Sensitivity of CPT I to malonyl-CoA in trained and untrained human skeletal muscle

EMMA C. STARRITT, RICHARD A. HOWLETT, GEORGE J. F. HEIGENHAUSER,
AND LAWRENCE L. SPRIET
Department of Human Biology and Nutritional Sciences, University of Guelph, Ontario N1G 2W1;
Department of Medicine, McMaster University, Hamilton, Ontario, Canada L8N 3Z5; and
Department of Physiology, The University of Melbourne, Parkville, 3052, Australia

Starritt, Emma C., Richard A. Howlett, George J. F. Heigenhauser, and Lawrence L. Spriet. Sensitivity of CPT I to malonyl-CoA in trained and untrained human skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* 278: E462–E468, 2000.—The present study examined the sensitivity of carnitine palmitoyltransferase I (CPT I) activity to its inhibitor malonyl-CoA (M-CoA), and simulated metabolic conditions of rest and exercise, in aerobically trained and untrained humans. Maximal CPT I activity was measured in mitochondria isolated from resting human skeletal muscle. Mean CPT I activity was 492.8 ± 72.8 and 260.8 ± 33.6 μmol·min^{-1}·kg wet muscle^{-1} in trained and untrained subjects, respectively (pH 7.0, 37°C). The sensitivity to M-CoA was greater in trained muscle; the IC$_{50}$ for M-CoA was 0.17 ± 0.04 and 0.49 ± 0.17 μM in trained and untrained muscle, respectively. The presence of acetyl-CoA, free coenzyme A (CoASH), and acetylcarnitine, in concentrations simulating rest and exercise conditions, did not release the M-CoA-induced inhibition of CPT I activity. However, CPT I activity was reduced at pH 6.8 vs. pH 7.0 in both trained and untrained muscle in the presence of physiological concentrations of M-CoA. The results of this study indicate that aerobic training is associated with an increase in the sensitivity of CPT I to M-CoA. Accumulations of acetyl-CoA, CoASH, and acetylcarnitine do not counteract the M-CoA-induced inhibition of CPT I activity. However, small decreases in pH produce large reductions in the activity of CPT I and may contribute to the decrease in fat metabolism that occurs during moderate and intense aerobic exercise intensities.

long-chain fatty acids; fatty acid transport; mitochondria; aerobic training; β-oxidation; carnitine palmitoyltransferase I; malonyl-coenzyme A

THE CARNITINE PALMITOYLTRANSFERASE (CPT) complex, consisting of CPT I, acylcarnitine translocase, and CPT II, has a pivotal role in the transport of long-chain fatty acids in the mitochondria for subsequent β-oxidation in skeletal muscle, as well as in other tissues (20). CPT I, located on the outer surface of 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. The acyl-CoA moiety is then reformed in the matrix of the mitochondria by the action of CPT II. This enzyme is located on the inner mitochondrial membrane and catalyses the transfer of the acyl group from carnitine to CoASH. The reformed acyl-CoA then enters the β-oxidation pathway (20).

CPT I is considered the rate-limiting step in the oxidation of long-chain fatty acids and is reversibly inhibited by malonyl-CoA (M-CoA), the first committed intermediate in fatty acid synthesis (21, 31). Thus there has been speculation as to the potential role for M-CoA in fuel selection in skeletal muscle. Decreases in muscle M-CoA during exercise in rat skeletal muscle have led to the suggestion that M-CoA levels regulate the rate of fatty acid oxidation in muscle (39). However, existing in vitro work with rat skeletal muscle suggests that CPT I would be fully inhibited at the M-CoA concentrations that exist in the intact cell (P. M. Berthon, R. A. Howlett, G. J. F. Heigenhauser, and L. L. Spriet, unpublished observation and Refs. 12, 21, 22). In addition, it has been demonstrated that M-CoA levels did not change during exercise in human skeletal muscle despite large increases in fatty acid oxidation rates (25, 26). These uncertainties regarding the interaction between CPT I activity and M-CoA inhibition during exercise suggest that in vitro investigations of CPT I in human skeletal muscle are warranted.

A number of animal studies have attempted to define the sensitivity of CPT I activity to M-CoA (12, 21, 23). However, this has not been attempted in human skeletal muscle. Many previous studies of the entire CPT complex in human skeletal muscle have used muscle homogenates or freeze-thawed mitochondria (1, 8, 17, 33, 37, 40, 41). Because the mitochondria are not preserved intact, a clear measurement of CPT I activity alone is not possible. However, Berthon et al. (3) recently modified the existing radiometric technique to allow for CPT I measurements in intact mitochondria isolated from needle biopsy samples of human skeletal muscle.

