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Regulation of CPT I activity in intermyofibrillar and subsarcolemmal mitochondria from human and rat skeletal muscle

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Bezaire, Veronic, George J. F. Heigenhauser, and Lawrence L. Spriet. Regulation of CPT I activity in intermyofibrillar and subsarcolemmal mitochondria from human and rat skeletal muscle. *Am J Physiol Endocrinol Metab* 286: E85–E91, 2004. First published September 3, 2003; 10.1152/ajpendo.00237.2003.—Carnitine palmitoyltransferase I (CPT I) is considered the rate-limiting enzyme in the transfer of long-chain fatty acids (LCFA) into the mitochondria and is reversibly inhibited by malonyl-CoA (M-CoA) in vitro. In rat skeletal muscle, M-CoA levels decrease during exercise, releasing the inhibition of CPT I and increasing LCFA oxidation. However, in human skeletal muscle, M-CoA levels do not change during moderate-intensity exercise despite large increases in fat oxidation, suggesting that M-CoA is not the sole regulator of increased CPT I activity during exercise. In the present study, we measured CPT I activity in intermyofibrillar (IMF) and subsarcolemmal (SS) mitochondria isolated from human vastus lateralis (VL), rat soleus (Sol), and red gastrocnemius (RG) muscles. We tested whether exercise-related levels (~65% maximal O_2 uptake) of calcium and adenylate charge metabolites (free AMP, ADP, and P_i) could override the M-CoA-induced inhibition of CPT I activity and explain the increased CPT I flux during exercise. Protein content was ~25–40% higher in IMF than in SS mitochondria in all muscles. Maximal CPT I activity was similar in IMF and SS mitochondria in all muscles (VL: 282 ± 46 vs. 280 ± 51; Sol: 390 ± 81 vs. 368 ± 82; RG: 252 ± 71 vs. 278 ± 44 nmol·min^{-1}·mg protein^{-1}). Sensitivity to M-CoA did not differ between IMF and SS mitochondria in all muscles (25–31% inhibition in VL, 52–70% in Sol and RG). Calcium and adenylate charge metabolites did not override the M-CoA-induced inhibition of CPT I activity in mitochondria isolated from VL, Sol, and RG muscles. Decreasing pH from 7.1 to 6.8 reduced CPT I activity by ~34–40% in both VL mitochondrial fractions. In summary, this study reports no differences in CPT I activity or sensitivity to M-CoA between IMF and SS mitochondria isolated from human and rat skeletal muscles. Exercise-induced increases in calcium and adenylate charge metabolites do not appear responsible for upregulating CPT I activity in human or rat skeletal muscle during moderate aerobic exercise.

carnitine palmitoyltransferase I; malonyl-coenzyme A; fat oxidation

THE CARNITINE PALMITOYLTRANSFERASE (CPT) complex consists of CPT I, acylcarnitine translocase, and CPT II. This complex facilitates the entry of long-chain fatty acid (LCFA) from the cytosol to the mitochondrial matrix, where LCFA undergo β-oxidation (16). CPT I, which spans the outer mitochondrial membrane, catalyzes the transfer of a variety of long-chain fatty acyl groups from free coenzyme A (CoASH) to carnitine. The generated acylcarnitine can then permeate the inner membrane via the acylcarnitine/carnitine translocase system. The acyl-CoA moiety is then reformed in the matrix of the mitochondria via CPT II.

CPT I is considered the rate-limiting step in the transfer of LCFA into the mitochondria and is reversibly inhibited by malonyl-CoA (M-CoA), the first committed intermediate of de novo fatty acid (FA) synthesis (17, 23). Some have speculated that M-CoA could be the key regulator of fat oxidation selection in skeletal muscle, and early work in rodent skeletal muscle supported this hypothesis (29). It has been shown that M-CoA levels are highest at rest, inhibiting CPT I activity and maintaining low rates of fat transport. During exercise, M-CoA levels decrease, releasing the inhibition of CPT I and allowing LCFA oxidation (29, 30). In human skeletal muscle, the situation is more complex, as M-CoA levels do not decrease during moderate-intensity exercise despite large increases in fatty acid oxidation rates (5, 20, 21). A possible explanation is that CPT I regulation is independent of M-CoA, as Kim et al. (11) have shown the presence of a M-CoA-resistant CPT I subfraction in rodent skeletal muscle. In any case, the biological mechanisms to explain how FA oxidation is increased in human muscle during exercise are not presently known. Taken together, these results suggest that M-CoA is not the sole regulator of CPT I activity and/or that M-CoA inhibition is overridden during exercise.

In searching for regulators of CPT I activity during exercise, we focused on calcium, the adenylate charge metabolites (free ADP, AMP and P_i), and pH. Important enzymes in the pathways that provide ATP, such as the carbohydrate-metabolizing enzymes glycogen phosphorylase and pyruvate dehydrogenase (PDH) and the tricarboxylic acid (TCA) cycle enzymes isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase, are regulated by calcium, adenylate charge metabolites, and pH. This mechanism of action leads to low activities at rest and high activities during exercise and led us to investigate the effects of calcium, the adenylate charge metabolites and pH on CPT I activity.

