Effects of high fat provision on muscle PDH activation and malonyl-CoA content in moderate exercise

L. MAUREEN ODLAND, GEORGE J. F. HEIGENHAUSER, AND LAWRENCE L. SPRIET
Department of Human Biology and Nutritional Sciences, University of Guelph, Guelph N1G 2W1; and Department of Medicine, McMaster University, Hamilton, Ontario, Canada L8N 3Z5

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Odland, L. Maureen, George J. F. Heigenhauser, and Lawrence L. Spriet. Effects of high fat provision on muscle PDH activation and malonyl-CoA content in moderate exercise. J Appl Physiol 89: 2352–2358, 2000.—This study examined the effects of elevated free fatty acid (FFA) provision on the regulation of pyruvate dehydrogenase (PDH) activity and malonyl-CoA (M-CoA) content in human skeletal muscle during moderate-intensity exercise. Seven men rested for 30 min and cycled for 10 min at 40% and 10 min at 65% of maximal 

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and fat metabolism during exercise will be heavily dependent on the balance between the activities of these key enzymes.

Several studies have reported significant sparing of muscle glycogen during exercise with elevated FFA concentration ([FFA]) in humans (for review see Ref. 36), but few have examined the mechanisms responsible for the reduced CHO utilization. Exercise findings at 85% of maximal 

FFA into the mitochondria, although little is known about the regulation of skeletal muscle CPT I during exercise, particularly in humans. Because in vitro CPT I activity is potently inhibited by malonyl-CoA (M-CoA) (4, 18, 31), it has been proposed that the increase in fatty acid oxidation in rat skeletal muscle during exercise may be due to a decrease in M-CoA content (40, 41). However, human muscle measurements revealed that M-CoA content did not correlate with changes in fat oxidation during cycle exercise (20, 22). In addition, in vitro CPT I activity is sensitive to small decreases in pH and the aerobic training status of the muscle (37). No study to date has determined whether an acute elevation of [FFA] alters human muscle M-CoA concentration at rest or during exercise.

The present investigation was to designed to determine the transport of FFA into the mitochondria, although little is known about the regulation of skeletal muscle CPT I during exercise, particularly in humans. Because in vitro CPT I activity is potently inhibited by malonyl-CoA (M-CoA) (4, 18, 31), it has been proposed that the increase in fatty acid oxidation in rat skeletal muscle during exercise may be due to a decrease in M-CoA content (40, 41). However, human muscle measurements revealed that M-CoA content did not correlate with changes in fat oxidation during cycle exercise (20, 22). In addition, in vitro CPT I activity is sensitive to small decreases in pH and the aerobic training status of the muscle (37). No study to date has determined whether an acute elevation of [FFA] alters human muscle M-CoA concentration at rest or during exercise.

The present investigation was to designed to determine the mechanism(s) responsible for the lower PDHa early in exercise (1 and 10 min at 40% and 10 min at 65% 

Address for reprint requests and other correspondence: L. L. Spriet, Dept. of Human Biology and Nutritional Sciences, Univ. of Guelph, Guelph, ON, Canada N1G 2W1 (E-mail: Ispriet@uoguelph.ca).
possibly contribute to the increased CPT I activity and fat oxidation in this condition.

METHODS

Subjects. Seven men volunteered to participate in the study. One subject was aerobically well trained, three were highly active, and three were untrained (age, 22.6 ± 0.8 yr; height, 179 ± 8 cm; mass, 83.2 ± 8.4 kg; \(\text{VO}_2\max\), 4.20 ± 0.23 l/min). Written consent was obtained from each subject after the experimental procedures and possible risks and benefits were clearly explained. The study was approved by the Human Ethics Committees of both universities.

