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Human skeletal muscle malonyl-CoA at rest and during prolonged submaximal exercise

L. MAUREEN ODLAND, GEORGE J. F. HEIGENHAUSER, GARY D. LOPASCHUK, AND LAWRENCE L. SPRIET

Department of Human Biology and Nutritional Sciences, University of Guelph, Guelph, Ontario N1G 2W1; Department of Medicine, McMaster University, Hamilton, Ontario L8N 3Z5; and Cardiovascular Disease and Lipid and Lipoprotein Research Groups, Department of Pediatrics and Pharmacology, Faculty of Medicine, University of Alberta, Edmonton, Alberta T6G 2S2, Canada

Odland, L. Maureen, George J. F. Heigenhauser, Gary D. Lopaschuk, and Lawrence L. Spriet. Human skeletal muscle malonyl-CoA at rest and during prolonged submaximal exercise. *Am. J. Physiol.* 270 (*Endocrinol. Metab.* 33): E541–E544, 1996.—Previous literature has indicated that contraction-induced decreases in malonyl-CoA are instrumental in the regulation of fatty acid oxidation during prolonged submaximal exercise. This study was designed to measure malonyl-CoA in human vastus lateralis muscle at rest and during submaximal exercise. Eight males and one female cycled for 70 min (10 min at 40% and 60 min at 65% maximal O_2 uptake). Needle biopsies were obtained at rest and at 10 min, 20 min, and 70 min of exercise. Malonyl-CoA content in preexercise biopsy samples determined by high-performance liquid chromatography (HPLC) was 1.53 ± 0.18 μmol/kg dry mass (dm). Malonyl-CoA content did not change significantly during exercise (1.39 ± 0.21 at 10 min, 1.46 ± 0.14 at 20 min, and 1.22 ± 0.15 μmol/kg dm at 70 min). In contrast, malonyl-CoA content determined by HPLC in perfused rat red gastrocnemius muscle decreased significantly during 20 min of stimulation at 0.7 Hz [3.44 ± 0.54 to 1.64 ± 0.23 nmol/g dm, (n = 9)]. We conclude that human skeletal muscle malonyl-CoA content *1*) is less than reported in rat skeletal muscle at rest, *2*) does not decrease with prolonged submaximal exercise, and *3*) is not predictive of increased fatty acid oxidation during exercise.

high-performance liquid chromatography; carnitine palmitoyltransferase I; fatty acid oxidation

MALONYL-CoA, the first committed intermediate produced during fatty acid synthesis, is a well-established regulator of fatty acid oxidation in the lipogenic tissues, adipose tissue and liver (10). It is produced in the cytoplasm by the action of acetyl-CoA carboxylase (ACC) and is a potent inhibitor of carnitine palmitoyltransferase I (CPT I) (4), the key regulatory enzyme responsible for the transfer of fatty acyl-CoA to the mitochondria for β-oxidation. Inhibition of CPT I by malonyl-CoA is not restricted to lipogenic tissues (15). An isoform of ACC has been found in heart muscle (3, 20), which appears to be regulated differently from hepatic ACC (14). Furthermore, measurable amounts of malonyl-CoA have been detected in the skeletal muscle of rats (18–20). Several recent investigations have promoted malonyl-CoA as a regulator of fatty acid oxidation in isolated cardiac myocytes (1), perfused working heart muscle (1, 14), and rat skeletal muscle during contractions (5, 18).

In rat skeletal muscle, malonyl-CoA levels declined with 30 min of submaximal treadmill exercise (18) and in response to electrical stimulation (5). This exercise-induced decrease in malonyl-CoA was postulated to be instrumental for increased fatty acid oxidation during prolonged exercise (18). Although increased malonyl-CoA content has been directly related to decreased fatty acid oxidation rates in perfused working heart muscle (14), only correlational data are currently available regarding the role of malonyl-CoA in skeletal muscle.

To date, there has been no reported attempt to measure malonyl-CoA content in human skeletal muscle. The presently used radiometric method to determine rat skeletal muscle malonyl-CoA content requires ≥200 mg of wet muscle (wm) (11). However, free and short-chain CoA esters can also be analyzed by high-performance liquid chromatography (HPLC), which requires less muscle tissue (9). The present investigation was designed to measure malonyl-CoA in human vastus lateralis muscle biopsy samples (50–100 mg wm) at rest and during prolonged aerobic exercise. It was hypothesized that a contraction-induced decrease in malonyl-CoA would occur in human skeletal muscle.