An additional level of complexity regarding LCFA transport and CPT I activity relates to the existence of mitochondria in two locations, intermyofibrillar (IMF) and subsarcolemmal (SS). Morphological and functional differences in IMF and SS mitochondria have been reported in rodent skeletal muscle (18). It has been suggested that the IMF and SS mitochondria have different functions related to metabolic compartmentalization (1, 19, 25). Some have also suggested that these two populations are at different stages of biogenesis (15). IMF mitochondria have been shown to be more efficient in ATP

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production, possibly to support the high ATP demand of muscle contraction and the regulation of Ca^{2+}-ATPase (4, 12, 14). SS mitochondria, which are nearer the capillaries, may also be involved in the transport of oxygen from the erythrocyte to the mitochondria (14). In rodent skeletal muscle, inner membrane and matrix enzymes of IMF mitochondria appear to have higher oxidative capacity than the SS mitochondria. (3, 4). CPT I activity has yet to be examined in IMF and SS mitochondria in human and rodent skeletal muscle. Moreover, most studies examining the characteristics of IMF and SS mitochondria were simply conducted in rat hindlimb muscle dominated by the fast glycolytic fiber type, disregarding possible fiber type differences.

Thus, in this study, we compared CPT I activity and CPT I sensitivity to M-CoA in both IMF and SS mitochondria from human skeletal muscle and rat soleus and red gastrocnemius muscles. We also investigated the effects of physiological concentrations of calcium, free AMP, ADP, and P_i, and pH in the presence of M-CoA on CPT I activity. We hypothesized that IMF mitochondria would have greater CPT I activity than SS mitochondria and that physiological concentrations of calcium and energy charge metabolites representative of 65% of peak pulmonary O_2 uptake ($\dot{V}O_{2\,max}$) would override M-CoA inhibition in all muscles.

METHODS

Subjects. Twelve healthy males volunteered for this study. Nine subjects were recreationally active, participating in moderate-intensity aerobic exercise three to five times a week, and three subjects were well trained with more frequent and longer training sessions (Table 1). Subjects were fully informed of the purpose of the experiments and of the possible risks before giving written consent to participate. The study was approved by the University of Guelph Ethics Committee.

Experimental protocol. Subjects visited the laboratory on two occasions. On the first occasion, $\dot{V}O_{2\,max}$ was measured with a metabolic cart (SensorMedics model) during incremental exercise on a cycle ergometer (Lode Instrument, Groningen, The Netherlands). On the second occasion, a resting-muscle sample was obtained from the vastus lateralis under local anesthesia (2% lidocaine without epinephrine) by use of the percutaneous needle biopsy technique described by Bergström (2). Visible fat and connective tissue were dissected free from the muscle, and the muscle was blotted to remove excess blood. The sample (~170 mg) was divided into two portions; the first (~160 mg) was used for immediate mitochondrial isolation for the determination of CPT I activity, and the second (~10 mg) was frozen in liquid N_2 for later analysis of citrate synthase (CS) activity as an index of mitochondrial volume in recreationally and well-trained subjects.

Animals and tissue extraction. Female Sprague-Dawley rats (n = 13) weighing on average 200 ± 9 g were used in the experiments. The animals were housed in a controlled environment with a 12:12-h light-dark cycle and fed Purina rat chow ad libitum. Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (6 mg/100 g body wt), and the soleus and red gastrocnemius muscles were excised as quickly as possible. This study was approved by the University of Guelph Animal Care Committee.

Isolation of mitochondria. The procedure for the isolation of IMF and SS mitochondria was adapted from Jimenez et al. (10). The entire procedure was performed at 0–4°C. The buffer solutions used contained the following: *solution 1*: 100 mM KCl, 5 mM $MgSO_4 \cdot 7H_2O$, 5 mM EDTA, and 50 mM Tris·HCl, pH 7.4; *solution II*: *solution 1* and 1 mM ATP, pH 7.4; *solution III*: 220 mM sucrose, 70 mM mannitol, 10 mM Tris·HCl, and 1 mM EDTA, pH 7.4. Muscle was immediately placed in ice-cold *solution I* and then blotted and weighed. Muscle was minced with scissors in 1 ml of *solution II* and transferred to an ice-cold glass Potter-Elvehjem homogenizer (Tri-R Stir-R model S63C; Fisher Scientific, Toronto, ON, Canada). Tissue was homogenized in 5 ml of *solution II* with a loose-fitting Teflon pestle (10 up-and-down strokes, 1,500 rpm). The homogenate was centrifuged at 700 *g* for 10 min at 4°C. The pellet was kept at 4°C for the extraction of IMF mitochondria. The supernatant was filtered through two layers of surgical gauze and then centrifuged at 12,000 *g* for 10 min at 4°C. The resulting SS mitochondrial pellet was resuspended in 1 μl/mg of tissue of *solution III*. The pellet from the initial spin was resuspended in 5 ml of *solution II* and homogenized with a tight-fitting homogenizer (1 min at 1,500 rpm + 1 min at 2,000 rpm). The strong mechanical disruption resulted in the release of the IMF mitochondria from the myofibrils. The resulting homogenate was centrifuged at 700 *g* for 10 min at 4°C. After filtration through two layers of surgical gauze, the supernatant was centrifuged at 12,000 *g* for 20 min at 4°C. The resulting IMF pellet was resuspend in 1 μl/mg of tissue of *solution III*.

