Evidence of a malonyl-CoA-insensitive carnitine palmitoyltransferase I activity in red skeletal muscle

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Because long-chain acyl-CoAs cannot diffuse freely across membranes, the mitochondrial carnitine shuttle system has an obligatory role in β-oxidation by permitting acyl-CoA translocation from the cytosol into the mitochondria in all mammalian cells (10). Carnitine palmitoyltransferase I (CPT I), which spans the outer mitochondrial membrane, catalyzes the initial step in this process by transferring acyl groups from CoA to carnitine. The acylcarnitines formed by CPT I traverse the inner membrane via a specific translocase that is coupled to carnitine palmitoyltransferase II (CPT II), which regenerates acyl-CoA upon transporting the fatty acyl groups into the mitochondrial matrix. Because CPT I represents the pace-setting reaction in the carnitine shuttle system, it is widely considered the most critical step in controlling fatty acid flux through the β-oxidative pathway (13).

CPT I is expressed as at least two isoforms, which are the products of different genes: a liver enzyme, CPT Iα (~88 kDa), and its smaller counterpart in cardiac and skeletal muscle, CPT Iβ (~82 kDa), each having distinctly kinetic properties (13). A distinguishing regulatory property of these isoenzymes is that both are inhibited by malonyl-CoA (15), which is produced in the cytosol by acetyl-CoA carboxylase (ACC) (26). In both liver and muscle, physiological alterations in malonyl-CoA concentrations correlate inversely with changes in β-oxidation. For example, starvation (15) and exercise (28) decrease tissue levels of malonyl-CoA, which presumably relieves inhibition of CPT I and increases fatty acyl-CoA entry into mitochondria (3). Conversely, carbohydrate feedings stimulate ACC activity and increase production of malonyl-CoA, which corresponds with a decrease in fatty acid oxidation and an increase in long-chain acyl-CoA accumulation (3). In some reports, physiological regulation of malonyl-CoA concentration appears to differ between red and white muscle (3, 29), suggesting that malonyl-CoA-mediated control of fatty acid metabolism might depend on muscle fiber type. However, the role of fiber type in modulating the malonyl-CoA/CPT I system has...
performed using muscles composed of dissimilar
function of muscle fatty acid oxidation might depend on the
fraction, and hypothesized that malonyl-CoA inhibi-
muscle might contain a malonyl-CoA-insensitive CPT I
ration (7, 15). We considered an alternative view, that
chondrial membrane enzyme that can be expressed if
nonsuppressible fraction to CPT II, the inner mito-
15, 23). Historically, investigators have attributed this
muscle mitochondria is uninhibited by malonyl-CoA (7,
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chondrial enigma.
Importantly, several investigators have reported
that a significant portion of CPT activity measured in
muscle mitochondria is uninhibited by malonyl-CoA (7,
15, 23). Historically, investigators have attributed this
nonsuppressible fraction to CPT II, the inner mito-
METHODS
Materials. The [3H]carnitine and the chemiluminescence
detection kit were from Amersham (Piscataway, NJ).
the calnexin antibody was from Santa Cruz (Santa Cruz, CA),
and the polyvinylidene difluoride (PVDF) membrane was
from Bio-Rad (Hercules, CA). All other reagents were ob-
horizontal
Animals. Male Sprague-Dawley rats from Harlan (Indi-
animals, IN) were fed a chow diet and water ad libitum. Rats
weighing ~300 g were used for all experiments.
Muscle homogenization. Rats were anesthetized by an
intra-peritoneal injection of ketamine-xylazine (90/10 mg/kg),
and skeletal muscles were removed. Muscle homogenates
and isolated mitochondria were prepared using white (WG)
and red (RG) gastrocnemius muscles and from soleus (SOL),
etrusor digitorum longus (EDL) and epitrochlearis (EPI)
muscles. Approximately 50–70 mg of tissue were minced
thoroughly with scissors in 300 μl of a modified sucrose–
EDTA medium (SET) containing 250 mM sucrose, 1 mM
EDTA, and 10 mM Tris·HCl, pH 7.4 (21). SET buffer was
added to a 20-fold diluted (wt/vol) suspension, and samples
were homogenized in a 3.0-ml Potter-Elvehjem glass homog-
izer at 10 passes across 30 s at 1,200 rpm with a Teflon
pestle. Protein concentrations in muscle homogenates ranged
from 0.9 to 2.3 mg/ml.
Isolation of mitochondria and microsomes. Muscles were
excised and immediately placed in ice-cold modified Chapell-