METHODS

Subjects. Eight males and one female volunteered to participate in the study. All were healthy and physically active (age, 24.5 ± 1.6 yr; height, 175 ± 9 cm; mass, 76.1 ± 9.6 kg). The experimental procedures, possible risks, and benefits were explained to each subject before written consent was obtained. The study received approval from the Human Ethics Committee of the University of Guelph and McMaster University.

Experimental protocol. Before the experiment, all participants performed an incremental maximal O_2 uptake ($\dot{V}O_{2max}$) test on a cycle ergometer. The mean $\dot{V}O_{2max}$ for the group was 3.96 ± 0.18 l/min. Each subject also participated in a practice trial to determine the power outputs required to elicit 40 and 65% $\dot{V}O_{2max}$.

On the morning of the experiment, subjects reported to the laboratory having eaten a meal high in carbohydrate 2–4 h

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before the experiment. Daily food records were kept for 48 h preceding the test session, and subjects were instructed to refrain from caffeine consumption and intense physical activity for 24 h before testing. These procedures were implemented to ensure high muscle and blood carbohydrate content, thus preventing elevated resting plasma free fatty acid concentration before exercise. One leg was prepared for percutaneous needle biopsy of the vastus lateralis with four incisions of the skin through to the deep fascia under local anesthesia (2% lidocaine without epinephrine) as described by Bergström (2). Special care was taken to ensure subjects 30 min of complete rest (i.e., no muscle contraction) before the preexercise muscle sample was obtained with the subject supine on a bed.

Exercise consisted of a single 70-min submaximal exercise bout on a cycle ergometer (10 min at 40% $\dot{V}O_{2max}$ and 60 min at 65–70% $\dot{V}O_{2max}$). In addition to the preexercise muscle biopsy, a biopsy was taken after 10 min of exercise at 40% $\dot{V}O_{2max}$, after 10 min at 65% $\dot{V}O_{2max}$ (20 min), and at the end of the exercise period. Muscle samples (50–150 mg wm) were frozen immediately in liquid N_2, removed from the needle while frozen, and stored in liquid N_2 until analyzed. O_2 uptake ($\dot{V}O_2$), CO_2 output ($\dot{V}CO_2$), and respiratory exchange ratio (RER) were determined throughout exercise with a metabolic cart (Q-plex 1, Quinton Instrument, Seattle, WA). The rate of fat oxidation (g/min) was calculated from $\dot{V}CO_2$ and $\dot{V}O_2$ by use of the nonprotein respiratory quotient (12).

Rodent skeletal muscle. Rat red gastrocnemius (RG) muscle was obtained from a larger study (7) in which perfused hindlimb musculature was stimulated at 0.7 Hz for 20 min. Muscle samples were freeze-clamped before and immediately after stimulation. Freeze-dried RG muscle was analyzed for malonyl-CoA content.

Analyses. A total of 35 human muscle samples were obtained, and in 9 sufficiently large samples (>100 mg wm), 20–60 mg of wet muscle were removed from the biopsy under liquid N_2 to allow for duplicate wet-to-dry analysis of malonyl-CoA. Remaining frozen muscle was freeze-dried, dissected free of blood and connective tissue, thoroughly powdered, and stored dry at −80°C. Rat muscle (~80–100 mg wm) was also freeze-dried and powdered. A portion of the human dry muscle (dm; 2–4 mg) was extracted in 0.5 M perchloric acid (PCA) and 1 mM EDTA, neutralized to pH 7.0 with 2.2 M $KHCO_3$, and analyzed for phosphocreatine (PCr), adenosine triphosphate (ATP), total creatine (TCr), and lactate. All human muscle metabolites were normalized to the highest TCr content for a given individual [mean ($\bar{x}$) TCr = 130.6 ± 4.4 mmol/kg dm] to correct for nonmuscle contamination.

Malonyl-CoA was extracted from freeze-dried and wet tissue with 0.5 M PCA. Wet tissue extraction occurred in 1.5 ml plastic Eppendorf microfuge tubes. Frozen tissue (~60 mg wm) was immersed in ice-cold PCA (PCA vol = 3 × mg wm), and muscle was homogenized to allow complete extraction. Aliquots of dry tissue (~8 mg dm) were placed in 12 × 75-mm glass tubes, and ice-cold PCA was added in the ratio of 10 µl/mg dm. Tubes were gently stirred to ensure all muscle was saturated before centrifugation at 3,500 revolutions/min for 10 min at 4°C. Supernatants were transferred to HPLC auto sample vials containing 1 M 3-(*N*-morpholino)propanesulfonic acid (pH 6.8, 10.9% total extract volume) and 0.32 M dithiothreitol (5.3% total extract volume) and analyzed immediately. CoA esters were separated and quantified using a previously described methodology (9, 14).

