 30 min at 37°C.

Mitochondrial yields using mechanical methodologies were 0.69 ± 0.04 vs. 0.91 ± 0.05 mg protein·g tissue−1 for deep red gastrocnemius muscle SS and IMF mitochondria, respectively, and 0.46 ± 0.05 vs. 0.44 ± 0.03 mg protein·g tissue−1 for superficial white gastrocnemius muscle SS and IMF mitochondria, respectively. Rates of palmitate oxidation were similar between IMF and SS mitochondria when normalized to mitochondrial protein (Fig. 1). Remarkably, however, palmitate oxidation was 8.9-fold higher (P < 0.05) in SS mitochondria and 5.3-fold higher (P < 0.05) in IMF mitochondria that were isolated from red compared with white gastrocnemius muscle, respectively, thus demonstrating striking fiber type-dependent differences in the fatty acid oxidative capacity of muscle mitochondria. In addition, in SS mitochondria from red gastrocnemius, 10 μM malonyl-CoA inhibited palmitate oxidation 89%, whereas the same dose of malonyl-CoA inhibited IMF mitochondria from red gastrocnemius by only 60% (P < 0.01; Fig. 2A). Conversely, when mitochondria were prepared from white gastrocnemius, malonyl-CoA inhibitable palmitate oxidation was similar in SS and IMF mitochondria (Fig. 2B). These data show that both SS and IMF mitochondria from type I muscle fibers display a markedly higher capacity to oxidize fatty acid compared with the respective subpopulations in type II fibers, and that malonyl-CoA-mediated inhibition of CPT1β and 0.46 vs. 0.44 mg protein·g tissue−1.

### Table 1. Characteristics of isolated muscle mitochondria

<table>
<thead>
<tr>
<th></th>
<th>% Remaining Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>100</td>
</tr>
<tr>
<td>+10 μM malonyl-CoA</td>
<td>16±6.5</td>
</tr>
<tr>
<td>−CoA</td>
<td>&lt;1±0.23</td>
</tr>
<tr>
<td>−Carnitine</td>
<td>&lt;1±0.24</td>
</tr>
<tr>
<td>−CoA/−carnitine</td>
<td>0</td>
</tr>
<tr>
<td>IMF (mechanical)</td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>100</td>
</tr>
<tr>
<td>+10 μM malonyl-CoA</td>
<td>7±1.2</td>
</tr>
<tr>
<td>−CoA</td>
<td>&lt;1±0.28</td>
</tr>
<tr>
<td>−Carnitine</td>
<td>&lt;1±0.01</td>
</tr>
<tr>
<td>−CoA/−carnitine</td>
<td>0</td>
</tr>
<tr>
<td>IMF (Nagarse)</td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>100</td>
</tr>
<tr>
<td>+10 μM malonyl-CoA</td>
<td>115±6.0</td>
</tr>
<tr>
<td>−CoA</td>
<td>ND</td>
</tr>
<tr>
<td>−Carnitine</td>
<td>ND</td>
</tr>
<tr>
<td>−CoA/−carnitine</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are means ± SE from 3 animals assayed in quadruplicate. ND, no data collected. Subsarcolemmal (SS) or intermyofibrillar (IMF) mitochondria were isolated from deep red quadriceps muscle and incubated 30 min at 37°C in a medium containing 150 μM [1-14C]palmitate (0.1 μCi per well) in the presence of either 0 or 10 μM malonyl-CoA and the presence (+) or absence (−) of coenzyme A (CoA) and carnitine (50 μM and 1 mM, respectively). IMF mitochondria were prepared using both mechanical and protease digestion (Nagarse) methods. The rate of 14CO2 production normalized to mitochondrial protein was used as an index of oxidation as described in MATERIALS AND METHODS. Oxidation rates were 78 ± 14, 49 ± 14, and 40 ± 5 nmol·mg protein−1·h−1 for SS, IMF (mechanical), and IMF (Nagarse) mitochondria, respectively.

---

**Fig. 1.** Palmitate oxidation capacity in red and white gastrocnemius. Subsarcolemmal (SS) or intermyofibrillar (IMF) mitochondria isolated from either deep red or superficial white rat gastrocnemius muscle were incubated for 30 min at 37°C in the presence of 150 μM [1-14C]palmitate (0.1 μCi per well). Data represent means ± SE normalized for mitochondrial protein from 8 animals per group. *P < 0.05 vs. the identical subpopulation in red muscle.

