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

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It is well established that a mixture of carbohydrate (CHO) and fat is used as fuel by skeletal muscle at rest and during exercise. Two of the main regulatory enzymes responsible for substrate choice for oxidation are pyruvate dehydrogenase (PDH) and carnitine palmitoyltransferase I (CPT I), which control the entry of CHO and free fatty acids (FFA), respectively, into the mitochondria. Therefore, the interaction between CHO 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 O₂ uptake (V蹲max) suggested that the FFA-induced glycogen sparing was regulated via glycogen phosphorylase (9, 10). At lower power outputs (40–65% V蹲max), we recently demonstrated that the downregulation of CHO metabolism occurred at two sites: decreased phosphorylase activity and reduced PDH activation (PDHα; 21). However, the mechanism for the reduced PDHα with elevated fat availability was not determined.

The activity of CPT I determines 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 PDHα early in exercise (1 and 10 min at 40% and 10 min at 65% V蹲max) in the presence of high FFA availability. We hypothesized that the decreased PDHα was related to reduced muscle pyruvate and/or increased mitochondrial NADH contents. A second purpose was to determine whether increased FFA availability would produce the hypothesized decrease in human skeletal muscle M-CoA content at rest and during exercise and fat metabolism during exercise will be heavily dependent on the balance between the activities of these key enzymes.
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; VO_2max, 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.

Preexperimental protocol. All subjects initially performed an incremental VO_2max 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% VO_2max. Daily food records were kept for 48 h preceding each test session, and subjects were instructed to refrain from caffeine consumption and intense physical activity for 24 h before testing. No difference was observed in subjects’ diets 48 h before each trial.

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, possibly contribute to the increased CPT I activity and fat oxidation in this condition.

Fig. 1. Schematic diagram of experimental protocol. VO_2, O_2 uptake; VO_2max, maximal VO_2.
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 (time 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.

RESULTS

Respiratory gas exchange. Whole body O2 uptake was similar in the two conditions and represented 44 ± 0.01 vs. 43 ± 0.01 and 68 ± 0.02 vs. 69 ± 0.02% Vo2max 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% Vo2max but was elevated at 20 min of exercise (65% Vo2max) 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% Vo2max) appeared to increase pyruvate during Int at 1 min, although the change was not significant (Fig. 4). However, a significant main effect for trial (Int > control) was present for pyruvate concentration ([pyruvate]) during steady-state exercise (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

Table 1. Respiratory gas exchange variables during 10 min of cycle exercise at 40% and 10 min at 65% Vo2max during Intralipid infusion or control

<table>
<thead>
<tr>
<th>Trial</th>
<th>4–7 min</th>
<th>14–17 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40% Vo2max</td>
<td>65% Vo2max</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V02, 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; V02, O2 uptake; Vo2max, maximal V02. *Significantly different from control.

Table 2. Whole blood glucose and lactate concentrations during 10 min of cycle exercise at 40% and 10 min at 65% Vo2max after Intralipid infusion or control

<table>
<thead>
<tr>
<th>Trial</th>
<th>-30 min</th>
<th>0 min</th>
<th>8 min</th>
<th>20 min</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Rest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40% Vo2max</td>
<td>65% Vo2max</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</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>
</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>
</tr>
<tr>
<td>Lactate</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>
</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>
</tr>
</tbody>
</table>

Values are means ± SE, n = 7. *Significantly different from 0 min. †Significantly different from rest (-30 and 0 min).
Fig. 5. Muscle NADH content at rest and during 20 min of cycle exercise at 40 and 65% VO₂max with Intralipid or control infusion. Values are means ± SE. *Significantly different from control; #significant main effect for rest-to-exercise transition.

**Table 3. Muscle metabolites during cycle exercise (10 min at 40% and 10 min at 65% VO₂max) in control and Intralipid trials**