Nine human biopsy samples were analyzed for malonyl-CoA content in both wet and freeze-dried tissue. The coefficient of variation (CV) between duplicate wet ($\bar{x}$ = 1.46 ± 0.37 µmol/kg dm) and dry ($\bar{x}$ = 1.39 ± 0.36 µmol/kg dm) samples was 2.36%. In addition, duplicate aliquots (8 mg dm) of freeze-dried muscle tissue were extracted separately. The CV for these duplicates was 2.16% (n = 14). The formula used to determine CV was

$$\sqrt{\Sigma(D - \bar{D})^2/[2(n - 1)]}/[(\bar{x}_1 + \bar{x}_2)/2]$$

where D is the difference between duplicate measures, and n = 2 for 2 measures.

Statistical analysis. Data are expressed as means ± SE. To determine significant differences between means over time, a one-way analysis of variance (ANOVA) was performed. Missing data points were generated automatically (MiniTab Release 10.0 for Windows). A Tukey post hoc analysis was used to determine the location of significant differences. Rat tissue malonyl-CoA content differences between control muscle and stimulated perfused muscle were compared using a one-tailed paired t-test. Results were considered significant at $P < 0.05$.

RESULTS

Human skeletal muscle. Preexercise malonyl-CoA concentration was 1.53 ± 0.18 µmol/kg dm (n = 9) (Fig. 1). During exercise, it was unchanged at 1.39 ± 0.21 µmol/kg dm after 10 min at 40% $\dot{V}O_{2max}$ (n = 7), 1.46 ± 0.14 µmol/kg dm after 10 min at 65% $\dot{V}O_{2max}$ (20 min, n = 8), and it reached 1.22 ± 0.15 µmol/kg dm at the end of exercise (70 min, n = 7). Repeated-measures ANOVA revealed no significant differences between mean values at any time point ($P > 0.05$).

ATP levels remained constant throughout exercise (Table 1), whereas PCr decreased significantly during exercise at 65% $\dot{V}O_{2max}$. There was no change in muscle lactate during exercise at 40% $\dot{V}O_{2max}$, but it increased significantly above rest levels after 10 min at 65% $\dot{V}O_{2max}$. By the end of the exercise protocol, lactate levels were not significantly different from those at rest.

RER increased significantly from 4–6 min (40% $\dot{V}O_{2max}$) to 14–16 min (65% $\dot{V}O_{2max}$), concomitant with the increase in workload at 10 min (Table 2). RER then

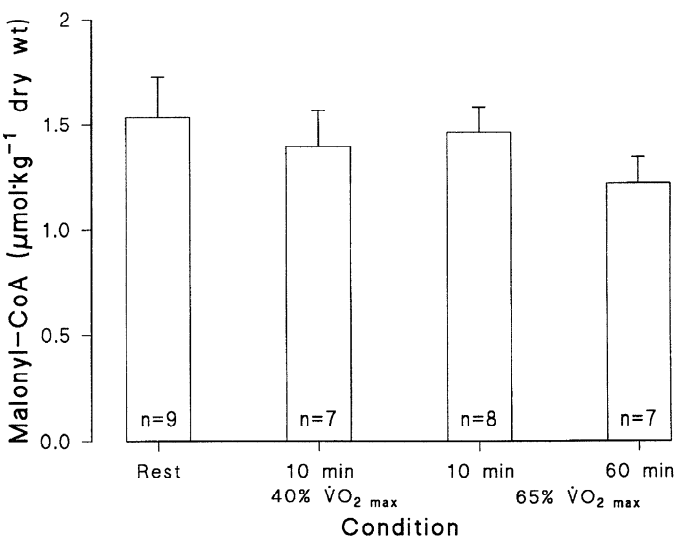

Fig. 1. Malonyl-CoA content in human skeletal muscle, at rest and after 10 min at 40% maximal O_2 uptake ($\dot{V}O_{2max}$), followed by 10 and 60 min at 65% $\dot{V}O_{2max}$.