**Fig. 2.** Palmitate oxidation and inhibition in SS and IMF mitochondria. 14CO2 was collected from either SS or IMF isolated from deep red (A) or superficial white (B) rat gastrocnemius muscle incubated with 150 μM [1-14C]palmitate (0.1 μCi/well) at 37°C for 30 min. Data represent means ± SE from 8 separate experiments. *P < 0.05 vs. the no malonyl-CoA condition. #P < 0.05 vs. identical subpopulation from red muscle. †P < 0.01 vs. malonyl-CoA inhibition in SS mitochondria from red muscle.

---

**AJP-Cell Physiol** • VOL 288 • MAY 2005 • www.ajpcell.org
Effects of endurance training on fatty acid oxidation. Because regular aerobic exercise is known to produce changes in muscle fiber composition, mitochondrial content, and fatty acid oxidation, we next evaluated subpopulation-specific metabolic responses to 10–12 wk of endurance exercise training. We chose to study mitochondria isolated from whole gastrocnemius muscle because this muscle contains both red and white fiber types and has been shown to undergo a robust training effect in response to treadmill running. Palmitate oxidation rates in IMF mitochondria from control animals were 35% greater (140.2 ± 21.9 vs. 103.8 ± 4.8 nmol 14CO2·mg protein−1·h−1) than those measured in SS mitochondria from the same muscle (Fig. 3). Endurance exercise training increased palmitate oxidation twofold in SS mitochondria to 207.0 ± 23.5 nmol 14CO2·mg protein−1·h−1 (P < 0.05). In comparison, the training regimen increased palmitate oxidation in IMF mitochondria only 46% (P < 0.05), consistent with previous studies showing that SS mitochondria exhibit greater metabolic plasticity than IMF mitochondria (5, 8, 21, 31).

Training-induced changes in mitochondrial enzymes. In an attempt to better characterize the biochemical basis for the training-induced adjustments in fatty acid oxidation, we evaluated changes in CPT1β activity as well as CS, a TCA cycle enzyme that is commonly used as a marker of mitochondrial content (Table 2). Mitochondrial yield was 53.7% and 50.8% greater in the SS mitochondria and IMF mitochondria fractions, respectively, harvested from trained muscles compared with untrained muscles. This finding suggested a robust training effect that stimulated mitochondrial biogenesis. Both CS and CPT1β activities were similar between the two subpopulations in control animals. When measured in whole muscle homogenates, which do not account for changes in mitochondrial composition, CS and CPT1β activities were 1.7-fold higher in preparations from trained compared with control muscles. However, when these activities were evaluated in isolated mitochondria and expressed relative to total mitochondrial protein, the only training effect observed was a 47% increase in CS specific activity of the SS mitochondrial subfraction.

Inhibitory responses to malonyl-CoA. In the presence of physiological concentrations of malonyl-CoA, IMF mitochondria from control animals generally maintained higher rates of palmitate oxidation than SS mitochondria (Fig. 4A). However, when these data were normalized to correct for differences in basal oxidation rates, malonyl-CoA-resistant palmitate oxidation in SS compared with IMF mitochondria was similar at all concentrations of the inhibitor (Fig. 4B). Likewise, in mitochondria from control animals, the IC50 values, representing malonyl-CoA sensitivity, and Hill coefficients, reflecting cooperativity between substrates and/or inhibitor binding, were similar between SS and IMF mitochondria.

![Fig. 3. Endurance exercise training increases fatty acid oxidation in SS and IMF mitochondria. SS or IMF (40–50 μg protein/well) isolated from rat gastrocnemius muscle were incubated for 30 min at 37°C in the presence of 150 μM [1-14C]palmitate (0.1 μCi/well). Data represent means ± SE from 5–6 animals per group. *P < 0.05 vs. the identical subpopulation in control animals.](image1)