In addition to the relationship between CPT I activity and M-CoA, other factors may be important in the regulation of CPT I and fuel selection at rest and during exercise. There is in vitro evidence in skeletal muscle that other metabolic intermediates, such as acetyl-CoA and CoASH, may affect the activity of CPT I (19, 22, 41). In addition, reductions in pH decreased the activity

of CPT I in rat skeletal muscle (Berthon et al., unpublished observation and Refs. 12, 23). Again, these effects have not been investigated in human skeletal muscle.

The aims of the present study were *1*) to characterize the sensitivity of skeletal muscle CPT I to M-CoA in resting biopsies from aerobically trained and untrained humans; *2*) to determine the effect of the metabolites acetyl-CoA, CoASH, and acetylcarnitine, present in concentrations simulating rest and exercise conditions, on CPT I activity in the presence of physiological levels of M-CoA; and *3*) to examine the effect of pH on CPT I activity in the presence of physiological levels of M-CoA.

METHODS

Subjects. Experiments were conducted on two subject groups. Six males and five females were classified as aerobically trained, and six males and six females were classified as untrained (Table 1). Trained subjects engaged in four or more aerobic workouts per week, whereas untrained subjects engaged in one workout per week or minimal activity. Subjects were fully informed of the purpose of the experiments and of any possible risk before giving written consent to participate. The study was approved by the University of Guelph Ethics Committee.

Experimental protocols. Before the experiment, peak pulmonary O$_2$ uptake (V̇O$_{2\,peak}$) was measured with a metabolic cart (SensorMedics model 2900; Yorba Linda, CA) during incremental exercise on a cycle ergometer (Excalibur, Quinton Instruments, Seattle, WA; Table 1). On the day of the experiment, a resting muscle sample was obtained from the vastus lateralis under local anesthesia (2% lidocaine without epinephrine) using the percutaneous needle biopsy technique described by Bergström (2). Visible fat and connective tissue were dissected free from the muscle, and it was blotted to remove excess blood. The muscle sample ($\sim$60–100 mg) was divided into two portions; the first ($\sim$50–90 mg) was used for the immediate isolation of mitochondria for the determination of CPT I activity, and the second ($\sim$10 mg) was frozen in liquid nitrogen for the later analysis of citrate synthase (CS) and β-hydroxyacyl-CoA-dehydrogenase (β-HAD) activity.

Isolation of mitochondria. The procedure for the mitochondrial isolation has been previously described by Jackman and Willis (15). The entire procedure was performed at 0–4°C. The buffer solutions used contained the following: *solution I*: 100 mM KCl, 40 mM Tris·HCl, 10 mM Tris base, 5 mM MgCl$_2$, 1 mM EDTA and 1 mM ATP, pH 7.4; *solution II*: 100 mM KCl, 40 mM Tris·HCl, 10 mM Tris base, 1 mM MgSO$_4$, 0.1 mM EDTA, 0.2 mM ATP, and 1.5% fatty acid free BSA, pH 7.4; *solution III*: as per *solution II* without BSA.

Muscle samples were weighed, finely minced, and gently homogenized with a manual glass homogenizer in 20 vol/wt of *solution I* and then centrifuged at 700 *g* for 10 min. The supernatant was centifuged at 14,000 *g* for 10 min, and the

Table 1. *Subject characteristics*

	n	Age, yr	Weight, kg	Height, cm	V̇O$_{2\,peak}$, ml·min^{-1}·kg^{-1}
Trained	11	24.4 ± 3.1	69.1 ± 10.1	173.0 ± 7.3	57.5 ± 8.8
Untrained	12	23.5 ± 5.3	73.7 ± 10.2	176.4 ± 7.1	46.8 ± 8.5*

Values are means ± SD; *n*, no. of subjects. V̇O$_{2\,peak}$, peak O$_2$ uptake. *Significant difference from trained.

pellet containing the mitochondria was then resuspended in 10 vol/wt of *solution II* and centrifuged at 7,000 *g* for 10 min. The pellet was resuspended in 10 vol/wt of *solution III* and centrifuged at 3,500 *g* for 10 min. This final pellet was resuspended in 1 vol/wt of a mannitol/sucrose buffer (220 mM sucrose, 70 mM mannitol, 10 mM Tris·HCl, and 1 mM EDTA, pH 7.4), gently rendered homogenous, and kept on ice for the subsequent determination of CPT I and intramitochondrial CS activities. This procedure extracts mainly subsarcolemmal mitochondria (as a protease was not used) that contain very low levels of contaminants, as previously described in heart (27) and skeletal muscle (4, 16).