Preexperimemtal protocol. All subjects initially performed an incremental \(\text{VO}_2\max\) test on a cycle ergometer. On a separate day, they participated in a practice trial to confirm the power outputs required to elicit 40 and 65% \(\text{VO}_2\max\). Written consent was obtained from each subject after the experimental procedures and possible risks and benefits were clearly explained. The study was approved by the Human Ethics Committees of both universities.

Experimental protocol. Each subject participated in two experimental trials, separated by 1–2 wk. On the morning of each trial, subjects reported to the laboratory having eaten a light meal high in CHO content ~2 h before arrival. In addition, all participants consumed a snack consisting of 2–3 bagels and 250–500 ml Gatorade 1 h before exercise in each trial to ensure low resting plasma [FFA].

Before exercise, a catheter was inserted into an arm vein for Intralipid-heparin or saline infusion, and blood samples were also drawn from this catheter (Fig. 1). A resting blood sample was taken just before the onset of the infusion (~30 min, Fig. 1), which involved a similar volume of saline (control) or a 20% triacylglycerol (TG) Intralipid-heparin solution (Int; Clintec Nutrition, Mississauga, ON). Intralipid is composed of 20% soybean oil (50% linoleic acid), 1.2% egg phospholipids, and 2.25% glycerol. The saline and Intralipid infusion rates were 100 ml/h for 30 min at rest and 20 min during exercise (Fig. 1). A total of 2,000 U of sterile heparin was administered in three doses during the rest portion of the Int trial to facilitate hydrolysis of the infused TG to FFA (Fig. 1).

In the following 30 min, one leg was prepared for percutaneous needle biopsy of the vastus lateralis muscle. Four incisions were made in the skin through to the deep fascia, under local anesthesia (2% lidocaine without epinephrine) as described by Bergström (3). Immediately before exercise (0 min), a resting muscle sample was obtained with the subject lying on a bed. The subject was then seated on the cycle ergometer (Quinton Excalibur, Quinton Instrument, Seattle, WA), and a second pre-exercise blood sample was drawn (0 min, Fig. 1). Subjects then cycled for 10 min at 40% \(\text{VO}_2\max\), followed by 10 min at 65% \(\text{VO}_2\max\). \(\text{O}_2\) uptake, \(\text{CO}_2\) output, and the respiratory exchange ratio (RER) were determined throughout exercise using a metabolic cart (Quinton Q-Plex 1, Quinton Instrument, Seattle, WA). Blood samples and muscle biopsies were taken at the time points indicated in Fig. 1.

Blood analyses. Blood samples (~3 ml) were drawn in heparinized plastic syringes and placed on ice. Plasma for FFA determination (Wako NEFA C test kit, Wako Chemical, Osaka, Japan) was obtained by immediate centrifugation of heparinized blood and incubation of 400 μl plasma with 100 μl 6 M NaCl at 56°C (10). This procedure denatures the lipoprotein lipase released into the blood by heparin injection and avoids TG hydrolysis and elevated FFA in the test tubes. A 200-μl aliquot of whole blood was deproteinized in 1.0 ml of 0.6 M HClO₄, and the supernatant was used for fluorometric determination of whole blood glucose and lactate.

Muscle analyses. Muscle samples were immediately frozen in liquid N₂. A small piece (10–15 mg) was chipped from each biopsy (under liquid N₂) for the measurement of PDH activity, as previously described (6). The remainder of the sample was freeze-dried, dissected free of blood and connective tissue, and powdered. A portion of dry muscle (~5 mg) was extracted in 0.5 M HClO₄ and 1 M EDTA, neutralized to pH 7.0 with 2.2 M KHCO₃, and analyzed for phosphocreatine (PCr), creatine, ATP, glucose-6-phosphate (G6P), NAD, pyruvate, and lactate (2, 13). A second portion of dry muscle (2 mg) was extracted in 0.5 M KOH, ethanol (50%), and 5 mM cysteine; neutralized with HCl; and assayed for NADH by using a previously described bioluminescent method (29, 34) and NADH monitoring kit (Bio-Orbit, Finland). Additional aliquots of dry muscle (8–12 mg) from the rest and final exercise biopsies (20 min) were assayed by HPLC for M-CoA content, as previously described (20, 22). The assay for M-CoA content requires a large amount of dry muscle (>10 mg), and this limited the analysis to resting and 20-min exercise samples. In addition, paired samples were only available for seven subjects at rest and for
five subjects at 20 min of exercise. To increase the sample size, M-CoA contents measured in a previous Intralipid study (Ref. 21; paired samples, n = 3 at rest and n = 5 at 20 min) were pooled with the present data. All aspects of the previous study (21) were identical to the present study including the same power outputs, biopsy sampling at exactly the same times, and similar venous FFA and RER differences between the high-FFA and control trials. All muscle measurements were normalized to the highest total creatine content for a given individual’s biopsies to correct for nonmuscle contamination.