The quality of the mitochondrial preparations (intact mitochondria) was determined first by measuring the CS activity of the extramitochondrial fraction in the suspension (1:20 dilution) and then by measuring the total CS activity of the suspension (1:20 dilution) after lysing the mitochondria with 0.04% Triton X-100 and repeated freeze-thawing. The difference in these activities equals the intramitochondrial fraction. CS activity was measured spectrophotometrically at 25°C as previously described (26).

Determination of CPT I activity. The forward radioisotope assay for the determination of CPT I (EC 2.3.1.21) activity from needle biopsy samples has been previously described (17, 27). Briefly, the reaction was run at a temperature of 37°C and started by the addition of 10 μl of mitochondrial suspension (1:3 dilution) to 90 μl of the following standard reaction medium: 117 mM Tris·HCl, pH 7.4, 0.28 mM reduced glutathione, 4.4 mM ATP, 4.4 mM $MgCl_2$, 16.7 mM KCl, 2.2 mM KCN, 40 mg/l rotenone, 0.5% BSA, 300 μM palmitoyl-CoA, and 5 mM L-carnitine with 1 μCi of L-[^{3}H]carnitine. The reaction was stopped after 6 min with the addition of 60 μl of ice-cold HCl. Palmitoyl-[^{3}H]carnitine formed during the reaction was extracted in 400 μl of water-saturated butanol in a process involving three washes with distilled water and subsequent recentrifugation to separate the butanol phase. Finally, radioactivity was assayed in 100 μl of the butanol phase in 5 ml of scintilation cocktail. Assays were performed in duplicate, and blanks were subtracted. IMF and SS measurements of CPT I activity were expressed relative to their mitochondrial protein contents. Protein concentration was measured using a bicinchoninic acid protein kit (Pierce, Rockford, IL).

When the effects of M-CoA and metabolites were tested in human skeletal muscle, the reaction mixture was modified to contain the following final concentrations of metabolites, representative of exercise at 65% $\dot{V}O_{2\,max}$: 100 μM $CaCl_2$, 0.65 μM AMP, 65 μM ADP, and 15 mM P_i (9). These metabolites were added individually and combined with 0.7 μM M-CoA, the physiological concentration of M-CoA in human skeletal muscle (21). An M-CoA sensitivity curve was also determined with the addition of M-CoA alone in concentrations of 0.2, 0.7, and 2.0 μM. The pH of the CPT I reaction mixture

Table 1. *Subject characteristics and human skeletal muscle enzyme activity*

Variables	Recreationally Active	Well Trained
Age, yr	19.3±1.1	22.7±2.0
Weight, kg	74.9±5.4	72.9±0.7
Height, cm	177.1±5.5	181.3±3.3
$\dot{V}O_{2max}$, ml·min^{-1}·kg^{-1}	46.1±2.2	64.0±1.2*

Values are means ± SE; n = 9 for recreationally active and n = 3 for well-trained subjects. $\dot{V}O_{2max}$, maximal O_2 uptake. *Significantly different from recreationally active, $P < 0.05$.

was adjusted to 7.1. When examining the effects of altered pH on CPT I activity, the pH of the reaction mixture was adjusted to 6.8.

In rat soleus and RG, the M-CoA concentrations used for the sensitivity curve were the same as in human muscle. Final concentrations of M-CoA in rat soleus and RG during simulated contractions were 0.43 and 0.29 μM M-CoA, respectively. Final concentrations of AMP_f, ADP_f, and P_{if} were 0.8 μM, 70 μM, and 6.2 mM in rat soleus and 2 μM, 116 μM, and 14.6 mM in RG, respectively (6, 13).

Statistics. All data are presented as means ± SE. The effect of metabolites and sensitivity to M-CoA were analyzed by a one-way ANOVA. When a significant *F*-ratio was obtained, post hoc analysis was completed using a Tukey's test. Paired *t*-tests were used to determine significance between all other treatments. Statistical significance was accepted at $P < 0.05$.

RESULTS

Human skeletal muscle. Characteristics of the recreationally active group and three well-trained individuals appear in Table 1. Protein content was ~1.6-fold higher in the IMF compared with the SS mitochondrial fractions of both the recreationally active group [1.68 ± 0.32 vs. 1.06 ± 0.21 mg/g wet muscle (WM)] and trained individuals (2.61 ± 0.44 vs. 1.65 ± 0.18 mg/g WM). CS activity was measured in all fractions to assess the quality of both mitochondrial preparations. The fraction of the CS activity inside the intact mitochondria was 85.2 ± 1.2 and 83.4 ± 1.1% in IMF and SS mitochondria, respectively.