![Fig. 4. Inhibition kinetics of malonyl-CoA on palmitate oxidation. SS and IMF mitochondria were incubated in the presence of 150 μM [1-14C]palmitate and concentrations of malonyl-CoA between 0 and 100 μM. Data were analyzed and plotted as either absolute 14CO2 production (A) or normalized to basal values (B). The 10−4 μM value represents oxidation rates when no malonyl-CoA was present. Neither Hill coefficients nor IC50 values were significantly different between subpopulations in control animals. *P < 0.05 vs. control SS.](image2)
Malonyl-CoA inhibition after exercise training. Compared with mitochondria from the control animals, SS and IMF mitochondria from muscle of exercise-trained rats maintained greater rates of $^{14}$CO$_2$ production at all submaximal doses of malonyl-CoA (Fig. 5, A and B). When normalized to account for basal differences in palmitate oxidation, the IC$_{50}$ values for both the SS and IMF mitochondria tended to increase with training, but these changes did not reach statistical significance. In SS mitochondria, the Hill coefficient was unaffected by exercise treatment (Fig. 5C). Interestingly, however, in IMF mitochondria, the training regimen reduced this coefficient by 51% ($P < 0.05$; Fig. 5D), suggesting that a greater change in malonyl-CoA was required to affect changes in palmitate oxidation. This result might reflect training-induced changes in the binding of malonyl-CoA and/or fatty acyl-CoA to CPT1.

To illustrate the inhibitory effects of malonyl-CoA on rates of palmitate oxidation within a linear range, we constructed modified Dixon plots (10) using the same data set presented in Fig. 5 (Fig. 6A). Figure 6B shows that we eliminated malonyl-CoA doses in the supraphysiological range, which introduced nonlinearity, before performing linear regression analyses. The slopes of the resulting regression lines, all of which exhibited $R^2$ values $> 0.97$, were more gradual when plotted using oxidation rates measured in either SS (0.0076 vs. 0.0044; $P < 0.001$) or IMF mitochondria (0.0079 vs. 0.0020; $P < 0.001$) from trained compared with untrained muscles. Thus, after exercise training, mitochondrial fatty acid oxidation was less responsive to a given change in malonyl-CoA when the concentrations were kept within a physiological range.

**DISCUSSION**

Skeletal muscles that are endurance trained and/or comprise predominately type I myofibers exhibit an enhanced capacity to oxidize lipid substrate, a feature that is thought to be attributable to increased mitochondrial number and protein content. The studies presented herein demonstrated that fiber type and exercise-related changes in muscle fatty acid oxidative capacity are mediated by qualitative as well as quantitative adaptations in specific mitochondrial subpopulations. Consistent with several earlier reports, we identified two biochemically distinct
mitochondrial subfractions in rat mixed gastrocnemius, as well as from the deep red and superficial white portions of the gastrocnemius muscle. Remarkably, oxidation rates measured in either SS or IMF mitochondria from red gastrocnemius were severalfold those measured in the respective subfractions from white gastrocnemius. This difference remained even on maximal inhibition with malonyl-CoA, a finding consistent with our previously published observations in skeletal muscle homogenates (29). These results are the first to show that the fatty acid oxidative potential of these distinct mitochondrial subpopulations depends on the fiber composition of the source muscle and is regulated by exercise training. We also found that in IMF, but not SS mitochondria, both muscle fiber type and exercise training affected malonyl-CoA-mediated inhibition of fatty acid oxidation. Taken together, these data suggest that the fatty acid oxidative properties of skeletal muscle depend not only on mitochondrial content but also on functional parameters that are discernibly conferred by distinct mitochondrial subpopulations.

In agreement with previous reports, we found that both CPT1β (4) and CS activities (8) were similar in IMF compared with SS mitochondria from rat gastrocnemius muscle. Our finding that training-induced increases in some oxidative enzyme activities were more robust when measured in whole homogenates than in isolated mitochondria is also consistent with previous reports (19, 40) and suggests that some changes in oxidative phosphorylation potential observed at the whole muscle level are in fact due largely to mitochondrial expansion. To the contrary, data from isolated mitochondria indicate that other adaptations, such as increased lipid oxidation, appear to reflect both an increase in mitochondrial number and a change in mitochondrial function.

Consistent with other studies reporting that SS mitochondria appear to be more responsive than the IMF population to endurance training (5, 31), we found that endurance exercise training increased palmitate oxidation rates 100% in SS but only 46% in IMF mitochondria from the gastrocnemius muscle. Thus the paradigm observed in our exercise studies, in which training markedly enhanced the fatty acid oxidation rates of mixed muscle, resembled that in the fiber type model, in which fatty acid oxidation capacity was ~9-fold higher in SS and ~5-fold higher in IMF mitochondria from red compared with white skeletal muscles. These observations are consistent with the notion that endurance training causes a metabolic remodeling of white and intermediate muscle fibers, which in turn produces a phenotype that more closely resembles that of red muscle.