Statistical analyses. Because one purpose of the paper was to examine the transition from rest to exercise, the data from the resting and 1 min (0- and 1-min) muscle samples were analyzed independently from the 10 and 20 min samples by two-way (rest or exercise vs. trial) ANOVA with repeated measures. The complete exercise data set (1, 10, and 20 min) was also analyzed by two-way (time vs. trial) ANOVA with repeated measures. When a significant F ratio was obtained, the Tukey A post hoc test was used to compare means. M-CoA contents were compared between conditions at rest and again at 20 min of exercise by using a one-tailed paired-samples t-test. Significance was accepted at P < 0.05.

RESULTS

Respiratory gas exchange. Whole body \( \dot{V}O_2 \) uptake was similar in the two conditions and represented 44 ± 0.01 vs. 43 ± 0.01 and 68 ± 0.02 vs. 69 ± 0.02% \( V_{O_2 \max} \) in the control and Int trials, respectively (Table 1). The RER was significantly lower during Int at both power outputs (Table 1).

Blood metabolites. Plasma [FFA] was unchanged at rest and during exercise in control but increased from 0.32 ± 0.05 to 0.91 ± 0.12 mM with Int infusion at rest (Fig. 2). Exercise [FFA] with Int was significantly higher than control at all time points. Whole blood glucose concentration was unaffected by Int but decreased significantly below resting levels at 8 and 18 min of exercise in both conditions (Table 2). Whole blood lactate concentration was similar between conditions and increased above rest levels at 8 and 18 min of exercise in both conditions (Table 2).

Muscle metabolites. The Int infusion had no effect on muscle PCr, G6P, and ATP contents at rest, during the rest-to-exercise transition, or during sustained exercise (Table 3). Muscle ATP content remained constant throughout exercise in both conditions, whereas PCr content declined significantly at 1 min of exercise and decreased further at 20 min in both trials (Table 3). G6P content did not increase significantly after 1 or 10 min of exercise at 40\( V_{O_2 \max} \) but was elevated at 20 min of exercise (65\( V_{O_2 \max} \)) in both conditions (Table 3).

Muscle lactate increased in both conditions during the transition from rest to exercise with no effect of the elevated FFA (Fig. 3). However, lactate was significantly elevated during steady-state exercise with Int. Muscle pyruvate was significantly lower at rest during Int infusion, but the rest-to-exercise transition (40% \( V_{O_2 \max} \)) appeared to increase pyruvate during Int at 1 min, although the change was not significant (Fig. 4).

NAD remained constant at rest and during exercise in both conditions (Table 3). NADH was significantly elevated at rest and at 1 min of exercise during Int, because NADH increased to the same extent during the rest-to-exercise transition in both conditions (Fig. 5). During steady-state exercise, however, no significant NADH differences existed between conditions.

