Long- and Medium-Chain Fatty Acid Oxidation Is Increased in Exercise-Trained Human Skeletal Muscle

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The purpose of this study was to test the hypothesis that skeletal muscle fatty acid oxidation is enhanced by increased entry into the mitochondria with exercise training. Muscle was obtained from young (≈ 24 years) sedentary (n = 13) and endurance-trained (n = 10) volunteers and oxidation studied by measuring $^{14}CO_2$ production from labeled medium-chain (MCFA) or long-chain (LCFA) fatty acids in muscle homogenate preparations. LCFA (palmitate) oxidation was ($P < .05$) approximately 34% higher in the trained than sedentary subjects (26.9 ± 3.0 *v* 17.8 ± 1.3 nmol CO_2/g · h). MCFA (octanoate) oxidation was also about 26% higher ($P < .05$) in the trained subjects (21.7 ± 2.1 *v* 16.1 ± 2.0 nmol CO_2/g · h). To examine the roles of carnitine-mediated transport and mitochondrial content, we also measured carnitine palmitoyltransferase I (CPT1), carnitine octanoyl transferase (COT), and citrate synthase (CS) activities. CPT1 and CS activity were significantly ($P < .05$) higher (≈25%) in the endurance-trained subjects; there was no difference in COT activity. These data suggest that adaptations at the level of CPT1 and processes distal to this step may contribute to increases in LCFA or MFCA oxidation with exercise training. In contrast, carnitine-mediated transport (COT) does not appear to contribute to an enhancement in MCFA oxidation with exercise training.

ENDURANCE TRAINING increases muscle lipid oxidation during submaximal exercise[1-4]; an increase in resting lipid oxidation has also been reported in some studies.[5,6] A variety of processes, such as lipolysis and lipid delivery, lipid transport across vascular, sarcollemal, and mitochondrial membranes, transport within the cell, and cellular oxidation within the mitochondria, could contribute to this enhancement in fat disposal.[1-4] In an effort to determine how exercise training enhances lipid oxidation, Sidossis et al[7] compared whole-body oleate and octanoate oxidation in endurance-trained versus sedentary subjects during submaximal exercise. Medium-chain fatty acids (MCFA) such as octanoate are assumed to traverse cell and organelle membranes through non–carrier-mediated means, while long-chain fatty acids (LCFA; ie, oleate, palmitate) must utilize a carnitine-acylcarnitine exchange system for mitochondrial entry.[1-4,8-10] These investigators[7] reported that oleate oxidation was higher in the endurance-trained group while octanoate oxidation did not differ and concluded that fatty acid oxidation was enhanced with exercise training, primarily via increased fatty acid entry into skeletal muscle mitochondria.

The purpose of the present study was to test the hypothesis that fatty acid oxidation is enhanced by increased fatty acid entry into the mitochondria with exercise training. Recent studies have documented the existence of fatty acid transporters and binding proteins that are involved in substrate translocation across the sarcolemmal and within the muscle cell.[4,11,12] To minimize these and other factors linked with lipid delivery we compared the oxidation of LCFA (palmitate) and MCFA (octanoate) in skeletal muscle homogenates from sedentary and endurance-trained subjects. We also measured carnitine palmitoyltransferase I (CPT1), carnitine octanoyl transferase (COT), and citrate synthase (CS) activities, in an effort to provide explanations for any observed differences in fatty acid oxidation with endurance training.

METHODS

Experimental Design and Subjects

Skeletal muscle from 13 sedentary (6 men, 7 women) and 10 exercise-trained (7 men, 3 women) volunteers were compared. Men and women were studied as there are no gender difference in CPT1 or other mitochondrial enzymes linked with lipid oxidation in human skeletal muscle.[13] The trained subjects were endurance runners who were training greater than 40 km/wk. Subjects were screened for metabolic abnormalities or medications that could affect lipid oxidation with a health history questionnaire. No subjects were smokers. Sedentary individuals had not participated in a regular exercise program in the previous 12 months. Subjects provided informed consent prior to participation and procedures were approved by the institutional review board.