Perry buffer (in mM: 100 KCl, 40 Tris·HCl, 10 Tris base, 5
MgSO4, 1 EDTA, and 1 ATP, pH 7.5) and separated into red,
white, or mixed gastrocnemius; only RG and WG were used
for these experiments. Muscles were blotted, weighed, and
placed into 2.0 ml (RG) or 4 ml (WG) of Chapell-Perry buffer.
Samples were minced thoroughly on ice, diluted 10-fold (wt/
vol) with Chapell-Perry buffer, and then homogenized twice
for 15 s with an Ultra-turrax at ~9,500 rpm. Tissue homog-
enates were centrifuged at 650 g for 10 min at 4°C. The
supernatant was gravity filtered through four layers of sur-
gical gauze and centrifuged at 8,500 g for 10 min at 4°C.
Microsomes were isolated from the supernatant by ultracen-
trifugation at 100,000 g for 1 h at 4°C. The mitochondrial
pellet from the 8,500-g spin was washed to remove erythro-
cytes, resuspended in 1.3 ml of Chapell-Perry buffer, and
centrifuged at 8,500 g for 10 min. Both the microsomal and
mitochondrial pellets were suspended in 1.0 ml of SET buffer
and used immediately for assaying CPT I specific activity
and palmitate oxidation. Proteins were determined by the bicin-
chonic acid method. For Western blots, 15 μg of protein
from mitochondrial and microsomal subfractions were sepa-
ared by 8–12% gradient SDS-PAGE, transferred onto PVDF
membranes, and probed for 2 h with calnexin antibody per
the manufacturer’s protocol. Proteins were visualized by
horseradish peroxidase-conjugated goat anti-rabbit immuno-
globulin G with a chemiluminescence Western blotting de-
tection kit.
Fatty acid oxidation. Palmitate oxidation rates were deter-
mined by measuring production of [1-14C]-labeled acid-soluble
metabolites (ASM), a measure of tricarboxylic acid (TCA)
cycle intermediates and acetyl esters (incomplete oxidation)
(27), and [14CO2] (complete oxidation), by use of a modified
48-well microtiter plate (Costar, Cambridge, MA), as previ-
ously described (11). Reactions were initiated by adding 40 μl
of whole homogenates or isolated mitochondria to 160 μl of
the incubation buffer (pH 7.4), yielding final concentrations
(in mM) of 0.2 palmitate ([1-14C]palmitate at 0.5 μCi/ml), 100
sucrose, 10 Tris·HCl, 5 potassium phosphate, 80 potassium
chloride, 1 magnesium chloride, 2 i-carnitine, 0.1 malate, 2
ATP, 0.05 CoA, 1 dithiothreitol, 0.2 EDTA, and 0.5% bovine
serum albumin. After incubation for 60 min at 30°C, reac-
tions were terminated by adding 100 μl of 4 N sulfuric acid,
and the CO2 produced during the incubation was trapped in
0.1 N sodium hydroxide that had been added to adjacent wells (11). The acidified medium was stored at 4°C
overnight, and then ASM were assayed in supernatants of
the acid precipitate (27). Radioactivity of CO2 and ASM was
determined by liquid scintillation counting by use of 4 ml of
Uniscint BD (National Diagnostics).
CPT I assay. CPT I activity was measured using 10–20 μg of
protein from whole homogenates. The assays were carried out
with 50 μM palmitoyl-CoA and 0.2 mM [3H]carnitine (0.5 μCi)
as previously described (11). After 10 min at 30°C, the
assay was terminated by adding 60 μl of 1.2 M ice-cold HCl.
The [3H]palmitoyl-carnitine formed was extracted with
water-saturated butanol and quantified by liquid scintillation
counting.
Ribonuclease protection assay analysis. RNA was isolated from skeletal muscle using TRIZol (GIBCO-BRL) reagent as
previously described (9). Ribonuclease protection assays
(RPA) were performed as described (31) with a complemen-
tary RNA probe generated by T7 polymerase from a linear-
ized template consisting of the rat CPT Iα2 cDNA fragment
extending from NeoI at nucleotide 238 (relative to the initia-
tion AUG) to EcoRI at nucleotide 423 subcloned into pBlue-
script.
Statistics. Statistical analyses were performed using JMP
Statistical Software (SAS, Cary, NC). The correlation be-
between malonyl-CoA-resistant activity and total oxidation

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rates was evaluated with a bivariate linear regression analysis, and significance was determined by ANOVA. Two- and three-way ANOVA was performed using a standard least squares model to test both the main and interaction effects of muscle type × incubation time × malonyl-CoA concentration (where appropriate) on palmitate oxidation, CPT I activity, or percent inhibition. In experiments consisting of a single time point and/or malonyl-CoA concentration, differences between red and white muscle were performed using a one-way ANOVA.