To our knowledge, this is the first study to specifically examine the lipid oxidative capacity of mitochondrial subpopulations from red, white, and mixed skeletal muscle. When normalized for mitochondrial protein, the oxidative capacities between subpopulations appeared similar in extreme red and similar or slightly higher in extreme white muscle IMF mitochondria. However, this was not the case in whole gastrocnemius muscle, in which the oxidative capacity of IMF mitochondria was higher than it was in SS mitochondria. The whole gastrocnemius consists of a more heterogeneous population of fiber types than either the red or white portions of the muscle. Delp and Duan (12) reported that the fiber type composition of extreme red gastrocnemius is 51, 35, 13, and 1% (types I, IIa, IId/x, and IIb), respectively, and that that of extreme white gastrocnemius is 0, 0, 8, and 92%. The fiber type distribution of whole gastrocnemius (3, 6, 34, and 57%) is closer to that of extreme white muscle but exhibits a higher proportion of type IId/x fibers than both extreme white and extreme red muscle. Interestingly, CS activities (36.2, 25.7, and 8.1 μmol·min⁻¹·g tissue⁻¹ for extreme red, whole, and extreme white gastrocnemius, respectively) suggest that whole gastrocnemius behaves metabolically more like red muscle, despite its fiber type distribution. Thus our results are in agreement with previous studies (41) showing that fiber type composition and metabolic activity are not perfectly correlated, and they suggest that the type IId/x fibers may contain a population of highly oxidative IMF mitochondria.

Our results showed that CPT1β activity was similar between the SS and IMF mitochondria subfractions and between mitochondria from control compared with trained muscles. However, under physiological conditions, this enzyme is largely controlled by cytosolic concentrations of malonyl-CoA. We therefore sought to determine whether the CPT1β enzymes residing in these two mitochondrial subfractions might be differentially resistant to malonyl-CoA. In a previous report (29), we demonstrated that red muscle exhibited a relative malonyl-CoA insensitivity compared with white skeletal muscle. In the present study, we show that the malonyl-CoA-resistant property of red muscle is most predominant in the IMF subfraction (Fig. 2A). Moreover, in the IMF fraction, exercise training altered malonyl-CoA inhibition of palmitate oxidation in a manner that lowered the Hill coefficient. These data suggest that fatty acid oxidation in IMF mitochondria of trained muscle is less responsive to changes in malonyl-CoA content, possibly because of adaptations that impart negative cooperativity (e.g., when binding of a substrate or inhibitor decreases an enzyme’s binding affinity for subsequent molecules of its substrate or inhibitor).

In the absence of malonyl-CoA, CPT1 dependence on palmitoyl-CoA has been shown to follow a hyperbolic relationship. The addition of malonyl-CoA produces a more sigmoid function, which is thought to reflect a more finely regulated system. Our understanding of CPT1 kinetics is further complicated by evidence suggesting that malonyl-CoA can act as both a competitive (38) and an allostERIC (11) inhibitor of the enzyme. Finally, because CPT1 has binding sites for carnitine, malonyl-CoA, and acyl-CoA, altered binding of any of these molecules could have contributed to the apparent negative cooperativity observed in IMF mitochondria from trained muscle. This finding might explain how exercise training provokes a disconnect between muscle malonyl-CoA levels and β-oxidative flux (22). One intriguing possibility is that exercise-mediated alterations in malonyl-CoA inhibition kinetics might shift the regulation of β-oxidation away from cytosolic levels of malonyl-CoA and toward substrate delivery of acyl-CoA, which is enhanced after exercise training. A complete understanding of the molecular mechanisms that regulate muscle CPT1 activity and sensitivity to malonyl-CoA is still unfolding. Interestingly, a recent report showed that phosphorylation of CPT1α (the liver isoform) by a cAMP-dependent kinase alters both enzyme activity and malonyl-CoA inhibition kinetics (27). The relevance of this mechanism in skeletal muscle is yet unknown.