Skeletal muscle (50 to 100 mg) was obtained from the vastus lateralis with the percutaneous needle biopsy technique.[14] Subjects were studied between 7 to 8 AM after a 12-hour, overnight fast (postabsorptive). They were instructed to consume their usual diet and perform normal daily activities for at least 3 days prior to the biopsy. The trained volunteers were studied 18 to 24 hours after their last exercise session. The trained subjects exercised virtually every day and testing was designed to represent their normal metabolic status. Body mass and height were obtained on the morning of the biopsy to the nearest 0.1 kg and 1.0 cm. Maximal oxygen consumption (VO_2max) was determined with an incremental treadmill test to voluntary exhaustion at least 4 days prior to muscle sampling. Body composition was obtained with the 7-site skinfold method.[15,16]

Biochemical Methods

These procedures have been used previously in this laboratory.[14,17] Briefly, fresh muscle was immediately placed into ice-cold medium containing 250 mmol/L sucrose, 1 mmol/L EDTA, and 10 mmol/L Tris-HCl (pH 7.4) (SETH). Muscle was then dried on blotting paper, weighed, and minced thoroughly with scissors in 19 μL of SETH

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medium per mg muscle. The mixture was homogenized in a glass homogenization tube with a motor-driven plastic pestle. This method has been found to provide intact mitochondria for metabolic studies.[17,18]

Muscle fatty acid oxidation rate was determined in the fresh muscle homogenate using a modification of the method of Dohm et al.[14,17] The oxidation rates of palmitate and octanoate were measured by collecting and counting the $^{14}CO_2$ produced during incubation in a separate homogenate mixture for each fatty acid. A 40-μL quantity of a 20-fold diluted muscle homogenate[14,17] was preincubated with 95% O_2-5% CO_2 mixture at 30°C for 15 minutes. A 160-μL reaction mixture (pH 7.4) was then added to the preincubated muscle homogenate. Final concentrations of the incubation mixture were in millimoles per liter: sucrose, 100; Tris-HCl, 10; potassium phosphate, 5; potassium chloride, 80; magnesium chloride, 1; L-carnitine, 2; malate, 0.1; adenosine triphosphate (ATP), 2; coenzyme A, 0.05; dithiothreitol, 1; EDTA, 0.2; and bovine serum albumin (BSA), 0.5%. The substrates used were either 0.2 mmol/L palmitate-1-^{14}C (0.5 μCi) or 0.2mmol/L octanoate-1-^{14}C (0.5 μCi) with 0.5% BSA. After 60 minutes of incubation at 30°C, 100 °L of 4N sulfuric acid was injected to stop the reaction. CO_2 produced during the 60-minute incubation was trapped with 200 μL of 2-mol/L sodium hydroxide. Briefly, the incubation wells and $^{14}CO_2$ trap consisted of modified 48-well microtiter plate (Costar, Cambridge, MA). Each system consisted of 2 adjacent wells with a fabricated grove between each to allow the acid driven $^{14}CO_2$ from the homogenate to be trapped by the sodium hydroxide. Adjoining well pairs were sealed from each other by a rubber gasket. A 150-μL quantity of the sodium hydroxide trap was counted for evolved $^{14}CO_2$ by liquid scintillation and oxidation rate expressed as nmol CO_2/g · h wet weight. This methodology[16,17] does not account for acid-soluble metabolites, which may result in an underestimation of absolute fatty acid oxidation rates.

Muscle CPT1 activity was measured using modification of the methods of McGarry et al[19] and Zierz and Engel.[20] This method measures the rate of formation of palmitoylcarnitine from palmitoyl-CoA and carnitine. Ten microliters of a 20-fold diluted muscle homogenate was preincubated for 20 minutes at 30°C in a microcentrifuge tube. Reactions were initiated when 90 μL reaction mixture was added to preincubated muscle homogenate at 30°C for 10 minutes. The incubation mixture (pH 7.4) contained 117 mmol/L Tris-HCl, 0.28 mmol/L reduced glutathione, 4.4 mmol/L ATP, 4.4 mmol/L $MgCl_2$, 16.7 mmol/L KCl, 2.2 mmol/L KCN, 40 mg/L rotenone, 0.1% BSA, and 50 μmol/L palmitoyl-CoA. The substrate was 0.2 mmol/L ^{3}H-carnitine (0.5 μCi). The reaction was terminated with 60 μL 1.2-mmol/L ice-cold HCl. The formed ^{3}H-palmitoylcarnitine was extracted with water-saturated butanol and counted by liquid scintillation. Carnitine octanoyl transferase (COT) activity was assayed using the same methodology as CPT1, except that 50 μmol/L octanoyl-CoA was substituted for palmitoyl-CoA .[21] Muscle CS activity was determined spectrophotometrically from the homogenate using the method of Srere.[22] Muscle protein content was determined using the bicinchoninic acid (BCA) method (Pierce, product 23225, Rockford, IL).