Maximal CPT I activity normalized for mitochondrial protein content was not significantly different between IMF and SS mitochondria (282 ± 46 vs. 280 ± 51 nmol·min^{-1}·mg protein^{-1}, respectively; Table 2). CS activity was significantly lower in IMF than SS mitochondria (0.073 ± 0.004 vs. 0.132 ± 0.016 mmol·min^{-1}·mg protein^{-1}; Table 2).

Inhibition with physiological levels of M-CoA (0.7 μM) was similar between the IMF and SS fractions of human muscle (31.2 ± 4.8 and 25.3 ± 4.5% inhibition; Table 2). Inhibition of CPT I activity with a range of M-CoA concentrations was also similar between the fractions (Fig. 1).

There was no effect of adding exercising levels of calcium (100 μM), free AMP (0.65 μM), ADP (65 μM), and P_i (15 mM) in the presence of 0.7 μM M-CoA, on CPT I activity in either the IMF or SS mitochondria (Fig. 2).

Lowering the pH of the assay medium from 7.1 to 6.8 reduced CPT I activity in both the IMF and SS mitochondrial fractions of human skeletal muscle to the same level (33.8 and 40.4%, respectively; Fig. 3). The presence or absence of physiological concentration of M-CoA had no effect on the pH-induced reduction of CPT I activity.

The addition of exercising levels of ADP (65 μM) by use of a submaximal concentration of palmitoyl-CoA (150 μM) in the presence of 0.7 μM M-CoA did not alter CPT I activity compared with palmitoyl-CoA and M-CoA alone in both the IMF and SS mitochondria (data not shown).

Similar to the results with the recreationally active individuals, the addition of exercising levels of calcium and the adenylate charge metabolites had no effect on muscle CPT I activity in well-trained individuals (data not shown).

Rat soleus and RG muscles. Protein content was 25% higher in the IMF vs. SS mitochondria (2.75 ± 0.31 vs. 2.19 ± 0.40 mg/g WM) in soleus muscle and 40% greater in the IMF than SS mitochondria of RG (3.15 ± 0.35 vs. 1.91 ± 0.19 mg/g WM, $P = 0.034$). Quality of the mitochondrial preparation was good, as the fraction of CS activity that was inside the intact mitochondria was 82.0 ± 4.0 and 84.2 ± 2.5% in IMF and SS

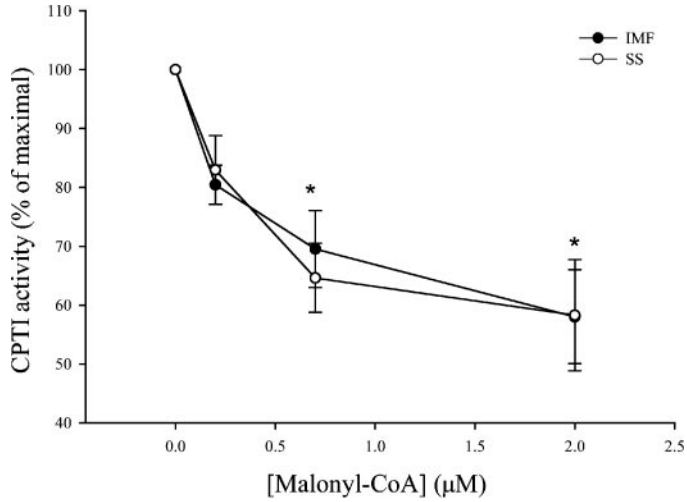

Fig. 1. Effects of malonyl-CoA (M-CoA) on carnitine palmitoyltransferase I (CPT I) activity of intermyofibrillar (IMF) and subsarcolammal (SS) mitochondria isolated from human vastus lateralis of recreationally active males. [M-CoA] = 0.2, 0.7, and 2.0 μM. Values are means ± SE; $n = 9$. *Significantly different from own control (no M-CoA).

Table 2. *Enzymatic activities and response to 0.7 μM M-CoA in IMF and SS mitochondria isolated from human vastus lateralis muscles of recreationally active subjects*

	IMF	SS
CPT I, nmol·min^{-1}·mg protein^{-1}	282±46	280±51
CS, mmol·min^{-1}·mg protein^{-1}	0.073±0.004	0.132±0.016*
Inhibition, %	31.2±4.8	25.3±4.5

Values are means ± SE; $n = 9$. M-CoA, malonyl-coenzyme A; IMF, intermyofibrillar; SS, subsarcolemmal; CPT I, carnitine palmitoyltransferase I; CS, citrate synthase. *Significantly different from IMF, $P < 0.05$.