RESULTS

Malonyl-CoA inhibition of palmitate oxidation in red and white muscle. To address the question of whether malonyl-CoA inhibits fatty acid oxidation equally in muscles of varied fiber compositions, we first examined the effects of a high but still physiological malonyl-CoA concentration (10 μM) on [14C]palmitate oxidation in homogenates of red and white muscles. Predictably, palmitate oxidation (ASM plus CO2) was greater in homogenates of red muscle (RG and SOL) than in homogenates of white muscle (EDL and WG) (Fig. 1A). Addition of 10 μM malonyl-CoA, which exceeds the IC50 of recombinant CPT I by ~100-fold, inhibited palmitate oxidation by 29, 39, 60, and 89% in RG, SOL, EDL, and WG, respectively (Fig. 1B). Relative inhibition by malonyl-CoA was similar between measures of complete oxidation (CO2) and total oxidation (CO2 plus ASM). Linear regression analyses indicated that the degree of malonyl-CoA resistance correlated positively ($r^2 = 0.678, P < 0.005$) with absolute rates of palmitate oxidation (Fig. 1C); thus highly oxidative red muscles were more resistant to malonyl-CoA than highly glycolytic white muscles. Adding protease inhibitors to the homogenization buffer did not eliminate the differences between red and white muscles (data not shown), suggesting that fiber type-specific malonyl-CoA resistance was not due to in vitro protease modification of CPT I that may have occurred during homogenate preparation and/or incubation. Because differences in malonyl-CoA resistance were most marked between RG and WG, these muscles were chosen for subsequent experiments.

Figure 2 shows the results of separate experiments that were conducted to determine whether malonyl-CoA inhibition of palmitate oxidation remains linear during the course of a 1-h incubation. In this experiment, 10 μM of malonyl-CoA inhibited palmitate oxidation by 67 and 92% ($P < 0.001$) in homogenates of RG and WG, respectively, and the relative inhibition was similar ($P = 0.71$) at 15-, 30-, 45-, and 60-min time points.
Malonyl-CoA sensitivity and palmitate oxidation in isolated mitochondria. Previous reports have suggested that the nonsuppressible component of CPT I activity might be related to mitochondrial damage. To address this possibility, we tested the integrity of mitochondria that were isolated from our homogenate preparations. Similar to the results in whole homogenates (Fig. 1), we found that oxidation rates were greater and the inhibitory effect of malonyl-CoA was less ($P < 0.001$) in mitochondria from RG compared with WG (Fig. 5). Importantly, in the isolated mitochondrial preparations, palmitate oxidation to CO$_2$ fully required the presence of both CoA and carnitine, respective substrates for the outer mitochondrial enzymes, ACS and CPT I, which catalyze the first two reactions of palmitate oxidation. These data support results obtained from whole homogenates (Table 1) and confirm that mitochondrial membranes were intact.

Additionally, others have suggested that contamination of mitochondria with sarcoplasmic reticulum (SR) might contribute to changes in malonyl-CoA sensitivity (17). We evaluated this possibility by assessing the presence of the SR-specific marker protein calnexin in mitochondrial compared with microsomal subfractions. Western blot analyses indicated that SR contamination inhibited only 32–51% in RG and 52–72% in WG ($P < 0.001$; Fig. 3B). When the malonyl-CoA-resistant fraction was subtracted from the total activity, as previously described (15), the IC$_{50}$ values appeared similar between red and white muscle (Fig. 3C), suggesting differences in efficacy but not in the potency of malonyl-CoA inhibition. Figure 4 shows that CPT I activity, measured in whole homogenates, was 1.7-fold greater ($P < 0.01$) in SOL (red) than in EPI (white) muscle. In EPI, 10 μM malonyl-CoA inhibited CPT I activity by 62% compared with only 34% in SOL ($P < 0.01$). These data recapitulate the results from oxidation experiments and demonstrate that fiber type-selective malonyl-CoA inhibition of CPT I activity is consistent with inhibition of palmitate oxidation, lending further support to our hypothesis that malonyl-CoA inhibition of muscle CPT I depends on fiber composition.