Determination of CPT I (EC 2.3.1.21) activity. The forward radioisotope assay for the determination of CPT I activity from needle biopsy samples has been previously described (3, 21). However, two significant changes were made to the method for the present study. The respective concentrations of the substrates palmitoyl-CoA and L-carnitine were increased to 300 µM and 5 mM, and the assay was performed at 37°C. Previous work with rat skeletal muscle suggested that these substrate concentrations were required for maximal CPT I activity and that 300 µM palmitoyl-CoA did not exert a detergent effect on the mitochondrial membranes (Berthon et al., unpublished observation and Ref. 24). Briefly, labeled palmitoyl-L-carnitine was measured after it was generated for 6 min after 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 7.0), 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 1 M HCl. Palmitoyl-[^{3}H]carnitine formed during the reaction was extracted in 400 µl of water-saturated butanol in a process involving 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 scintillation cocktail. Assays were performed in duplicate, and blanks were subtracted.

To express CPT I activity in terms of the whole muscle (µmol·min^{-1}·kg wet muscle^{-1}), CPT I activity in the mitochondrial suspension was referenced to the ratio of CS activity in the intact mitochondria in suspension to total muscle CS activity (see below). When the effects of pH, M-CoA, and additional metabolites were studied, the reaction medium was modified to contain 117 mM Tris·HCl (pH 6.8) or M-CoA and the various metabolites in addition to the standard reaction medium outlined above. The concentration of M-CoA representative of the physiological level in human skeletal muscle at rest is 0.7 µM (26). To mimic the conditions of rest and exercise in muscle, acetyl-CoA, CoASH, and acetylcarnitine were added individually and then all together to the standard reaction medium (including 0.7 µM M-CoA) to give final concentrations of 4 µM, 17 µM, and 2 mM, respectively, for the rest condition and 12 µM, 9 µM, and 6 mM, respectively, for the exercise condition (7, 10, 29).

Determination of CS (EC 4.1.3.7) and β-HAD (EC 1.1.1.35) activities. CS activity was assayed spectrophotometrically at 25°C as previously described (36). Total muscle CS activity was assayed in a portion of the original muscle sample ($\sim$10 mg) and homogenized in 100 vol/wt of a 100 mM potassium phosphate buffer solution. CS activity of intact mitochondria in the suspension was determined by first assaying the extramitochondrial fraction in the suspension (1:20 dilution) and then assaying the total CS activity of the suspension (1:20 dilution) after lysing the mitochondria with 0.04% Triton X-100 and repeated freeze thawing. The difference in
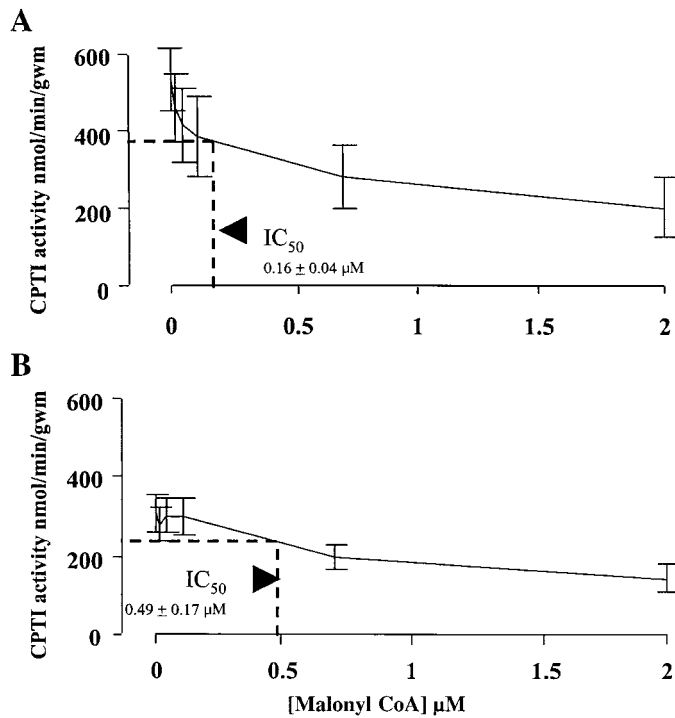
Fig. 1. Effect of malonyl-CoA (M-CoA) concentration on carnitine palmitoyltransferase (CPT) I activity (pH 7, 37°C) in trained (*A*) and untrained (*B*) subjects. The IC$_{50}$ values shown were calculated from plots of CPT I activity vs. log M-CoA concentration ([Malonyl CoA]).

these activities gives the intramitochondrial fraction. β-HAD was assayed spectrophotometrically at 25°C as previously described (18).