Our present study also shows that exercise training had no effect on the IC₅₀ value of malonyl-CoA in either mitochon-
evaluated malonyl-CoA-mediated regulation of a multistep physiological outcome (e.g., complete fatty acid oxidation to CO₂). Importantly, this methodology eliminated inadvertent measurement of CPT2, which resides on the inner mitochondrial membrane and is insensitive to malonyl-CoA (37). In previous reports (37), residual CPT activity in muscle mitochondria challenged with high concentrations of malonyl-CoA was considered to be an artifactual activity resulting from exposure of CPT2 during mitochondrial processing. However, exposed CPT2 cannot explain our results, because we evaluated malonyl-CoA-mediated inhibition of palmitate oxidation. We consider it unlikely that mitochondrial damage could have occurred in a manner that differed systematically between mitochondrial preparations and in such a way as to permit CPT1β-independent palmitoyl-CoA entry into the matrix without coincident disruption of both the β-oxidative and TCA pathways. In addition, polargraphy experiments that used succinate as a substrate showed similar respiratory coupling ratios (RCR) between SS mitochondria (RCR = 6.4) and IMF mitochondria (RCR = 6.6) (data not shown), and we demonstrated that palmitate oxidation was fully dependent upon the presence of both carnitine and CoA (Table 1). Together, these data provide strong evidence that our isolation protocols maintained an intact outer membrane and malonyl-CoA responsiveness in both SS mitochondria and IMF mitochondria.

A fundamental question that arises from this study is why mitochondria from exercise trained muscle, and/or muscles rich in type I fibers, exhibit an inherent enhanced capacity to oxidize lipid substrate. Perhaps these functional distinctions relate to properties of the mitochondrial membrane environment, potentially imparted by fiber type- or subpopulation-specific differences in membrane lipid composition and/or import of proteins such as UCP3 and CD36 (7, 23, 32). Another intriguing aspect of skeletal muscle mitochondrial biology centers on speculation that these two subfractions perform specific bioenergetic roles in providing ATP for compartmentalized energy requirements. For example, SS mitochondria might exist near the sarcolemma to provide ATP for specialized functions related to membrane function and transport, whereas IMF mitochondria might support contractile activity (34). Evidence from the current study as well as previous work indicates that the two subpopulations are differentially responsive to common stimuli, thus implying the existence of distinct, compartmentalized signaling pathways that link biogenesis of specific mitochondrial subpopulations to localized ATP demands. Notably, SS mitochondria were specifically expanded in a transgenic mouse model that is characterized by muscle fiber type conversions resembling those that occur in response to exercise training (52). In these animals, muscle-specific expression of a constitutively active form of calcium/calmodulin-dependent kinase IV results in the robust induction of peroxisome proliferator-activated receptor-γ co-activator-1α (PGC1α), a master transcriptional co-activator that promotes mitochondrial biogenesis. PGC1α levels have also been shown to be increased in more oxidative muscle fibers. Both calcium-mediated signals and PGC1α have been implicated in regulating metabolic adaptations and fiber type conversion in muscle due to increased contractile activity. It is therefore tempting to speculate that the functional changes in SS mitochondria that we observed in response to training might be specifically mediated by exercise-induced activation of the calmodulin-dependent kinase/PGC1α pathway.

The broader and perhaps more therapeutically relevant implications of this study relate to the emerging link between muscle lipid homeostasis, mitochondrial function, and whole body metabolic fitness. Human studies have shown that metabolic diseases such as obesity and diabetes are associated with a low percentage of type I (red) muscle fibers (16). Conversely, with their wild-type counterparts, mice that are genetically engineered to have a high number of type I fibers are more insulin sensitive and less susceptible to diet-induced obesity (46). In rodents (44, 47) and humans (3), both age-related and diet-induced insulin resistance have been associated with elevated or dysregulated muscle levels of malonyl-CoA. Genetic manipulations that lower muscle malonyl-CoA content increase lipid catabolism and protect against obesity (1). Together with our data, these observations suggest that the insulin-sensitizing effects of regular physical activity might be attributable, at least in part, to an increase in type I fibers and the corresponding expansion of a mitochondrial population that is less responsive to malonyl-CoA.