Exercise Testing

Maximal oxygen consumption was determined during an incremental treadmill test to voluntary exhaustion. Initial speed was 10 km · h^{-1} and increased by 1.6 km · hr^{-1} every 3 minutes to a speed of 12.8 (sedentary) or 14.4 km · h^{-1} (exercise-trained). Treadmill grade then increased by 2% every 2 minutes until voluntary exhaustion was achieved. Heart rate (12-lead electrocardiogram) and expired gases (Sensor Medics 2900, Yorba Linda, CA) were monitored continuously. Criteria for a maximal test were attainment of at least 2 of the following: (1) voluntary exhaustion; (2) a respiratory exchange ratio greater than 1.1; (3) a plateau in oxygen consumption despite an increase in workload; and/or (4) attainment of at least 85% of age-predicted maximal heart rate.

Table 1. Subject Characteristics (mean ± SD)

Variable	Sedentary	Exercise-Trained
Age (yr)	23.4 ± 1.0	25.4 ± 1.4
Height (cm)	169.3 ± 2.7	174.2 ± 0.9
Body mass (kg)	72.0 ± 3.3	75.7 ± 2.9
Body mass index (kg/m^2)	25.2 ± 1.2	24.9 ± 0.9
Body fat (%)	20.0 ± 1.7	13.2 ± 1.5*
Maximal oxygen consumption		
L · min^{-1}	2.7 ± 0.3	3.9 ± 0.2*
mL · kg^{-1} · min^{-1}	35.9 ± 4.0	52.7 ± 2.4*

*$P \leq .01$ *v* sedentary.

Statistics

Descriptive data, muscle lipid oxidation, and muscle enzymes in the sedentary and exercise-trained groups were compared with factorial analysis of variance (ANOVA). Data were also combined and examined with Pearson product correlations. Statistical significance was denoted at $P < .05$.

RESULTS

Subjects

Subject characteristics are presented in Table 1. The exercise-trained group was leaner (body fat percentage) and possessed a higher maximal oxygen consumption than the sedentary group. There were no differences in body mass, stature, or age between the sedentary and exercise-trained groups.

Muscle Lipid Oxidation and Enzymes

Muscle lipid oxidation was elevated in the vastus lateralis of the exercise-trained versus the sedentary group. As presented in Fig 1, palmitate oxidation was significantly ($P < .005$) higher, by approximately 34%, in the trained versus sedentary individuals (17.8 ± 1.3 *v* 26.9 ± 3.0 nmol CO_2/g · h, for sedentary *v* trained, respectively). Octanoate oxidation was also elevated ($P < .05$) in the trained subjects, by approximately 26% (16.1 ± 2.0 *v* 21.7 ± 2.1 nmol CO_2/g · h). There was a positive relationship between palmitate and octanoate oxidation ($r = 0.66$, $P < .001$).

As presented in Fig 2, with exercise training there were increases in the activities of 2 enzymes involved with lipid oxidation in skeletal muscle. Protein content for the assays did not differ between groups (54.2 ± 3.5 *v* 48.3 ± 3.4 mg/g for sedentary *v* exercise-trained, respectively). CPT1 activity was elevated ($P < .05$) by approximately 20% in the trained subjects (29.4 ± 2.0 *v* 36.9 ± 3.7 nmol/g · min). CS activity was also elevated ($P < .05$) by about 30% in the trained subjects (53.1 ± 7.5 *v* 76.5 ± 7.8 μmol/g · h). Palmitate oxidation was significantly and positively related to CPT1 activity ($r = 0.62$, $P < .005$) and CS activity ($r = 0.43$, $P < .05$) (Fig 3). Palmitate oxidation relative to mitochondrial content (palmitate oxidation/CS activity) was not different between groups (0.5 ± 0.1 *v* 0.4 ± 0.1 arbitrary units for sedentary *v* trained, respectively). Octanoate oxidation also did not differ between the sedentary and trained groups when expressed relative to CS activity (0.3 ± 0.1 *v* 0.3 ± 0.1). CPT1 activity expressed