### Table 1. Peroxisomal and carnitine-dependent palmitate oxidation in muscle homogenates

<table>
<thead>
<tr>
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<th>Red gastrocnemius</th>
<th>White gastrocnemius</th>
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<tr>
<td></td>
<td>Palmitate Oxidation, nmol·g$^{-1}$·h$^{-1}$</td>
<td>Palmitate Oxidation, nmol·g$^{-1}$·h$^{-1}$</td>
</tr>
<tr>
<td></td>
<td>ASM</td>
<td>CO$_2$</td>
</tr>
<tr>
<td>Control</td>
<td>2,615 ± 270</td>
<td>969 ± 79</td>
</tr>
<tr>
<td>ETS inhibitors</td>
<td>686 ± 43</td>
<td>ND</td>
</tr>
<tr>
<td>No carnitine</td>
<td>261 ± 31</td>
<td>ND</td>
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Whole muscle homogenates were incubated 1 h at 29°C in a medium containing 200 μM [$^{14}$C]palmitic acid (0.1 μCi/well) in the presence or absence of mitochondrial electron transport system (ETS) inhibitors (2.2 mM KCN and 40 mg/l rotenone) and 1 mM L-carnitine. Production of [$^{14}$C]-labeled CO$_2$ and acid-soluble metabolites (ASM) was assayed as described in METHODS. Data are means ± SE from 3 animals assayed in quadruplicate. ND, [$^{14}$C]-CO$_2$ production over background could not be detected.

Peroxisomal oxidation in red and white muscle. Others have shown that both mitochondria and peroxisomes contribute to total palmitate oxidation in muscle homogenate systems (18). To quantify the contribution of peroxisomes to palmitate oxidation in red and white muscle, we blocked mitochondrial respiration by incubating muscle homogenates in the presence of the electron transport chain inhibitors KCN and rotenone.

In both RG and WG, peroxisomes accounted for ~20% of total palmitate oxidation (Table 1), similar to previous reports (18). These results suggest that fiber type-dependent differences in malonyl-CoA resistance are unrelated to differences in peroxisomal oxidation. Table 1 also shows that palmitate oxidation to CO$_2$ depended fully on the presence of carnitine, indicating that mitochondrial membrane integrity was not compromised during homogenate preparations. Similarly, subtracting carnitine from the buffer diminished production of ASM by 90%; however, since a small portion of the [$^{14}$C]-labeled acid-soluble products appeared to be carnitine independent, oxidation results from subsequent experiments are presented as CO$_2$ only.

Malonyl-CoA sensitivity in homogenates of RG and WG. To better evaluate fiber type-dependent differences in malonyl-CoA sensitivity, palmitate oxidation to CO$_2$ was studied in the presence of increasing malonyl-CoA in whole homogenates of RG and WG (Fig. 3A). In the presence of 1 and 5 μM malonyl-CoA, concentrations that fall within the range previously reported in rat muscle (3), palmitate oxidation was inhibited only 32–51% in RG and 52–72% in WG ($P < 0.001$; Fig. 3B). When the malonyl-CoA-resistant fraction was subtracted from the total activity, as previously described (15), the IC$_{50}$ values appeared similar between red and white muscle (Fig. 3C), suggesting differences in efficacy but not in the potency of malonyl-CoA inhibition. Figure 4 shows that CPT I activity, measured in whole homogenates, was 1.7-fold greater ($P < 0.01$) in SOL (red) than in EPI (white) muscle. In EPI, 10 μM malonyl-CoA inhibited CPT I activity by 62% compared with only 34% in SOL ($P < 0.01$). These data recapitulate the results from oxidation experiments and demonstrate that fiber type-selective malonyl-CoA inhibition of CPT I activity is consistent with inhibition of palmitate oxidation, lending further support to our hypothesis that malonyl-CoA inhibition of muscle CPT I depends on fiber composition.

![Graph showing time course of malonyl-CoA resistance in red and white muscle.](image-url)
tion was similarly low in mitochondria from red and white muscles (Fig. 6A). Taken together, the data shown in Figs. 5 and 6 provide evidence that mitochondrial CPT I contributes to malonyl-CoA resistance in red muscle and that this fiber type-dependent property is unlikely to be an artifact due to SR contamination. Interestingly, we found that the CPT I specific activity in microsomes was similar to that in mitochondria (Fig. 6B). Palmitate oxidation to CO₂ was undetectable
in our microsomal preparations, indicating negligible levels of mitochondrial contamination (data not shown). The high CPT I activities in muscle microsomes, which is consistent with previous observations in liver microsomes, indicate that both mitochondrial and SR CPT I could have contributed to malonyl-CoA resistance in our homogenate system. How acylcarnitines that are synthesized in extramitochondrial organelles might be subsequently shuttled to the mitochondria for oxidation remains uncertain.