Data analysis. All data are reported as means $\pm$ SE. The IC$_{50}$, defined as the M-CoA concentration causing 50% of the maximal inhibition of CPT I activity, was calculated from plots of CPT I activity vs. log[M-CoA] for each subject (12). The effect of each additional metabolite on CPT I activity was compared with the activity of CPT I in the presence of 0.7 μm M-CoA with a paired *t*-test. Comparisons between groups were made with unpaired *t*-tests. Linear regression analysis was performed using the method of least squares. The reliability of the assay was assessed with the coefficient of variation (CV) of CPT I activities determined from the same extracted mitochondria. The formula used to determine CV was

$$\sqrt{\Sigma\,(D - \overline{D})^2/[2(N - 1)]/[(\overline{X}_1 + \overline{X}_2)/2]}$$

where D is the difference between duplicate measures, *N* is the total number of observations, and $\overline{X}_1$ and $\overline{X}_2$ are the means of each measurement. The CV for CPT I activity determined from the same extracted mitochondria was 3.7% ($n = 4$). The CV for CPT I activity determined from two different biopsies from the same muscle on the same day (4.4%) and on different days (7.0%) have previously been determined (3). Significance was accepted at the $P < 0.05$ level.

RESULTS

$\dot{V}O_{2\,peak}$ was significantly greater in the trained group vs. the untrained group (Table 1). The activities of CS (23.7 $\pm$ 3.5 vs. 16.1 $\pm$ 4.2 mmol/kg wet muscle) and β HAD (3.98 $\pm$ 0.96 vs. 2.15 $\pm$ 0.92 mmol/kg wet muscle)

were also significantly higher in the trained vs. untrained groups. The maximal CPT I activity measured in resting biopsies was significantly higher in the trained group ($n = 11$) than in the untrained group ($n = 12$; 492.8 $\pm$ 72.8 vs. 260.8 $\pm$ 33.6 μmol·min^{-1}·kg wet muscle^{-1}, respectively). There were significant correlations between CPT I activity and relative $\dot{V}O_{2\,peak}$ ($r = 0.46$, $P = 0.02$), CS activity ($r = 0.62$, $P = 0.003$), and β-HAD activity ($r = 0.75$, $P = 0.002$)

M-CoA sensitivity. The mean IC$_{50}$ for M-CoA was significantly lower in the trained than in the untrained group (0.17 $\pm$ 0.04 vs. 0.49 $\pm$ 0.17 μM, respectively; Fig. 1).

Effects of additional metabolites on CPT I activity. There was no effect of adding resting levels of acetyl-CoA (4 μM) and CoASH (17 μM) and exercise levels (acetyl-CoA: 12 μM, CoASH: 9 μM) on the activity of CPT I in the presence of 0.7 μM M-CoA in either trained or untrained groups (Figs. 2 and 3). Resting levels of acetylcarnitine (2 mM) alone and resting levels of all three metabolites together reduced the activity of CPT I below values measured in the presence of 0.7 μM M-CoA in both trained and untrained subjects (Fig. 2). However, exercise levels of acetylcarnitine (6 mM) alone and exercise levels of all three metabolites together had no effect (Fig. 3).