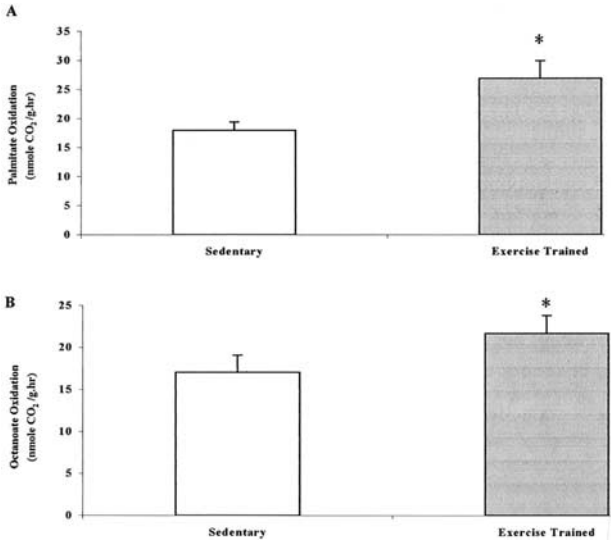

Fig 1. Oxidation of ^{14}C-labeled (A) palmitate and (B) octanoate in skeletal muscle homogenates from sedentary (n = 13) and exercise-trained (n = 10) subjects. *Significantly ($P < .05$) different between sedentary and exercise-trained.

relative to mitochondrial content (CPT1/CS activity) was not different ($P = .23$) between groups (0.7 ± 0.1 *v* 0.5 ± 0.1 arbitrary units for sedentary *v* trained, respectively). Octanoate oxidation was significantly and positively related to CS activity ($r = 0.54$, $P < .001$) (Fig 3). There was no significant difference in COT activity (43.8 ± 3.9 *v* 48.7 ± 3.3 nmol/g · h for sedentary *v* trained, respectively) between the groups (Fig 2). There was no relationship for octanoate oxidation with COT activity.

Relationships Between Muscle Lipid Oxidation and Cardiovascular Fitness

Maximal oxygen consumption (mL · kg^{-1} · min^{-1}) was positively related to muscle lipid oxidation (palmitate, $r = 0.62$, $P < .001$; octanoate, $r = 0.56$, $P < .005$) and CPT1 activity ($r = 0.58$, $P < .005$). Relationships were also evident for absolute (L · min^{-1}) maximal oxygen consumption with palmitate oxidation ($r = 0.61$, $P < .01$), octanoate oxidation ($r = 0.58$, $P < .01$), and CPT1 activity ($r = 0.54$, $P < .01$). CS activity was positively related to both relative ($r = 0.56$, $P < .01$) and absolute ($r = 0.61$, $P < .01$) maximal oxygen consumption.

DISCUSSION

The main finding of the current study was that both LCFA (palmitate) and MCFA (octanoate) oxidation were enhanced in skeletal muscle homogenates from endurance-trained compared to sedentary subjects (Fig 1). If some basic assumptions are accepted, these data may provide insight into possible mechanisms that contribute to the increase in fat oxidation in skeletal muscle with exercise training. Octanoate has been demonstrated to freely traverse the inner mitochondrial membrane prior to entering subsequent catabolic pathways.[8-10] Palmitate oxidation is, however, at least partially dependent on mitochondrial uptake at the level of the mitochondrial membranes.[8-10] Our observed increases in both LCFA and MCFA oxidation (Fig 1) suggest that adaptations involving entry into the mitochondria in conjunction with other downstream processes promote enhanced fatty acid oxidation in skeletal muscle with exercise training. This information is important, as there are limited data detailing the cellular adaptations in fat oxidation that occur with endurance training, specifically in human skeletal muscle.[7,13,22-24] It should be mentioned, however, that the cross-sectional design of the present study may possibly reflect genetic, as well as training-induced, differences in muscle metabolism.