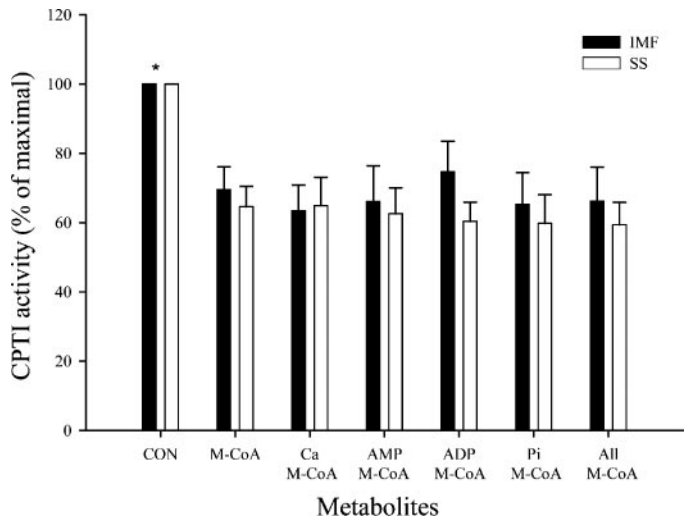

Fig. 2. CPT I activity of IMF and SS mitochondria isolated from human vastus lateralis of recreationally active males in the presence of physiological concentrations [65% maximal O_2 uptake ($\dot{V}O_{2max}$)] of calcium, free AMP, ADP, and P_i, with 0.7 μM M-CoA. Values are means ± SE; $n = 9$. *Significantly different from own M-CoA (0.7 μM).

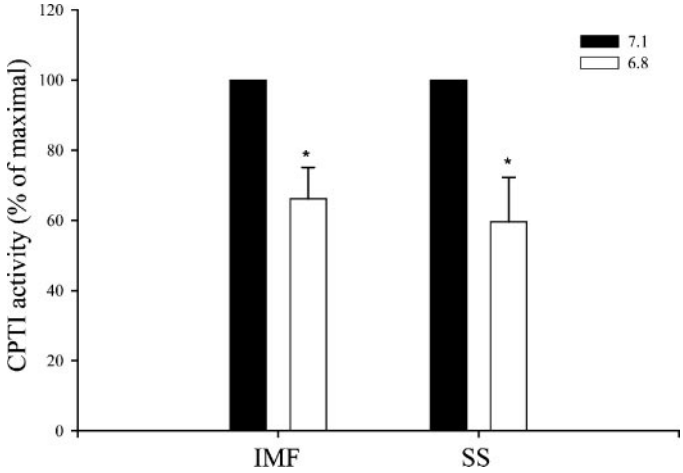

Fig. 3. Effect of pH on CPT I activity of IMF and SS mitochondria extracted from human vastus lateralis of recreationally active males. Filled bars, pH 7.1; open bars, pH 6.8. Values are means ± SE; $n = 9$. *Significantly different from pH 7.1.

mitochondria of rat soleus muscle and 83.4 ± 3.2 and 80.4 ± 5.1% in RG.

CS activity was lower in IMF than in SS mitochondria in soleus, but no significant difference was observed between the fractions in RG (Table 3). Maximal CPT I activity was similar between IMF and SS mitochondrial populations in both rat muscle groups.

In soleus and RG muscles, IMF and SS mitochondria were inhibited to the same degree with the addition of various concentrations of M-CoA (Fig. 4, *A* and *B*). In soleus, M-CoA inhibition (2 μM) resulted in a 70.0 ± 10.7 and 55.1 ± 9.3% reduction in CPT I activity in IMF and SS mitochondria. The M-CoA inhibition was similar in RG for IMF vs. SS (52.8 ± 10.3 vs. 57.9 ± 12.9%; Table 3).

No effect was observed when metabolites representative of moderate-intensity exercise were added to IMF and SS mitochondrial fractions of rat soleus and RG in the presence of 0.7 μM M-CoA (Fig. 5, *A* and *B*). The metabolites were also added to 2.0 μM M-CoA, but no effect was noted (data not shown).