Table 1. *Muscle metabolite contents during prolonged submaximal exercise*

	Time, min			
	0	10	20	70
ATP	24.03 ± 1.08	23.77 ± 0.78	24.38 ± 1.26	24.44 ± 1.03
PCr	81.2 ± 3.6	73.1 ± 3.1	47.9 ± 2.0*†	42.2 ± 5.2*†
Lactate	7.9 ± 2.2	9.5 ± 1.9	28.7 ± 7.15*†	21.3 ± 3.9

Values are means ± SE of 7 human muscle samples expressed in mmol/kg dry mass. PCr, phosphocreatine. *Significantly different from rest (0 min); † significantly different from 10 min.

decreased during 60 min at 65% $\dot{V}O_{2max}$ such that the values at 30–32, 46–48, and 64–66 min were significantly less than the value at 14–16 min. In addition, RER values at 30–32 and 46–48 min were significantly higher than the value at 4–6 min. Similarly, the rate of fat oxidation increased during the 65% $\dot{V}O_{2max}$ workload (Table 2). Fat oxidation rates at 30–32, 46–48, and 64–66 min were significantly greater than the rate at 14–16 min, and the rate at 64–66 min was also significantly greater than at 4–6 min.

Rat skeletal muscle. Malonyl-CoA content in control and stimulated perfused muscle was 3.44 ± 0.54 and 1.64 ± 0.23 nmol/g dm, respectively (n = 9). This corresponded to a 52% decrease in malonyl-CoA from the resting value in response to stimulation at 0.7 Hz ($P < 0.05$).

DISCUSSION

The primary purpose of this investigation was to measure malonyl-CoA in human skeletal muscle biopsy samples at rest and during prolonged submaximal exercise. The mean resting content in vastus lateralis muscle was 1.53 ± 0.18 μmol/kg dm, and this content did not change in the first 20 min of exercise. At the completion of exercise, malonyl-CoA declined to 80% of the preexercise content (1.22 ± 0.15 μmol/kg dm), but this decrease was not statistically significant. Therefore our hypothesis was rejected, because two intensities of submaximal exercise did not decrease malonyl-CoA in human skeletal muscle. This is in contrast to rat skeletal muscle data, where a decrease in malonyl-CoA level was observed after stimulation that paralleled previous reports [see Winder et al. (18)].

Table 2. *Workload, RER, and fat utilization at various time points during prolonged submaximal cycle exercise*

Time, min	%$\dot{V}O_{2max}$	RER	Fat, g/min
4–6	41.1 ± 0.7	0.88 ± 0.02*	0.33 ± 0.05
14–16	64.3 ± 2.2	0.95 ± 0.02	0.20 ± 0.10
30–32	67.8 ± 2.5	0.91 ± 0.02*†	0.39 ± 0.09*
46–48	69.2 ± 2.1	0.91 ± 0.02*†	0.42 ± 0.08*
64–66	69.1 ± 2.7	0.89 ± 0.02*	0.50 ± 0.13*†

Values are means ± SE of 8 muscle samples. Workload, % maximal O_2 uptake ($\dot{V}O_{2max}$). RER, respiratory exchange ratio. Exercise consisted of 10 min at 40% $\dot{V}O_{2max}$ followed by 60 min at 65% $\dot{V}O_{2max}$. *Significantly different from 14–16 min; † significantly different from 4–6 min.

It has been suggested that decreases in malonyl-CoA may be important in regulating the rate of fatty acid oxidation in skeletal muscle during exercise because of decreased inhibition of CPT I (18, 20). This hypothesis is based solely on correlational data from rat skeletal muscle, in which significant decreases in malonyl-CoA have been reported after prolonged exercise (18, 19). To date, no study has attempted to demonstrate a causal relationship between malonyl-CoA content and fatty acid oxidation rates in skeletal muscle during exercise. Human muscle malonyl-CoA measured in the present study is less than that reported in rat muscle, and this content remained constant throughout exercise. This occurred despite a significant decrease in RER and a significant increase in the calculated rate of fat oxidation during exercise at 65% $\dot{V}O_{2max}$. Furthermore, previous literature (13) has also reported an increase in fat oxidation and free fatty acid turnover during prolonged exercise at 65% $\dot{V}O_{2max}$ measured with indirect calorimetry and tracer technology, respectively. Therefore our data suggest one or both of two possibilities: *1*) malonyl-CoA is not a primary regulator of fatty acid oxidation in human skeletal muscle, and/or *2*) malonyl-CoA levels are not predictive of the fatty acid oxidation rate in human skeletal muscle during prolonged exercise. It should be noted that there was a 16% decrease in malonyl-CoA from 10 to 70 min of cycling at 65% $\dot{V}O_{2max}$. Although this decrease was not statistically significant, it remains uncertain whether the change is biologically significant. In addition, the time course of this 16% decrease is unknown except that it occurred sometime between 10 and 70 min. A more detailed time course analysis is required to answer this question.