Findings concerning the oxidation of MCFA in human skeletal muscle with exercise training are controversial. With an increase in mitochondrial content after training (Fig 2),[13,24] it seems logical to hypothesize that lipid catabolism could function at an increased rate and increase MCFA oxidation.[1,2,4,25] Our report of an increase in octanoate oxidation (Fig 1) is thus not surprising. However, these data are not in agreement with other findings. Sidossis et al[7] reported enhanced LCFA, but similar MCFA oxidation in endurance-trained and sedentary subjects when infusing labeled substrates. One interpretation of our findings and those of Sidossis et al[7] is that some factor(s) may limit MCFA delivery to skeletal muscle mitochondria in

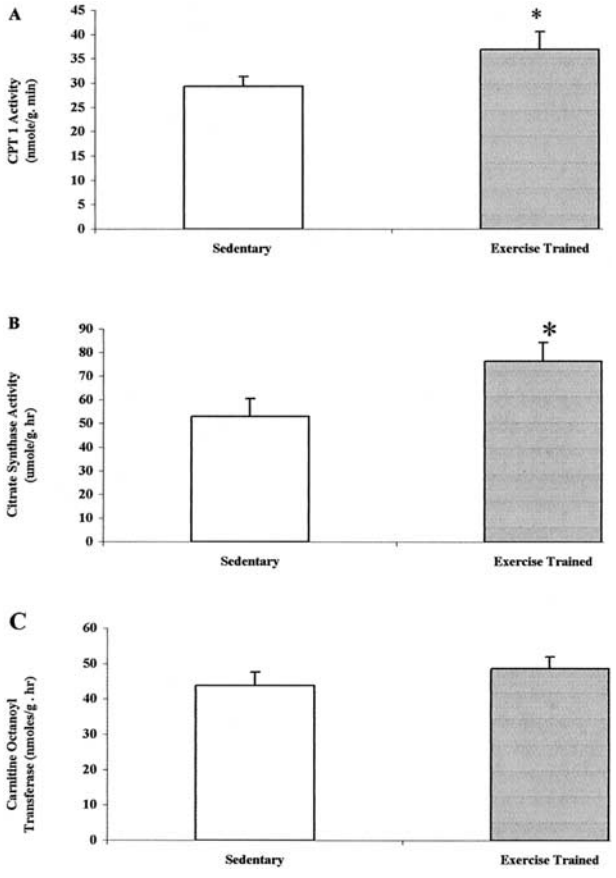

Fig 2. (A) Carnitine palmitoyl transferase (CPT1), (B) citrate synthase, and (C) carnitine octanoyl transferase activities in sedentary (n = 13) and exercise-trained (n = 10) subjects. *Significantly ($P < .05$) different between sedentary and exercise-trained.