In addition to their principal tasks of substrate oxidation and ATP generation, mitochondria are also a primary source of reactive oxygen species (ROS) and play major roles in antioxidant defense and programmed cell death. Increased oxidative stress can lead to the production of ROS, which have been implicated in the pathogenesis and subsequent complications of aging (43). Cross-sectional studies have found that muscle lipid peroxidation (a marker of oxidative stress) is lower in endurance-trained subjects compared with either obese or insulin-resistant subjects, despite similarly high intramuscular triglyceride levels among the three groups (45). In addition, both acute (35) and chronic exercise (17) have been shown to elicit adaptations that boost antioxidant defense mechanisms, thereby conferring protection against oxidative stress and ROS-induced cellular damage. For example, exercise causes the robust induction of the muscle uncoupling proteins UCP2 and UCP3, which are thought to function in a protective manner by limiting the generation of ROS (48). Notably, one study (23) also reported that UCP3 protein content is greater in SS compared with IMF mitochondria. In the aggregate, these results support the notion that exercise-mediated changes in mitochondrial numbers and/or function might serve to protect against oxidative stress (45) and, moreover, suggest that the SS and IMF mitochondrial subpopulations may indeed play distinct roles in ROS generation and/or antioxidant defense.

In conclusion, we have demonstrated that distinct mitochondrial subpopulations display both quantitative and qualitative metabolic differences that are dependent upon muscle fiber type and training status. Our results, which support the idea that SS mitochondria and IMF mitochondria might perform distinct bioenergetic functions within the muscle, now prompt
provocative new questions with respect to the potential roles of these two subpopulations in mediating exercise-induced protec-
tion against age- and disease-related metabolic dysfunction (13, 26).

ACKNOWLEDGMENTS

The authors acknowledge the outstanding technical expertise and contri-
butions of student intern Amanda D. Carpenter to control experiments leading to
these studies.

REFERENCES

1. Abu-Elheiga L, Oh W, Kordari P, and WakiI SJ. Acetyl-CoA carbox-
  ylase 2 mutant mice are protected against obesity and diabetes induced by
  high-fat/high-carbohydrate diets. Proc Natl Acad Sci USA 100: 10207–

2. Baldwin KM, Klinkerfuss GH, Terjung RL, Mole PA, and Holloszy
  JO. Respiratory capacity of white, red, and intermediate muscle: adaptive

3. Bavenholm PN, Kuhi J, Pigon J, Saha AK, Ruderman NB, and
  Efendic S. Insulin resistance in type 2 diabetes: association with truncal
  obesity, impaired fitness, and atypical malonyl coenzyme A regulation.

4. Bezare V, Heigenhauser GJ, and Spriet LL. Regulation of CPTI
  activity in intermyofibrillar and subsarcolemmal mitochondria isolated
  from human and rat skeletal muscle. Am J Physiol Endocrinol Metab 286:

5. Bizeau ME, Willis WT, and Hazel JR. Differential responses to endur-
  ance training in subsarcolemmal and intermyofibrillar mitochondria. AJP

  Cardiac and skeletal muscle mitochondria have a monocarboxylate trans-

7. Campbell SE, Tandon NN, Woldegorgis G, Luiken JJ, Glatz JF, and
  Bonen A. A novel function for fatty acid translocase (FAT/CD36):
  involvement in long chain fatty acid transfer into the mitochondria. J Biol

8. Cogswell AM, Stevens RJ, and Hood DA. Properties of skeletal muscle
  mitochondria isolated from subsarcolemmal and intermyofibrillar regions.

9. Constable SH, Favier RJ, McLane JA, Fell RD, Chen M, and Holloszy
  JO. Energy metabolism in contracting rat skeletal muscle: adaptation to

10. Cook GA. Differences in the sensitivity of carnitine palmitoyltransferase
  I to inhibition by malonyl-CoA are due to differences in K values. J Biol

11. Cook GA, Mynnat RL, and Kashfi K. Yonetani-Theorell analysis of
  hepatic carnitine palmitoyltransferase-I inhibition indicates two distinct

12. Delp MD and Dugan C. Composition and size of type I, IIa, IIDX, and
  IIB fibers and citrate synthase activity of rat muscle. J Appl Physiol 80:

13. Emerit J, Edeas M, and Bricaire F. Neurodegenerative diseases and

14. Gollnick PD and King DW. Effect of exercise and training on mitochond-

15. Guzman M and Castro J. Effects of endurance exercise on carnitine
  palmitoyltransferase I from rat heart, skeletal muscle and liver mitochon-

  JO, O’Shea LB, Don IJ, and Mole PA. Mitochondrial citric acid
  cycle and related enzymes: adaptive response to exercise. Biochem Bio-