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Table 1. Respiratory gas exchange variables during 10 min of cycle exercise at 40% and 10 min at 65% \( V_{O_2 \max} \) during Intralipid infusion or control

<table>
<thead>
<tr>
<th>Trial</th>
<th>4–7 min ( 40% V_{O_2 \max} )</th>
<th>14–17 min ( 65% V_{O_2 \max} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \dot{V}O_2 ) l/min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.85 ± 0.11</td>
<td>2.92 ± 0.14</td>
</tr>
<tr>
<td>Intralipid</td>
<td>1.82 ± 0.12</td>
<td>2.95 ± 0.13</td>
</tr>
<tr>
<td>RER</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.87 ± 0.01</td>
<td>0.95 ± 0.01</td>
</tr>
<tr>
<td>Intralipid</td>
<td>0.83 ± 0.01*</td>
<td>0.92 ± 0.02*</td>
</tr>
</tbody>
</table>

Values are means ± SE, \( n = 7 \). RER, respiratory exchange ratio; \( \dot{V}O_2 \), \( O_2 \) uptake; \( V_{O_2 \max} \), maximal \( V_{O_2} \). \*Significantly different from control.

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Table 2. Whole blood glucose and lactate concentrations during 10 min of cycle exercise at 40% and 10 min at 65% \( V_{O_2 \max} \) after Intralipid infusion or control

<table>
<thead>
<tr>
<th>Trial</th>
<th>–30 min</th>
<th>Rest</th>
<th>0 min</th>
<th>8 min</th>
<th>20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_{O_2 \max} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.13 ± 0.27</td>
<td>4.57 ± 0.55</td>
<td>3.34 ± 0.44*</td>
<td>3.21 ± 0.31*</td>
<td></td>
</tr>
<tr>
<td>Intralipid</td>
<td>3.39 ± 0.49</td>
<td>3.72 ± 0.12</td>
<td>2.93 ± 0.22*</td>
<td>3.07 ± 0.17*</td>
<td></td>
</tr>
<tr>
<td>Lactate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.40 ± 0.08</td>
<td>0.52 ± 0.03</td>
<td>1.69 ± 0.27†</td>
<td>6.07 ± 0.46†</td>
<td></td>
</tr>
<tr>
<td>Intralipid</td>
<td>0.61 ± 0.14</td>
<td>0.64 ± 0.10</td>
<td>1.82 ± 0.18†</td>
<td>5.76 ± 0.59†</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE, \( n = 7 \). †Significantly different from 0 min. \*Significantly different from rest (–30 and 0 min).
Consequently, the NAD-to-NADH ratio during Int was significantly lower than control at rest and 1 min of exercise but not different between conditions during sustained exercise (Table 3).

The Int infusion did not affect PDHa at rest, but the activation in response to exercise was significantly reduced at 1 min of exercise and remained lower throughout exercise (Fig. 6).

The 30 min of Int infusion significantly decreased M-CoA at rest (control vs. Int; 1.43 ± 0.17 vs. 1.12 ± 0.22 μmol/kg dm, n = 10) and after 20 min of exercise (1.84 ± 0.17 vs. 1.33 ± 0.16 μmol/kg dm, n = 10).

DISCUSSION

This study examined the effect of increased fat availability on whole body substrate utilization, the regulation of skeletal muscle PDHa, and muscle M-CoA content during moderate exercise. At rest, an acute increase in [FFA] increased muscle NADH content, decreased the NAD\(^+\)-to-NADH ratio, and decreased pyruvate and M-CoA contents. The Int infusion reduced PDHa during the rest-to-exercise transition (40% \(V\dot{O}_2\)max), most likely because of a lower muscle [pyruvate] at rest and higher muscle NADH concentration ([NADH]) at rest and 1 min of exercise. With sustained exercise (40 and 65% \(V\dot{O}_2\)max), RER was lower in the high-fat trial vs. control, and muscle PDHa remained blunted by an unexplained mechanism, while the [NADH] returned to control values, and muscle pyruvate was higher than control. Muscle M-CoA content was also lower after 20 min in the high-fat trial, which may have reduced the inhibition on muscle CPT I activity and contributed to the increased fat oxidation.
The transition to exercise. In the first minute of exercise, the higher [NADH] was maintained, whereas [pyruvate] actually increased above the control trial. It therefore appears that the higher NADH in the Int trial was the primary factor inhibiting PDH at the onset of exercise. In vitro evidence suggests that PDH kinase is stimulated by NADH and inhibited by NAD$^+$ (24), supporting the suggestion that PDH inhibition by PDH kinase was not relieved to the same extent at the onset of exercise in the Int trial.