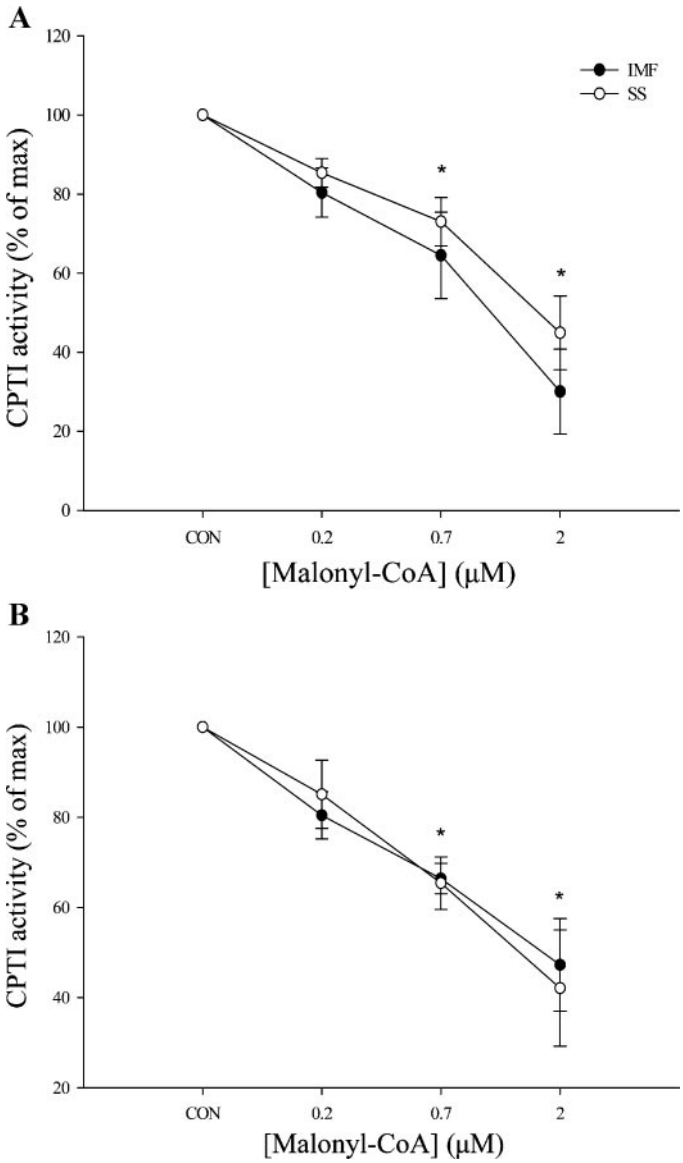

Fig. 4. Effects of M-CoA on CPT I activity of IMF and SS mitochondria isolated from rat skeletal muscle. [M-CoA] = 0.2, 0.7, and 2.0 μM. CON, CPT I activity with no M-CoA present. *A*: soleus ($n = 8$). *B*: red gastrocnemius ($n = 5$). Values are means ± SE. *Both SS and IMF mitochondria significantly different from own CON (no M-CoA).

DISCUSSION

To our knowledge, this is the first report to isolate IMF and SS mitochondria in human skeletal muscle and measure maximal CPT I activity and sensitivity to M-CoA in both fractions of human and rat skeletal muscle. Both fractions had similar CPT I activities and responses to the inhibitor M-CoA. Physiological concentrations of calcium and adenylate charge metabolites had no effect on in vitro CPT I activity in human or rat muscles in the presence of M-CoA. Decreasing pH from 7.1 to 6.8 reduced CPT I activity by ~34–40% in both mitochondrial fractions of human skeletal muscle.

Table 3. *Enzymatic activities and response to 2 μM M-CoA in IMF and SS mitochondria of rat Sol and RG muscle*

	Tissue	IMF	SS
CPT I, nmol·min^{-1}·mg protein^{-1}	Sol	390±81	368±82
	RG	252±71	278±44
CS, mmol·min^{-1}·mg protein^{-1}	Sol	0.044±0.008	0.095±0.012*
	RG	0.086±0.003	0.093±0.015
Inhibition, %	Sol	70.0±10.7	55.1±9.3
	RG	52.8±10.3	57.9±12.9

Values are means ± SE; $n = 5$. Sol, soleus; RG, red gastrocnemius. *Significantly different from IMF mitochondria, $P < 0.05$.

IMF and SS mitochondria extraction. We adapted a mitochondrial extraction protocol (10) to maximize mitochondrial IMF and SS yield when using ~160 mg of muscle obtained from human needle biopsies. Our mitochondrial protein yield with recreationally active and well-trained subjects (~2.7 and ~4.8 mg protein/g WM, SS and IMF total) was similar to the results of Wibom and Hultman (28), who also extracted mitochondria from human skeletal muscle (~2.2 for sedentary, ~2.8 for moderately active, and ~5.5 mg/g WM for trained subjects). However, the mitochondrial protein yield from another human study was higher at ~8–13 mg/kg WM (22). This