Expression of CPT I splice variants in red and white muscle. Next, we investigated the possibility that our observations might be related to fiber type-selective mRNA abundance of a recently identified CPT I splice variant that is preferentially expressed in rat heart and skeletal muscle (30, 31). Relative expression of alternatively spliced mRNAs in different muscles was quantified by RPA (Fig. 7). Expression of the predominant splice variant CPT Iβ1 was approximately three-fold greater in red muscles (SOL and RG) than in white muscles (EDL and WG) (Fig. 7B). Likewise, expression of the alternatively spliced mRNA species CPT Iβ2 was also more abundant in red muscles (Fig. 7C). Thus CPT I gene expression across fiber types is consistent with the higher enzyme activity and fatty acid oxidative capacity measured in red compared with white muscles. However, because the ratio of CPT Iβ1 to CPT Iβ2 abundance was similar between red and white muscles, mRNA expression of CPT Iβ2 appeared to be unrelated to fiber type-selective differences in malonyl-CoA resistance. In separate experiments, we used cRNA probe for CPT Iα and found that abundance of this isoform was negligible in skeletal muscle (data not shown), as previously reported (7).

DISCUSSION

Malonyl-CoA is presumed to be a key regulator of muscle fatty acid oxidation by virtue of its potent inhibition of CPT I and because muscle malonyl-CoA content changes reciprocally with β-oxidation (3). Unexplained, however, is how fatty acid oxidation proceeds despite muscle concentrations of malonyl-CoA that should completely inhibit CPT Iβ. This enigma
might be at least partly reconciled by our data, which provide evidence of a malonyl-CoA-insensitive CPT I activity in red skeletal muscle. Similar to previous reports, we found that, in homogenates of WG, physiological concentrations of malonyl-CoA inhibited palmitate oxidation by 75–90%. Conversely, in RG, these same concentrations inhibited palmitate oxidation by only 35–54%. Furthermore, 100 μM malonyl-CoA, which exceeds the reported IC50 by several orders of magnitude, inhibited palmitate oxidation by only 62%, suggesting that CPT I in red muscle is resistant to full inhibition by malonyl-CoA. Additionally, a comparison across muscle types showed that malonyl-CoA resistance correlated positively with the muscle’s fatty acid oxidative capacity. These results provide the first reported evidence of fiber type-specific differences in malonyl-CoA-mediated regulation of muscle lipid oxidation.

Although the present investigation did not evaluate fiber type by parameters other than fatty acid oxidative capacity, a recent study of rat hindlimb muscles provided detailed analyses of fiber composition based on the protein expression profile of four distinct myosin heavy-chain isoforms (22). Investigators found that RG, SOL, EDL, and WG consisted of 24, 84, 5, and 0.8% type I fibers, respectively. Type II fibers were further classified into IIA, IIB, IIC, IID, IIAD, and IIDB subtypes. We combined their data on fiber type with our results presented in Fig. 1 to evaluate the relationship between histochemical fiber type and malonyl-CoA resistance. The only significant correlation detected by linear regression ($r^2 = 0.63, P < 0.01$) was between malonyl-CoA resistance and the proportion of type IIA fibers, which was 18, 9, 15, and <1% in RG, SOL, EDL, and WG, respectively (22). Surprisingly, these correlative findings across studies suggest that expression of the malonyl-CoA-resistant CPT I subfraction might be more closely linked to type IIA than type I fibers, although we acknowledge that this result should be interpreted cautiously because of the small number of animals that was used for the analysis. Still, in experiments using larger populations (data not shown), both fatty acid oxidative capacity and malonyl-CoA resistance were consistently greater in RG than in SOL (other muscle types were not evaluated), again implying that these metabolic properties are unrelated to the proportion of type I fibers. This finding is reflective of the poor association that is often reported between histochemical fiber type, delineated by expression of specific myosin isoforms, and the metabolic fiber type of a muscle.