<table>
<thead>
<tr>
<th></th>
<th>Preexercise</th>
<th>40% VO₂max</th>
<th>65% VO₂max</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 min</td>
<td>10 min</td>
</tr>
<tr>
<td>ATP</td>
<td>29.1±0.8</td>
<td>28.3±0.8</td>
<td>28.2±0.9</td>
</tr>
<tr>
<td></td>
<td>28.1±1.0</td>
<td>26.4±0.7</td>
<td>27.9±1.0</td>
</tr>
<tr>
<td>PCr</td>
<td>92.8±1.9</td>
<td>78.2±2.0†</td>
<td>76.3±1.7</td>
</tr>
<tr>
<td></td>
<td>88.5±2.4</td>
<td>75.2±3.9†</td>
<td>75.5±2.6</td>
</tr>
<tr>
<td>G6P</td>
<td>0.68±0.08</td>
<td>1.12±0.16</td>
<td>1.39±0.21</td>
</tr>
<tr>
<td></td>
<td>0.87±0.20</td>
<td>1.16±0.25</td>
<td>1.03±0.16</td>
</tr>
<tr>
<td>NAD⁺/NADH</td>
<td>1.77±0.09</td>
<td>1.89±0.07</td>
<td>1.89±0.05</td>
</tr>
<tr>
<td></td>
<td>1.78±0.09</td>
<td>1.84±0.13</td>
<td>1.78±0.09</td>
</tr>
<tr>
<td>G6P</td>
<td>17.4±1.6</td>
<td>12.6±0.9‡</td>
<td>11.9±2.6</td>
</tr>
<tr>
<td></td>
<td>14.6±2.1*</td>
<td>10.1±1.5†</td>
<td>12.0±2.0</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmol/kg dry muscle. PCr, phosphocreatine; G6P, glucose-6-phosphate. *Significantly different from control; †significantly different from rest; ‡significantly different from 40% VO₂max.

Fig. 3. Muscle lactate content at rest and during 20 min of cycle exercise at 40 and 65% VO₂max with Intralipid or control infusion. Values are means ± SE. *Significant main effect for rest-to-exercise transition; †significant main effect for condition during exercise. dm, Dry muscle.

**Fig. 4. Muscle pyruvate content at rest and during 20 min of cycle exercise at 40 and 65% VO₂max with Intralipid or control infusion. Values are means ± SE. *Significantly different from control; #significant main effect for condition during exercise.**
Regulation of PDHa during exercise. Skeletal muscle PDH, located within the inner mitochondrial membrane, is a multienzyme complex comprising three main catalytic subunits (PDH (E1), dihydrolipoamide acetyltransferase (E2), and dihydrolipoamide dehydrogenase (E3)) and two regulatory subunits (PDH kinase and PDH phosphatase) (15, 23, 28). A phosphorylation (PDH phosphatase)-dephosphorylation (PDH kinase) cycle controls the movement between the deactivated (PDHb) and activated (PDHa) states. The balance between the kinase and phosphatase activities (allosteric control) determines the state of PDH at any point in time. Ca\(^{2+}\) is a potent PDH phosphatase activator, whereas PDH kinase is inhibited by pyruvate and activated by high NADH-to-NAD\(^{+}\), ATP-to-ADP, and acetyl-CoA-to-CoA ratios (6, 7, 14, 23, 24, 28). Earlier work reported that high FFA provision reduced PDHa during steady-state low- and moderate-intensity exercise (21). The present study examined both the regulation of the decreased PDHa and the activation time course during the rest-to-exercise transition.

It was assumed that the exercise Ca\(^{2+}\) concentration was similar between trials, because the exercise power outputs were identical. This implies that the lower PDHa in the high FFA trial was due to factors that regulate PDH kinase. Several studies have demonstrated that the acetyl-CoA-to-CoA ratio is not involved in the regulation of PDH kinase and PDHs during exercise (9, 10, 14, 21, 26). Measurements of resting and exercise muscle ATP contents and calculated free ADP levels (see Dudley et al., Ref. 8) were also similar, such that the ATP-to-ADP ratio decreased to the same extent in both trials in the initial minute of exercise (data not shown). However, there was a lower muscle pyruvate content and a higher NADH content at rest in the high-FFA trial. Although these changes had no effect on resting PDHa, they may have increased PDH kinase activity and contributed to lower PDHa during 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 PDHa 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 PDHa 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 PDHa 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 PDHa 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 PDHa 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-
sation 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% \( V_{O_2} \text{max} \). 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 acetylcarnitine, 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% \( V_{O_2} \text{max} \) 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 \( \sim 40\% \) when pH decreased from 7.0 to 6.8 (37). Estimated pH (muscle lactate and pyruvate contents) in the present study decreased from \( \sim 7.0 \) at rest in both trials to 6.87–6.91 at 40% and 6.74–6.76 at 65% \( V_{O_2} \text{max} \). 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% \( V_{O_2} \text{max} \) as previously reported during steady-state exercise (21). The present study also examined the regulation of the PDHa decrease 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% \( V_{O_2} \text{max} \) PDHa 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% \( V_{O_2} \text{max} \) in both trials inhibited CPT I activity and contributed to lower fat oxidation rates compared with exercise at 40% \( V_{O_2} \text{max} \).

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