Effect of pH on CPT I activity. CPT I activity was markedly decreased when the pH of the reaction medium was reduced from 7.0 to 6.8 in the presence of 0.7
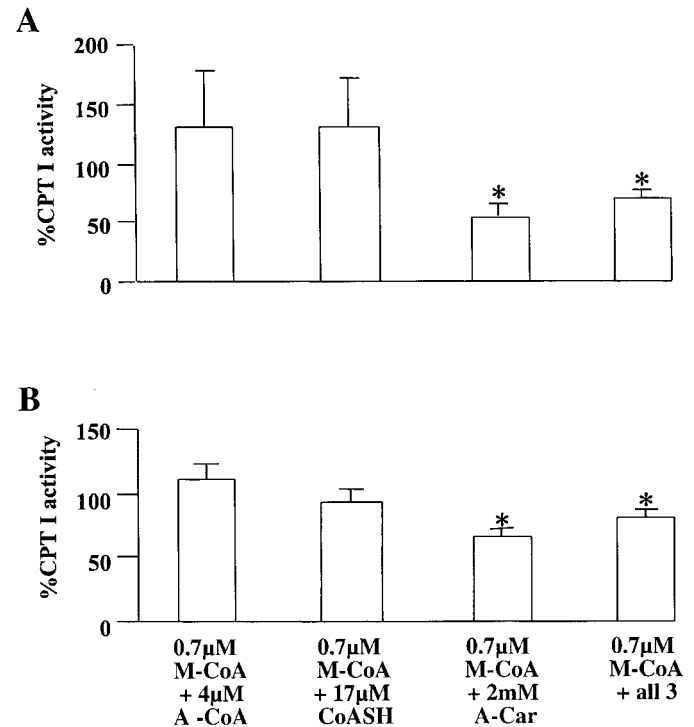

Fig. 2. Effect of resting levels of acetyl-CoA (A-CoA), free CoA (CoASH), and acetylcarnitine (A-Car) on CPT I activity in trained (*A*) and untrained (*B*) subjects. Enzyme activity is expressed as %CPT I activity measured in the presence of 0.7 μM M-CoA. *Difference ($P < 0.05$) from CPT I activity determined in the presence of 0.7 μM M-CoA only.
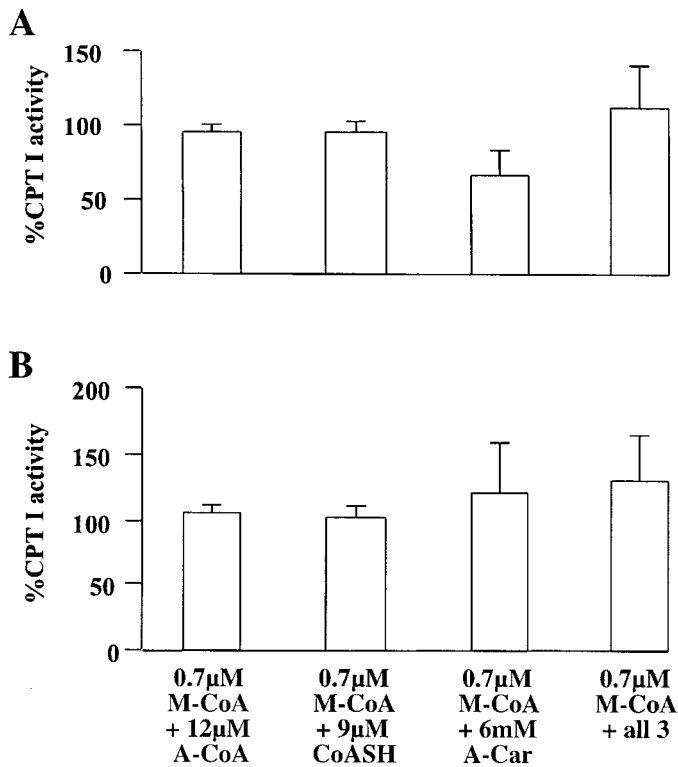
A

B

Fig. 3. Effect of exercise levels of acetyl-CoA, CoASH, and acetylcarnitine on CPT I activity in trained (*A*) and untrained (*B*) subjects. Enzyme activity is expressed as %CPT I activity measured in the presence of 0.7 µM M-CoA.

µM M-CoA in both trained (7.0: 406.5 ± 71.5 vs. 6.8: 251.0 ± 67.9) and untrained muscle (7.0: 195.6 ± 30.2 vs. 6.8: 116.3 ± 20.7 $\mu mol \cdot min^{-1} \cdot kg$ wet muscle^{-1}; Fig. 4).

DISCUSSION

The results of the present cross-sectional study indicate a clear relationship between the training status of the individual and the activity of CPT I measured in intact subsarcolemmal mitochondria from resting human skeletal muscle biopsy samples. This is in agreement with previous findings (3). The sensitivity of CPT I to M-CoA was higher in aerobically trained vs. untrained subjects. However, there was no clear influence of resting or exercise concentrations of acetyl-CoA, CoASH, or acetylcarnitine on the inhibition of physiological levels of M-CoA (0.7 µM) on CPT I activity. Only resting levels of acetylcarnitine (2 mM) caused a further reduction in CPT I activity in both trained and untrained individuals. Finally, the pH of the reaction medium had a marked affect on CPT I activity. A small reduction in pH from 7.0 to 6.8 reduced the activity of CPT I by ~40% in both trained and untrained individuals in the presence of physiological levels of M-CoA.