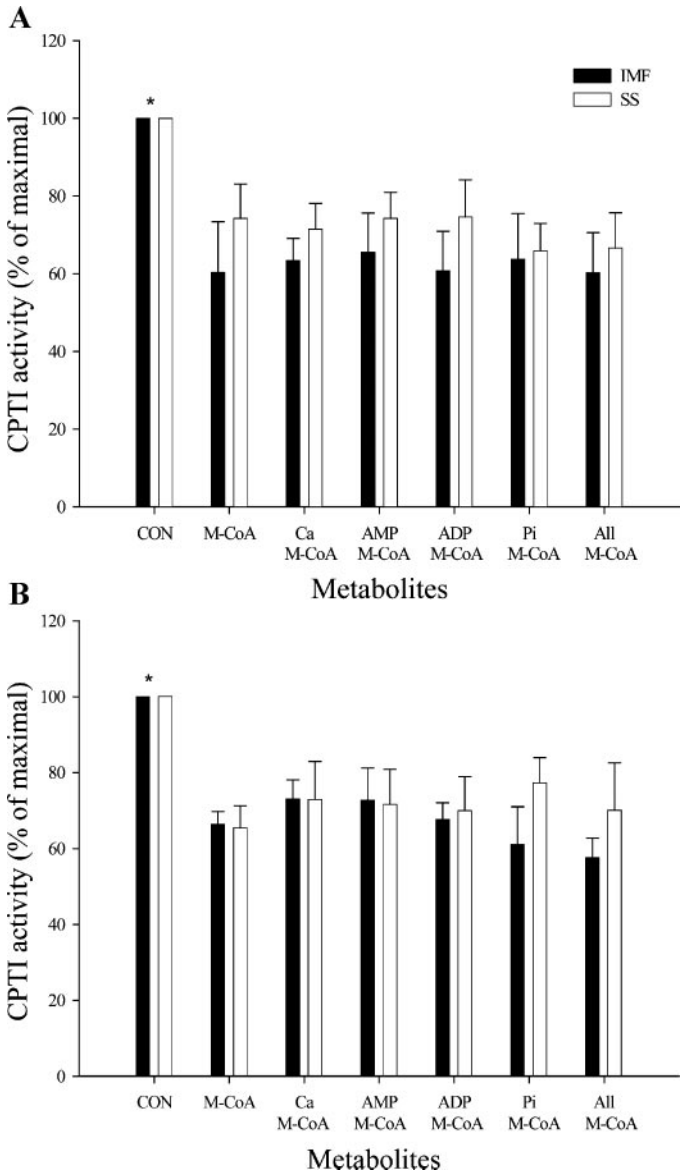

Fig. 5. CPT I activity of IMF and SS mitochondria isolated from rat skeletal muscle in the presence of physiological concentrations (65% $\dot{V}O_{2max}$) of calcium, free AMP, ADP, and P_i with M-CoA. *A*: soleus: [M-CoA] = 0.43 μM (n = 5). *B*: red gastrocnemius: [M-CoA] = 0.23 μM. (n = 5). Values are means ± SE. *All conditions significantly different from own CON (no M-CoA) for both SS and IMF mitochondria.

may have been due to the proteinase treatment used to isolate the mitochondria, which was not used in our study or by Wibom and Hultman.

Our protein yield in rat muscles was similar to what was previously reported for rat muscle. The quality of the preparations was good, as ~82–85% of the mitochondria extracted from all muscles were intact.

CPT I activity in human muscle IMF and SS mitochondria. Our results revealed no differences in CPT I activity or M-CoA sensitivity between IMF and SS fractions of human skeletal muscle. We measured a 25–31% inhibition of CPT I activity by using physiological concentrations of M-CoA. These data are consistent with previous findings from our laboratory and suggest that only ~30% of CPT I could be regulated by M-CoA under normal conditions (27). Similar in vitro CPT I activities and sensitivity to M-CoA in the IMF and SS mitochondria suggest that CPT I activity between the two fractions would be similar in vivo unless M-CoA levels differed in IMF and SS populations. However, there is no indication to date of such differences.

Calcium and adenylate charge metabolites are powerful activators of several enzymes in the pathways that metabolize carbohydrate, including the TCA cycle. Calcium is released upon muscle contraction, and increases in free AMP, ADP, and P_i occur at the onset of exercise and during prolonged moderate-intensity exercise. We tested the possibility that these metabolites could override M-CoA inhibition of CPT I or directly increase CPT I activity. The results demonstrated that this was not the case in human skeletal muscle, as CPT I activity remained at ~70% of maximal in the presence of M-CoA with and without these potential regulators. The results were similar in muscles from well-trained subjects despite trained muscles having tighter metabolic control and greater sensitivity to small changes in cell energy status (7, 8). Our in vitro conditions resulted in maximal CPT I activity. In an attempt to better simulate in vivo conditions, we tested the effect of free ADP and M-CoA by using submaximal concentrations of palmitoyl-CoA but again without stimulatory effects on CPT I activity.

We previously determined that exercising levels of acetyl-CoA, CoASH, and acetylcarnitine had no effect on CPT I activity (27). However, Starritt et al. (27) reported a 40% inhibition of CPT I activity when pH was reduced from 7 to 6.8 in a mixture of IMF and SS mitochondria. We extended this finding by demonstrating that the IMF and SS fractions responded in a similar fashion to a change in pH. A change in pH could be the link between increasing carbohydrate and decreasing fat oxidation when exercise intensity increases from moderate to high. Muscle pH values have been estimated at ~6.9 and ~6.6 during cycle exercise at 65 and 90% $\dot{V}O_{2\,max}$, respectively (24).