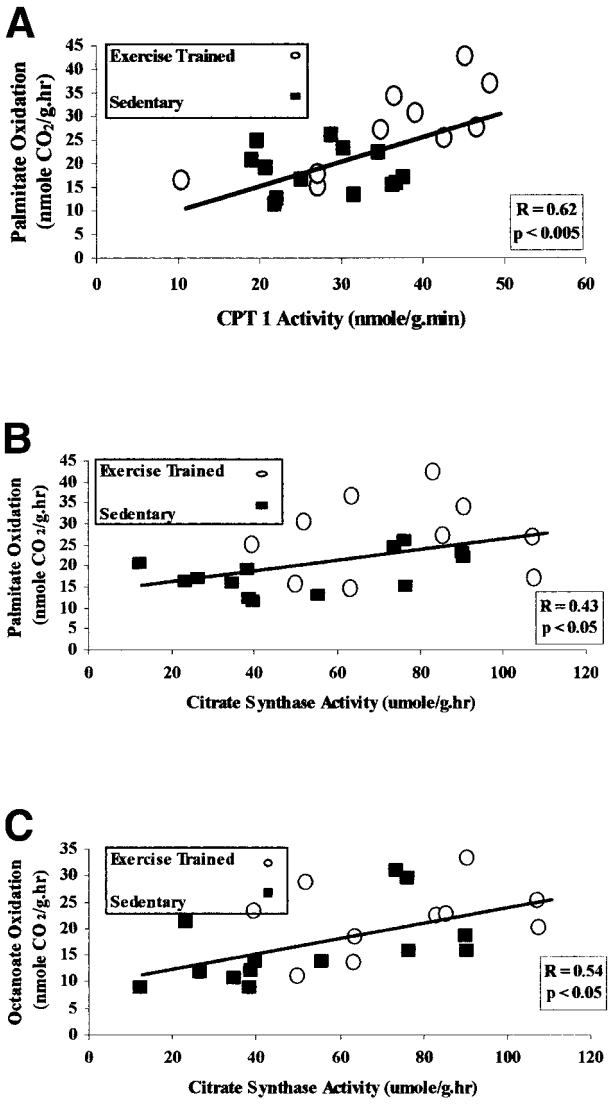

Fig 3. Relationship of muscle palmitate oxidation with (A) CPT1 and (B) citrate synthase activities and (C) the relationship of octanoate oxidation with citrate synthase activity in skeletal muscle from exercise-trained (n = 10, ○) and sedentary (n = 13, ●) subjects.

vivo.[34] If this premise is correct, it may explain why a similar whole-body, MCFA oxidation rate has been reported in sedentary versus trained individuals,[7] despite the increased mitochondrial capacity for fatty acid oxidation in endurance-trained individuals we observed (Fig 1). It is important to consider that the present study was designed to test the hypothesis that the increase in muscle fatty acid oxidation with endurance training is attributable to increased fatty acid entry into the mitochondria. In our homogenate system we therefore eliminated factors linked with lipid transport across and within the cell by disrupting the sarcolemma. Our data suggest that the muscle mitochondria of endurance-trained individuals are capable of oxidizing both LCFA and MCFA at an elevated rate. However, other regulatory factors may regulate fat oxidation in vivo, explaining differences in the present in vitro versus other whole-body studies.[7]

A regulatory step in the oxidation of fatty acids is the transfer of nonpermeable, LCFA across mitochondrial membranes. This process is regulated by CPT1.[3,8-10] CPT1 activity was enhanced with training in the present study (Fig 2), in agreement with others.[13,23,24] However, the increase in both palmitate and octanoate oxidation in the trained subjects indicates that events downstream of mitochondrial transport may also contribute to the increase in fat oxidation.

To provide a possible explanation for post-CPT1 events, we measured CS activity. CS activity is in direct proportion to muscle mitochondrial content[25,26] and was significantly elevated in the vastus lateralis of the trained subjects (Fig 2). When palmitate and octanoate oxidation and CPT1 activity were expressed relative to CS activity, there were no differences between the control and trained groups (see Results). These findings support the data of Berthon et al,[13] suggesting that the higher CPT1 activity in trained subjects is a result of increased mitochondrial content.

Other factors, however, can affect CPT1 activity. Malonyl-CoA can inhibit CPT1 and decrease fatty acid oxidation.[19,27] Reduced malonyl-CoA concentrations are evident in the skeletal muscle of rodents during exercise, which may explain the accompanying increase in fat oxidation.[28-30] Although there is increased sensitivity of CPT1 to malonyl-CoA in trained individuals,[24] to our knowledge there is no information on malonyl-CoA concentrations in endurance-trained human skeletal muscle at rest. In addition, the response of malonyl-CoA to exercise in human skeletal muscle is controversial as no change[31,32] or a decrease[33] has been reported. In our muscle homogenate system, it cannot be determined if malonyl-CoA concentration differed between the trained and untrained groups.