Measurement of in vitro CPT I activity. The absolute values for maximal CPT I activity for both untrained and trained groups were two- to threefold higher in the present study compared with previously reported values from our laboratory (3). The reason for the higher values in the present study relates to alterations in the

conditions used in the assay to measure CPT I activity. The previous study employed substrate concentrations of 100 µM palmitoyl-CoA and 0.4 mM L-carnitine and a temperature of 30°C (3). In the present study, the palmitoyl-CoA concentration was increased to 300 µM, and the carnitine concentration was increased to 5 mM, because previous experiments with rodent skeletal muscle indicated that these concentrations were required to achieve maximal CPT I activity (Berthon et al., unpublished observation). The temperature employed in the assay was also increased from 30 to 37°C to simulate the in vivo condition. Although the altered assay conditions resulted in higher CPT I activities, the linear relationship between measures of the training status of the individual (β-HAD, CS activities) and maximal CPT I activity remained. The correlations between CPT I activity and CS activity and also between CPT I and relative O_2 uptake were not as strong in the present study, possibly due to a smaller range of O_2 uptake values (37–65 ml $O_2 \cdot min^{-1} \cdot kg$ body mass^{-1}) in the present subjects. In the previous study, we purposely included subjects at the extreme ends of the O_2 uptake spectrum (28–72 ml $O_2 \cdot min^{-1} \cdot kg$ body mass^{-1}), and this appears to have strengthened the above relationships.

Sensitivity of CPT I to M-CoA. The existing data on the relationship between CPT I activity and M-CoA concentration in skeletal muscle comes mainly from rodent tissue. IC_{50} values for M-CoA in rat skeletal muscle have been reported ranging from 0.015 to 0.22 µM (Berthon, unpublished observation and Refs. 12, 21, 23). This wide range is likely due to a combination of varying factors, such as the pH and temperature of the
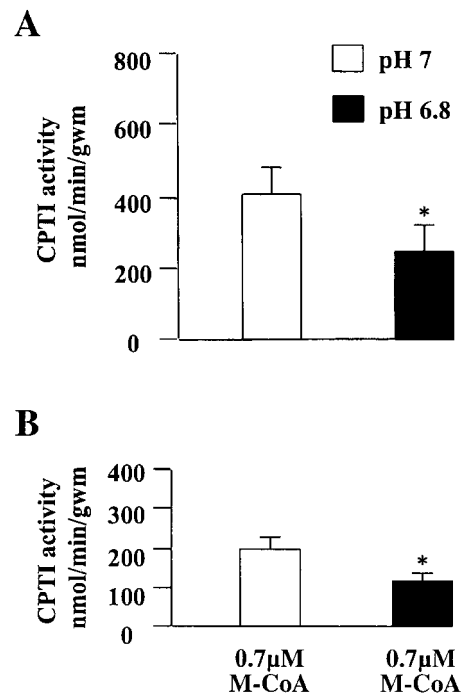
A

B

Fig. 4. Effect of pH on the activity of CPT I measured in the presence of 0.7 µM M-CoA in trained (*A*) and untrained (*B*) subjects. *$P < 0.05$ vs. pH 7.0.

assay medium (12) and the different muscles (fiber types) examined. Berthon et al. (unpublished observation) reported an IC_{50} for M-CoA of 0.22 ± 0.04 µM in untrained rat soleus muscle at pH 7.0. It appears that CPT I from human skeletal muscle is less sensitive to M-CoA than rat skeletal muscle. The IC_{50} for M-CoA in untrained humans reported in the present study was 0.49 ± 0.17 µM, which is approximately twofold higher than reported for untrained rat skeletal muscle.

In the present study, the IC_{50} for M-CoA in aerobically trained human muscle is ~70% lower than that from untrained muscle. This finding suggests that aerobic training is associated with an increase in the sensitivity of skeletal muscle CPT I to M-CoA. In the presence of physiological levels of M-CoA (0.7 µM), the activity of CPT I was 284.2 ± 75.9 in the trained group and 196.0 ± 30.2 µmol·min^{-1}·kg wet muscle^{-1} in the untrained group (Fig. 1). Although these activities are not statistically different, the inhibition imposed by this concentration of M-CoA represents a 47% decrease in the maximal activity of CPT I in trained individuals compared with a 36% decrease in untrained individuals. Despite an increased sensitivity to M-CoA, the activity of CPT I remained higher at a given M-CoA concentration in trained muscle (Fig. 1).