Consistent with our findings, previous studies, including those that first described the kinetic properties of muscle CPT I (15), have also reported residual CPT activity in muscle mitochondria exposed to high concentrations of malonyl-CoA. Investigators have attributed this activity to inner mitochondrial membrane CPT II, which can be exposed during mitochondrial isolation (7). However, exposure of CPT II cannot explain our data because, unlike previous investigations, the present study evaluated malonyl-CoA inhibition of palmitate oxidation. We consider it unlikely that mitochondrial damage occurred in a manner that differed systematically between red and white muscle, and in such a way as to permit CPT I-independent acyl-CoA entry into the matrix without accompanying disruption of the β-oxidative and TCA pathways. Thus our observation that palmitate oxidation to CO2 was retained indicates that mitochondria were physiologically intact. Additionally, demonstration that palmitate oxidation was fully dependent on the presence of both carnitine and CoA further confirms the integrity of mitochondrial membranes.

Importantly, the present investigation evaluated the inhibitory effects of malonyl-CoA in whole muscle homogenates. This represents another key distinction between our data and previous studies, because skeletal muscle possesses two mitochondrial subpopulations, intermyofibrillar and subsarcolemmal, which exhibit distinct biochemical properties and respond differently to physiological stimuli (12). Thus, in contrast to previous studies, our experiments using whole homogenates eliminated potential problems associated with poor mitochondrial yield and disproportionate recovery of mitochondrial subpopulations, either of which might result in mischaracterization of muscle mitochondrial enzymes. In a homogenate system, both mitochondria and peroxisomes contribute to total oxidative capacity (18). We found that peroxisomes contributed equally (~19%) to total oxidation in red compared with white muscle, suggesting that differences in peroxisomal oxidation cannot explain fiber type-selective malonyl-CoA resistance.

Taken together, our findings provide strong evidence that red muscle expresses a malonyl-CoA-resistant CPT I subfraction. CPT Iβ has not been isolated in a catalytically active form that would allow kinetic characterization of the purified enzyme; however, the full-length cDNA has been expressed in yeast (20) and mammalian COS cells (25). The recombinant enzyme is completely inhibited by 1.0–10 μM malonyl-CoA (20, 25), which appears to contradict our finding that, in RG, 10–100 μM malonyl-CoA inhibited palmitate oxidation by only 35–62%. This discrepancy suggests that red muscle might express a novel CPT I isoform that confers a modified malonyl-CoA-regulatory site, a possibility that is supported by evidence indicating that both rat (31) and human (30) muscle expresses multiple mRNA splice variants of the CPT Iβ transcript. The deduced polypeptide sequence of CPT Iβ2, a novel mRNA species that is expressed in rat muscle (31), predicts an isoform of the enzyme that omits putative malonyl-CoA- regulatory regions. In the present investigation, we found that both the CPT Iβ1 and CPT Iβ2 transcripts were about three times more abundant in red than in white muscles. To our knowledge, these data are the first to show that differences in CPT I gene expression across muscle fiber types are consistent with similar differences observed in enzyme activity and fatty acid oxidation rates. However, relative expression of the two CPT I mRNA species was similar in red and white muscles and thus appeared to be unre-

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related to fiber type-specific differences in malonyl-CoA sensitivity. These results do not exclude the possibility that protein expression of β2 relative to β1 might differ among muscle fiber types, although direct demonstration that the CPT Iβ2 transcript is in fact translated into a distinct isoenzyme is still lacking. Protein expression and characterization of the catalytic and regulatory properties of novel CPT1 splice variants should provide further insight into their potential role in conferring malonyl-CoA insensitivity.

The emerging model of CPT I topology predicts that the catalytic site resides in the large carboxyl-terminal domain facing the cytosol and that the smaller cytosolic amino-terminal domain is crucial for maintaining a confirmation that permits optimal malonyl-CoA binding and inhibition (10). When CPT Iβ is expressed in yeast, deletion of the conserved first 28 NH2-terminal amino acids abolishes malonyl-CoA sensitivity and increases catalytic activity 2.5-fold, indicating that an intact NH2-terminal domain is required for malonyl-CoA inhibition (20). Furthermore, several investigators have proposed that it is the interaction between the COOH and NH2 domains that determines malonyl-CoA sensitivity (25). According to this model, post-translational modification of either domain and/or other factors that alter CPT I confirmation might contribute to physiological modulation of malonyl-CoA sensitivity in vivo. Consistent with this premise, malonyl-CoA sensitivity of hepatic CPT I decreases in response to physiological stresses that increase malonyl-CoA sensitivity in vivo. Consistent with this premise, malonyl-CoA sensitivity of hepatic CPT I decreases in vivo. Consequently, malonyl-CoA content and fatty acid oxidation in rat muscle and liver in vivo. Am J Physiol Endocrinol Metab 278: E259–E265, 1999.


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