As a possible explanation for increased octanoate oxidation in trained subjects, we measured COT activity as some data suggest that octanoate is transported into the mitochondria by a carnitine-dependent step.[21,34-36] COT activity did not differ between control and trained subjects (Fig 2), nor was there a relationship between COT and octanoate oxidation (see Results). It is not evident why COT activity did not increase in conjunction with other mitochondrial enzymes with the training stimulus. It is possible that the muscle sampled (vastus lateralis) did not reflect the training adaptations induced by distance running to a similar degree as perhaps another muscle group such as the gastrocenmius; however, the vastus lateralis has been the muscle group sampled in other studies of fat oxidation and exercise training.[13,24] Regardless, these data imply that it is unlikely that a carnitine-dependent transport system specific to octanoate is enhanced with exercise training in human skeletal muscle; no other studies, to our knowledge, have determined if COT is altered with endurance training in humans.

In summary, the main finding of the present study was that the oxidation of both LCFA (palmitate) and MCFA (octanoate) were elevated in the skeletal muscle of endurance-trained subjects compared to their sedentary counterparts. When the assumed properties of LCFA and MCFA are considered, these data suggest that adaptations at the level of transport into the mitochondria and processes distal to this step, such as intracellular oxidation, can contribute to the increase in muscle lipid

oxidation with exercise training. In support of this hypothesis, muscle CPT1 activity was higher in endurance-trained subjects, as was CS activity. In contrast, carnitine-mediated transport does not appear to contribute to the enhancement in MCFA with exercise training. Our in vitro findings of an increase in MCFA oxidation are not in agreement with other in vivo data,[7] which suggests that several mechanisms may control whole-body lipid oxidation.

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REFERENCES

1. Holloszy JO, Coyle EF: Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. J Appl Physiol 56: 831-838, 1984

2. Coggan AR, Williams BD: Metabolic adaptations to endurance training: Substrate metabolism during exercise, in Hargreaves M (ed): Exercise Metabolism. Champaign, IL, Human Kinetics, 1995, pp 177-210

3. van der Vusse GJ, Reneman RS: Lipid metabolism in muscle, in Handbook of Physiology. Regulation and Integration of Multiple Systems. Bethesda, MD, American Physiological Society, 1996, pp 952-994

4. Turcotte LP, Richter EA, Kiens B: Lipid metabolism during exercise in Hargreaves M (ed): Exercise Metabolism. Champaign, IL, Human Kinetics, 1995, pp 99-130

5. Calles-Escandon J, Goran MI, O'Connell M, et al: Exercise increases fat oxidation at rest unrelated to changes in energy balance or lipolysis. Am J Physiol 270:E1009-E1014, 1996

6. Poehlman ET, Gardner AW, Aciero PJ, et al: Effects of endurance training on total fat oxidation in elderly persons. J Appl Physiol 76:2281-2287, 1994

7. Sidossis LS, Wolfe RR, Coggan AR: Regulation of fatty acid oxidation in untrained vs trained men during exercise. Am J Physiol 274:E510-E515, 1998

8. McGarry JD: The mitochondrial carnitine palmitoyl transferase system: Its broadening role in fuel homeostasis and new insights into its molecular features. Biochem Soc Trans 23:321-324, 1995

9. McGarry JD, Brown NF: The mitochondrial carnitine palmitoyltransferase system from concept to molecular analysis. Eur J Biochem 244:1-14, 1997

10. McGarry JD, Foster DW: The metabolism of (-) octanoylcarnitine in perfused livers from fed and fasted rats: Evidence for a possible regulatory role of carnitine acyltransferase in the control of ketogenesis. J Biol Chem 249:7984-7990, 1974

11. Bonen A, Dyck DJ, Luiken JJFP: Skeletal muscle fatty acid transport and transporters, in Richter EA, Kiens B, Galbo H, et al (eds): Skeletal Muscle Metabolism in Exercise and Diabetes. New York, NY, Plenum, 1998, pp 193-205

12. Hamilton JA, Kamp F: How are free fatty acids transported in membranes? Diabetes 48:2255-2269, 1999

13. Berthon PM, Howlett RA, Heigenhauser GJF, et al: Human skeletal muscle carnitine palmitoyltransferase I activity determined in isolated intact mitochondria. J Appl Physiol 85:148-153, 1998

14. Jong-Yeon K, Hickner RC, Cortright RL, et al: Lipid oxidation is reduced in obese human skeletal muscle. Am J Physiol 279:E1039-E1044, 2000