The lower PDHα reported in the first minute of exercise was maintained during exercise continued for 10 min at 40% and 10 min at 65% $V_{O_{2\text{max}}}$ as previously reported (21). Interestingly, muscle NADH content was no longer different from the control trial at 10 and 20 min of exercise. The only difference in the acute regulators of PDH between trials was the higher muscle [pyruvate], which would predict a higher PDHα during the high-FFA trial. Clearly, other regulatory mechanism(s) must be involved in PDH regulation during low- and moderate-intensity exercise in the presence of high FFA. It should also be noted that high FFA provision did not blunt PDHα during the onset of exercise and steady-state intense exercise at 80–85% $V_{O_{2\text{max}}}$ (9, 10). It appeared that the higher $Ca^{2+}$ concentration at these power outputs activated PDH by stimulating PDH phosphatase and dominated the inhibition by PDH kinase to the same extent in both the control and high-FFA trials.

Additional regulatory mechanisms, such as multisite phosphorylation of the E1 component of the PDH complex, may also be affected by high FFA provision. PDH kinase deactivates PDH by phosphorylating three sites on the E1 component (sites 1, 2, and 3), whereas PDH phosphatase activates PDH by dephosphorylating at these sites. PDH can be further regulated via a sequential pattern of phosphate addition and removal at sites 1, 2, and 3 (35). Although PDHα is the result of phosphorylation-dephosphorylation at site 1 (inactivating site), sites 2 and 3 serve as barriers to restrict the access of PDH phosphatase to site 1. PDH kinase activity initially increases site 1 occupancy on the E1 components. As phosphorylation continues and increases site 2 and then site 3 occupancy, the inhibition increases. Hence, PDH phosphatase must dephosphorylate sites 3, 2, and 1 before activation occurs. It is not known whether high FFA exposure increases the kinase-induced phosphorylation and/or decreases the phosphatase-induced dephosphorylation.

High FFA provision, M-CoA content, and fat metabolism. We have previously argued that high fat availability leads to increased FFA uptake and oxidation and increased mitochondrial NADH content at rest and during the onset of exercise (5, 9). The findings of From et al. (11) using Langendorff perfused rat hearts support this suggestion. They reported that the provision of short-chain fatty acids, which rapidly cross cell membranes, increased fat oxidation and estimated mitochondrial NADH content and improved the cell energy state. Normally, the rates of FFA transport into the cell and the mitochondria appear to limit fat metabolism during exercise. However, extra FFA provi-
sion with acute fat infusion and caffeine administration may partially circumvent this problem (5, 9, 10).

The decreased RER during exercise with high FFA in this study suggested that skeletal muscle FFA oxidation was increased. M-CoA is a potent inhibitor of skeletal muscle CPT I, the enzyme responsible for FFA entry into the mitochondria (4, 17, 18, 31). Studies in rat muscle suggest that reductions in M-CoA during exercise are responsible for increased fat oxidation (40, 41). However, human studies report no decrease in M-CoA during aerobic exercise of varying power outputs and rates of fat oxidation (20, 22) and that CPT I is sensitive to additional regulators (37). Therefore, we suggested that, although the M-CoA-CPT I interaction was important for fuel selection at rest (30), it was not important for fuel selection in human skeletal muscle during exercise. However, the present results demonstrate that, despite no exercise-induced changes in M-CoA concentration, an acute increase in FFA provision decreased resting M-CoA content, and this effect persisted throughout exercise at 65% \( \overline{V}O_2 \) max. The decreased M-CoA content correlated with increased fat oxidation and reduced PDHa, and it may have increased fat use by reducing CPT I inhibition and increasing FFA transport into the mitochondria.