M-CoA has been proposed to be a regulator of fatty acid catabolism in rat skeletal muscle by virtue of its relationship with CPT I (39). M-CoA levels decrease during exercise in rat skeletal muscle, suggesting a mechanism for increased fatty acid oxidation during prolonged exercise (39). However, contrary to the observations made in rat skeletal muscle, Odland et al. (25, 26) demonstrated no change in M-CoA levels at a variety of exercise power outputs and time points in human skeletal muscle. Furthermore, the present study suggests that training increases the sensitivity of CPT I to M-CoA. This finding seems paradoxical if M-CoA levels do not change during prolonged exercise given that the proportion of fatty acids oxidized during prolonged exercise is greater in trained individuals (38). Taken together, these findings suggest that the role of M-CoA as a regulator of CPT I activity may be more complicated than originally proposed in rat skeletal muscle.

Recently Hutber et al. (14) have shown that training attenuated the exercise-induced decline in M-CoA levels observed in rat skeletal muscle. It is unknown whether the sensitivity of CPT I to M-CoA is altered by training in rat skeletal muscle. Potentially, if the sensitivity is increased with training, as in human skeletal muscle, then the decrease in the muscle M-CoA level during exercise in rat skeletal muscle required to maintain the same level of inhibition would be less. The influence of training on the sensitivity of CPT I to M-CoA in rat skeletal muscle needs to be evaluated before the significance of this observation can be determined.

Effect of acetyl-CoA, CoASH, and acetylcarnitine on CPT I activity. There is some evidence in the literature that compounds structurally related to M-CoA such as acetyl-CoA and CoASH can inhibit CPT activity in rat skeletal muscle (19, 22, 41). In addition to binding at their own sites with an inhibitory effect that is not as great as M-CoA, these compounds appear to also compete with M-CoA at its binding site on CPT I (22). It is possible that the presence of these compounds in the muscle cell might have a role in the regulation of CPT I activity by competing with M-CoA for the binding site on CPT I. In the presence of M-CoA, they may act as partial agonists, increasing CPT I activity since they are less potent inhibitors than M-CoA. The present study examined the effect of acetyl-CoA, CoASH, and acetylcarnitine on the activity of CPT I in the presence of physiological levels of M-CoA in concentrations simulating rest (4 µM, 17 µM, and 2 mM, respectively) and exercise conditions in the muscle cell (12 µM, 9 µM, and 6 mM, respectively). However, there was no effect of any of these compounds on CPT I activity, except for 2 mM acetylcarnitine. This further reduced the activity of CPT I below that of 0.7 µM M-CoA alone, in both trained and untrained muscle. However, when the concentration of acetylcarnitine was increased to 6 mM, the effect was not maintained in either group.

The physiological significance of this acetylcarnitine effect is unclear. The level of acetylcarnitine increases in muscle during exercise (10, 29). Perhaps the additional inhibition imposed on CPT I by lower levels of acetylcarnitine at rest is relieved during moderate exercise, and this contributes to the increased catabolism of fatty acids observed during exercise at this intensity (30). However, the increase in acetylcarnitine is less during submaximal exercise at the same absolute workload after training (28) and therefore would seem to be an unlikely contributor to the increased reliance on fatty acid oxidation in trained individuals during exercise.

Another factor confounding the interpretation of the metabolite results is the uncertainty regarding the cytoplasmic and mitochondrial concentrations of acetylcarnitine, acetyl-CoA, CoASH, and M-CoA. We predict that most of the acetylcarnitine is outside the mitochondria, and most of the CoASH and acetyl-CoA are inside the mitochondria. We would also expect that most of the M-CoA is cytoplasmic, given that acetyl-CoA carboxylase, the enzyme that produces most of the cellular M-CoA, is cytoplasmic. However, to our knowledge, this information is not available in rodent or human skeletal muscle. Therefore, the concentrations used in our assays may be higher than the true cellular cytoplasmic concentrations. However, since no effects were found with these concentrations, it seems unlikely that there would be any effects at lower concentrations.