In the course of assessing the quality of the mitochondrial extractions, we also determined that CS activity was significantly lower in IMF than in SS mitochondria. CS is a key TCA cycle enzyme, which serves both the carbohydrate and fat metabolic pathways. Because CPT I activity did not differ between the mitochondrial fractions, it is possible that the higher CS activity in the SS fraction is needed for higher carbohydrate flux through PDH and the TCA cycle. However, to our knowledge, these data are unavailable, and future measurements of maximal activities and regulation of key regulatory enzymes in the two populations of mitochondria are needed to answer these questions.

CPT I activity in rat muscle IMF and SS mitochondria. CPT I activity was similar between the two fractions in rat soleus and RG muscles but was higher in the soleus due to the greater proportion of oxidative fibers. The similar CPT I activities are puzzling, as IMF mitochondria have higher inner-membrane oxidative capacity, higher states III and IV respiration, and are more coupled (10, 14). Thus they are more efficient at generating ATP, possibly to support muscle contraction. However, it is possible that CPT I differences on the outer mitochondrial membrane are not necessary since the IMF protein content is significantly greater than SS protein. This may imply that the

differential regulation of oxidative metabolism lies distal to the transport of LCFA into the mitochondria.

Although few outer-membrane enzymes have been measured in IMF and SS mitochondria, several inner-membrane enzyme activities have been examined with ambiguous results. Krieger et al. (12) reported a 65% greater succinate dehydrogenase (SDH) activity in IMF vs. SS mitochondria, whereas Cogswell et al. (4) found a 40% greater SDH activity in SS mitochondria. Cogswell et al. also reported slightly higher cytochrome oxidase (COX) activity (13%) in IMF mitochondria of mixed rat hindlimb muscle, whereas Jimenez et al. (10) reported higher COX activity in SS mitochondria of RG (43%) and no difference in the soleus and tibialis anterior muscles. Cogswell et al. also found no difference in cytochrome *b*, c_1, *c*, and *a* protein content of IMF and SS mitochondria. The matrix enzyme isocitrate dehydrogenase activity was 70% higher in IMF than in SS mitochondria, whereas CS activity did not differ (3, 4). We also found similar CS activities between SS and IMF fractions in RG muscle. However, it should be noted that both mixed rat hindlimb muscle and RG contain ~33% slow and fast oxidative fibers and ~67% fast glycolytic fibers. We did observe higher CS activity in SS mitochondria of rat soleus muscle, which may be related to the presence of more oxidative fibers. The lack of consistency in these findings makes it difficult to draw firm conclusions regarding the potential for oxidative metabolism in the two mitochondrial fractions. Specific measurements from the mitochondrial fractions of the predominant fiber types in rodent skeletal muscle are needed.

Regulation of CPT I activity. M-CoA is thought to be the main regulator of CPT I activity in rodent skeletal muscle, since a decrease in M-CoA correlates with increased fat oxidation during contractions. Because no difference was observed in M-CoA sensitivity between the IMF and SS fractions, we combined the results for the purpose of this discussion. CPT I activity was inhibited by ~53–58% in soleus and ~55–70% in RG muscles when only 2 μM M-CoA was used. Kim et al. (11) recently suggested the presence of an M-CoA-insensitive CPT I isoform that correlated with the proportion of type IIA fibers in rat skeletal muscle. Using 10 μM malonyl-CoA, they reported only 39 and 29% inhibition in soleus and RG muscles. The two data sets differ, as we reported a broader range and more powerful inhibition of CPT I activity in the RG vs. soleus, which argues against the type IIA-to-M-CoA insensitivity correlation (11). We also report a 1.4- to 2-fold greater inhibition in CPT I activity in both muscles when using only 20% of the M-CoA concentration used by Kim et al. The magnitude of the inhibition may depend on the different protocols, because we isolated mitochondria and measured CPT I activity directly, whereas Kim et al. used whole muscle homogenates and measured palmitate oxidation to estimate CPT I activity. It may be that M-CoA does not interact fully with CPT I in a whole muscle homogenate as it may in isolated mitochondria. A similar theory may explain the lack of response to M-CoA in vivo, as it may be bound to a protein or be compartmentalized, making it less accessible to CPT I (16). Future experiments using intact soleus strips and isolated mitochondria may answer these questions.

In summary, this study is the first to measure CPT I activity in IMF and SS mitochondria extracted from needle biopsy samples of human skeletal muscle. CPT I activity and inhibition by M-CoA were similar between fractions in human muscle and also in rat soleus and RG IMF and SS fractions. Physiological concentrations of calcium and adenylate charge metabolites had no effect on CPT I activity in the presence of M-CoA in all muscles. CS activity was lower in IMF compared with SS mitochondria of human and rat soleus muscle but not in RG muscle. The present findings suggest a similarity in the maximal ability to transport LCFA into the mitochondrial fractions in human skeletal muscle. Decreasing pH from 7.1 to 6.8 reduced CPT I activity by ~34–40% in both mitochondrial fractions of human skeletal muscle. Additional measurements of IMF and SS oxidative enzymes and their regulation are needed to understand their roles in the oxidative production of energy in skeletal muscle.

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