The mechanism for the reduction in M-CoA content at rest and during exercise was not investigated in this study but must be related to inactivation of acetyl-CoA carboxylase (ACC), the enzyme responsible for catalyzing the production of M-CoA and/or activation of M-CoA decarboxylase, the enzyme that degrades M-CoA. The regulation of ACC in resting and contracting rat skeletal muscle has been recently reviewed (30, 42). A large body of work has determined that inactivation of ACC can result from AMP kinase-induced phosphorylation, mediated by increases in muscle calcium and free AMP and decreases in PCr (16, 19, 25, 27, 39). ACC activity may also be reduced by 1) a lack of cytoplasmic acetyl-CoA, a substrate for the enzyme (or citrate and acetylcarboxenitine, potential providers of cytoplasmic acetyl-CoA), 2) a decrease in citrate content, an activator of ACC (32, 33, 38), and 3) direct inhibition of ACC by fatty acyl-CoA (1, 33, 38). It may be argued that an increased fatty acyl-CoA content would be the most logical candidate to explain the decreased M-CoA in the high-FFA condition. It has also been suggested that activation of M-CoA decarboxylase may contribute to decreases in M-CoA content in certain situations in heart and skeletal muscle (1, 12). However, to date, none of the above issues has been examined in human skeletal muscle at rest or during exercise.

An additional factor may have contributed to the regulation of fat metabolism in the present study. The RER increased and fat metabolism decreased when the exercise intensity was increased from 40 to 65% \( \overline{V}O_2 \) max in both trials (Table 1). Recent work in human skeletal muscle suggested that the reduced fat metabolism at higher power outputs may be partially mediated by a decrease in muscle pH, as in vitro mitochondrial CPT I activity decreased by ~40% when pH decreased from 7.0 to 6.8 (37). Estimated pH (muscle lactate and pyruvate contents) in the present study decreased from ~7.0 at rest in both trials to 6.87–6.91 at 40% and 6.74–6.76 at 65% \( \overline{V}O_2 \) max. Therefore, it is likely that the increasing acidosis contributed to the lower fat metabolism during the higher power output in both trials.

Lastly, it must be remembered that the present interpretations based on human skeletal muscle samples and previous interpretations based on rat muscle samples may be confounded by the uncertainty regarding the cytoplasmic and mitochondrial concentrations of key regulators, including NADH, pyruvate, M-CoA, citrate, and several others. Although compartment concentrations can be predicted, this issue has not been resolved in rat and human skeletal muscle.

In summary, an acute increase in FFA provision decreased PDHa in human skeletal muscle at the onset of exercise (1 min) at 40% \( \overline{V}O_2 \) max as previously reported during steady-state exercise (21). The present study also examined the regulation of the PDH complexes and attributed it to lower muscle pyruvate and increased NADH contents at rest and increased NADH during the first minute of exercise. With sustained exercise at 40 and 65% \( \overline{V}O_2 \) max PDH remained lower in the high-FFA trial, by some unexplained mechanism, as muscle [NADH] returned to control values. This study is also the first to demonstrate that elevated FFA provision decreased RER throughout exercise and decreased human skeletal muscle M-CoA content at rest and after 20 min of exercise. The lower M-CoA may have contributed to the increased fat oxidation by reducing CPT I inhibition and increasing mitochondrial FFA transport. Lastly, in vitro work suggests that decreases in muscle pH at 65% \( \overline{V}O_2 \) max in both trials inhibited CPT I activity and contributed to lower fat oxidation rates compared with exercise at 40% \( \overline{V}O_2 \) max.

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