15. Jackson AS, Pollock ML: Generalized equations for predicting body density of men. Br J Nutr 40:497-504, 1978

16. Jackson AS, Pollock ML, Ward A: Generalized equations for predicting body density of women. Med Sci Sports Exerc 12:175-182, 1980

17. Dohm GL, Huston RL, Askew EW, et al: Effects of exercise on activity of heart and muscle mitochondria. Am J Physiol 223:783-787, 1972

18. Sholte HR, Yu Y, Ross JD, et al: Rapid isolation of muscle and heart mitochondria, the lability of oxidative phosphorylation and attempts to stabilize the process in vitro by taurine, carnitine and other compounds. Mol Cell Biochem 174:61-66, 1997

19. McGarry JD, Mills SE, Long CS, et al: Observations on the affinity for carnitine, and malonyl-CoA sensitivity, of carnitine palmitoyltransferase I in animal and human tissues. Biochem J 214:21-28, 1983

20. Zierz S, Engel AG: Different sites of inhibition of carnitine palmitoyltransferase by malonyl Co-A, and by acetyl-CoA and CoA in human skeletal muscle. Biochem J 245:205-209, 1987

21. Saggerson ED, Carpenter CA: Carnitine palmitoyltransferase and carnitine octanoyltransferase activities in liver, kidney cortex, adipocytes, lactating mammary gland, skeletal muscle, and heart. FEBS Lett 129:229-232, 1981

22. Srere PA: Citrate synthase. Meth Enzymol 13:3-26, 1969

23. Costill DL, Fink WJ, Getchell LH, et al: Lipid metabolism in skeletal muscle of endurance-trained males and females. J Appl Physiol 47:787-791, 1979

24. Starritt EC, Howlett RA, Heigenhauser GJF, et al: Sensitivity of CPT1 to malonyl-CoA in trained and untrained human muscle. Am J Physiol 278:E462-E468, 2000

25. Gollnick PD, Saltin B: Significance of skeletal muscle oxidative enzyme enhancement with endurance training: hypothesis. Clin Physiol 2:1-12, 1982

26. Howald H, Hoppeler H, Claasen H, et al: Influence of endurance training on the ultrastructural composition of the different muscle fiber types in humans. Pfluegers Arch 403:369-376, 1985

27. McGarry JD, Mannaerts GP, Foster DW: A possible role for malonyl-CoA in the regulation of hepatic acid oxidation and ketogenesis. J Clin Invest 60:265-270, 1977

28. Hutber CA, Rasmussen BB, Winder WW: Endurance training attenuates the decrease in skeletal muscle malonyl-CoA with exercise. J Appl Physiol 83:1917-1922, 1997

29. Winder WW, Arogyasami J, Barton RJ, et al: Muscle malonyl-CoA decreases during exercise. J Appl Physiol 67:2230-2233, 1989

30. Winder WW, Arogyasami J, Elayan IM, et al: Time course of exercise-induced decline in malonyl-CoA in different muscle types. Am J Physiol 259:E266-E271, 1990

31. Odland LM, Heigenhauser GJF, Lopaschuk GD, et al: Human skeletal muscle malonyl-CoA at rest and during prolonged exercise. Am J Physiol 270:E541-E544, 1996

32. Odland L, Howlett RA, Heigenhauser GJF, et al: Skeletal muscle malonyl-CoA content at the onset of exercise at varying power outputs in humans. Am J Physiol 275:E1080-E1085, 1998

33. Dean D, Daugaard JR, Young ME, et al: Exercise diminishes the activity of acetyl-CoA carboxylase in human muscle. Diabetes 49: 1295-1300, 2000

34. Coggan AR, Del Corral P, Lynch NA, et al: Oxidation of ^{14}C-octanoate during moderate intensity exercise: muscle vs other tissues. FASEB J 14:A429, 2000 (abstr)

35. Binnert C, Pachiaudi C, Beylot M, et al: Influence of human obesity on the metabolic fate of dietary long- and medium-chain triacylglycerols. Am J Clin Nutr 67:595-601, 1998

36. Rossle C, Carpentier YA, Richelle M, et al: Medium-chain triglycerides induce alterations in carnitine metabolism. Am J Physiol 258:E944-E947, 1990