McGarry et al. (10) determined that the concentration of malonyl-CoA required for 50% inhibition of enzyme activity (I_{50}) for human semitendinosis muscle CPT I was 0.025 μM. The calculated concentration of malonyl-CoA measured in the present study ranged between ~2 and 9 μM, which, if located entirely in the cytoplasm, should be sufficient to completely inhibit CPT I. However, the I_{50} determination was in vitro, and it is highly probable that, in vivo, other regulators are present that override inhibition from malonyl-CoA. Additionally, it is possible that only a fraction of the total malonyl-CoA content measured is available to interact with CPT I. Although it is expected that the bulk of hepatic malonyl-CoA is present extramitochondrially (10), the distribution of malonyl-CoA in skeletal muscle is uncertain. It has been established that propionyl-CoA carboxylase (present in muscle mitochondria) can nonspecifically use acetyl-CoA to produce malonyl-CoA within the mitochondria (8). Thus there is potential for the majority of malonyl-CoA present in human skeletal muscle to be within the mitochondria. Furthermore, rat skeletal muscle mitochondria possess substantial numbers of low-affinity binding sites for malonyl-CoA (16). Thus the possibility exists that overall measurements of tissue malonyl-CoA are not predictive of the concentration of malonyl-CoA seen by CPT I.

Malonyl-CoA content in human skeletal muscle ranged from 0.68 to 2.72 μmol/kg dm (n = 35). These

values are considerably lower than previously reported rat skeletal muscle data, which range between ~3 and 12 nmol/g dm depending on the muscle analyzed (19). The difference between rat and human values is most likely due to species differences. Human skeletal muscle is heterogeneous with respect to muscle fiber type, whereas rat skeletal muscle samples are more homogeneous. Human vastus lateralis muscle is comprised of ~50% type I, 40% type IIa, and 10% type IIb fibers (17). In addition, the difference in malonyl-CoA levels could be due to differences in mitochondrial levels (i.e., the need for propionyl-CoA carboxylase and anaplerosis in human vs. rat skeletal muscle).

Rat skeletal muscle. Rat skeletal muscle malonyl-CoA was measured by HPLC in an attempt to confirm previous work with rat muscle. Malonyl-CoA in rat RG muscle at rest ranged from 1.36 to 6.30 nmol/g dm ($\bar{x}$ = 3.44 ± 0.54 nmol/g dm, n = 14) and decreased to 1.63 ± 0.23 nmol/g dm after stimulation at 0.7 Hz (~73% of twitch $\dot{V}O_{2max}$). Malonyl-CoA contents in the present investigation (RG) are lower than those reported for the red quadriceps muscle (~11 nmol/g dm) when the radiometric technique is used (19). However, the present 52% decrease in malonyl-CoA with stimulated contraction is similar to the 42% decrease reported previously with electrical stimulation (5 Hz for 5 min) (5) and the 64% decrease with prolonged treadmill exercise (30 min, 21 m/min, 15% grade) (18). Although the present results agree with those previously reported on a relative basis, it is apparent that a comparison between the two measurement techniques is required to reconcile the different absolute values.

In summary, skeletal muscle malonyl-CoA was measured in human and rat tissue by HPLC. Rest and exercised human muscle malonyl-CoA content was less than that determined in rat tissue. Human malonyl-CoA was not affected by exercise. The present results suggest that *1*) species differences exist in malonyl-CoA content between rat and human skeletal muscle, *2*) human muscle malonyl-CoA does not decrease with 10 min of cycle ergometry at 40% and 60 min at 65% $\dot{V}O_{2max}$, and *3*) overall tissue levels of malonyl-CoA are not predictive of fatty acid oxidation rate in human muscle during exercise. The data in the present study suggest that a more detailed time course of the malonyl-CoA response to exercise at 65% $\dot{V}O_{2max}$ is required. In addition, further studies are necessary to determine the response of human skeletal muscle malonyl-CoA to exercise of different intensities and durations.

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Address for reprint requests: L. M. Odland, Dept. of Human Biology and Nutritional Sciences, Univ. of Guelph, Guelph, Ontario N1G 2W1, Canada.

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