Effect of pH on CPT I activity. A small decline in pH from 7.0 to 6.8 in the present study resulted in a significant decrease (~50%) in the activity of CPT I in both trained and untrained muscle in the presence of physiological levels of M-CoA. We were not able to measure whether M-CoA binding to CPT I (IC_{50}) was decreased at pH 6.8 in the trained or untrained groups of this study, as the availability of muscle was limited. However, although a portion or all of the pH effect could be explained by decreased M-CoA binding to CPT I , we

found no difference between IC_{50} values at pH 7 vs. 6.8 in rat skeletal muscle (Berthon, unpublished observation).

It seems likely that the small decline in pH that normally occurs during moderate and intense aerobic exercise would cause a significant reduction in the activity of CPT I in both trained and untrained individuals. Howlett et al. (13) reported muscle lactate levels of 38 and 108 mmol/kg dry muscle in untrained men after only 10 min of cycle exercise at 65 and 90% $\dot{V}O_{2\,peak}$, respectively. According to the equations of Sahlin et al. (32), corresponding muscle pH values would be ~6.9 and 6.6 at these power outputs. This decrease in muscle pH during intense exercise could contribute to the reduction in fatty acid oxidation that has been observed to occur during intense aerobic exercise (>85% $\dot{V}O_{2\,peak}$; see Refs. 30, 34), by reducing the activity of CPT I under these conditions. It may also contribute to decreased fatty acid use in moderate power outputs between 65 and 85%, where the pH decrease may be 0.1–0.3 units.

The effect of pH on CPT I activity may also contribute to the explanation of the glucose-fatty acid cycle reversal hypothesis proposed to explain the reciprocal relationship between the oxidation of carbohydrate and fat in resting (35) and exercising skeletal muscle (9, 34). These authors suggest that glycolytic flux is the primary regulator of fatty acid oxidation. An increase in glycolytic flux is associated with a reduction in the oxidation of long-chain fatty acids, which is proposed to involve the entry of the long-chain fatty acids into the mitochondria. It is possible that an increase in muscle lactate and decrease in pH could provide the link between the increased glycolytic flux and decreased fatty acid oxidation. Because increases in glycolytic flux are associated with decreased pH values during moderate and intense aerobic power outputs (13), this could lead to decreased CPT I activity and therefore decreased long-chain fatty acid oxidation.

The significance of the link between pH and CPT I activity could be magnified during exercise after an overnight fast, where rates of fatty acid oxidation are much higher than during the postprandial situation. Higher fatty acid oxidation rates and lower glycolytic activity during exercise after an overnight fast may decrease the pH effect on CPT I. This effect might also be reduced in trained individuals. After training, the decrease in pH during exercise is attenuated when exercising at the same absolute power output. Muscle pH remains higher (lower muscle lactate) during moderate and intense aerobic power outputs after as little as 5–7 days of aerobic training (5, 11, 28). Perhaps this delayed decrease in muscle pH in trained individuals contributes to the increased reliance on fatty acid oxidation at a given power output during exercise in trained individuals.

In summary, this investigation reports that the sensitivity of CPT I to M-CoA was greater in aerobically trained vs. untrained human skeletal muscle. Furthermore, the sensitivity to M-CoA was less than that reported for rat skeletal muscle. There were no effects of the physiological compounds acetyl-CoA and CoASH on the inhibition imposed on CPT I by physiological levels of M-CoA. However, low levels of acetylcarnitine further reduced the activity of CPT I. This effect was abolished when the concentration of acetylcarnitine was increased to levels expected to occur in the muscle cell during exercise. A small decline in pH (7.0–6.8) resulted in a significant reduction in CPT I activity. This effect is of particular physiological significance, given that pH decreases in this range occur during moderate and intense aerobic exercise. The pH inhibition of CPT I activity may help explain the reduction in fatty acid oxidation that has been observed to occur during moderate to intense aerobic exercise. In addition, in trained individuals, the decrease in muscle pH that occurs during moderate and intense aerobic exercise is attenuated, favoring the oxidation of fatty acids. Last, the present results were obtained by examining primarily subsarcolemmal mitochondria extracted from human skeletal muscle. Future work should extend these measurements to intermyofibrillar mitochondria.

Address for reprint requests and other correspondence: Dr. Lawrence L. Spriet, Department of Human Biology and Nutrition Science, University of Guelph, Guelph, Ontario, N1G 2W1, Canada (E-mail: lspriet.ns@aps.uoguelph.ca).

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