Skeletal muscle fat metabolism after exercise in humans: influence of fat availability

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A potential factor regulating both plasma and intramuscular-derived skeletal muscle FA oxidation is malonyl-CoA, an allosteric inhibitor of the carnitine palmitoyltransferase I (CPTI) enzyme complex, which regulates long-chain FA (LCFA) translocation into the mitochondria for oxidation. In skeletal muscle and heart tissue, malonyl-CoA concentration in the cytoplasm is under the dual control of synthesis by acetyl-CoA carboxylase 2 (ACC2) (1) and degradation by malonyl-CoA decarboxylase (MCD) (2). Reduced malonyl-CoA is associated with increased FA oxidation in rodent skeletal muscle during exercise (49); however, decreases in malonyl-CoA do not appear to fully explain the increased FA oxidation during moderate-intensity exercise in human skeletal muscle (9, 29, 35). Furthermore, a malonyl-CoA-resistant CPTI subfraction has been detected in rodent skeletal muscle (18), suggesting that malonyl-CoA is not the sole regulator of CPTI activity at rest or during exercise. Indeed, despite constant or elevated malonyl-CoA levels, CPTI activity can increase mitochondrial FA transport during moderate-intensity exercise of up to 2 h in duration (16). Taken together, these findings suggest that the regulation of CPTI activity is more complex than simple changes in malonyl-CoA levels and that a yet-unknown mechanism in skeletal muscle alters the sensitivity of CPTI for malonyl-CoA (16).

Regulation of malonyl-CoA concentration and skeletal muscle fat metabolism during postexercise recovery could be mediated by the metabolic fuel sensor 5’AMP-activated protein kinase (AMPK) in rodents (36). Exercise-induced activation of AMPK appears to regulate decreased ACC and in-

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creased MCD activities coordinately after exercise (31), which lowers malonyl-CoA concentrations, resulting in increased FA oxidation and decreased FA synthesis (31, 36). However, recent evidence indicates that in many instances, there is a mismatch among AMPK activation, ACC2 Ser221 phosphorylation (ACC2 Ser221-P), and FA oxidation during skeletal muscle contraction (41). For example, malonyl-CoA content, FA oxidation rates, and ACC2 activity are dissociated in ACC2 knockout mice (30), and liver kinase B1 (LKB1)-deficient mice, with no activation of AMPKα2 during muscle contraction, have only a modest decline in ACC2-P (44). Collectively, these studies and an increasing body of evidence suggest that the AMPK-ACC2 signaling pathway may not be mandatory for exercise-induced increases in FA oxidation and that alternate enzymes, such as MCD, may have more critical roles in regulating malonyl-CoA production.

Whether the increase in FA oxidation observed with glycogen-lowering exercise in human skeletal muscle during recovery is associated with AMPK- and ACC2-mediated mechanisms has received little attention and remains unclear. Recent evidence demonstrates that reduced skeletal muscle malonyl-CoA content after 4 h of postexercise recovery in a fasted state is associated with a sustained increase in ACCβ-P; however, an increase in AMPK-P and activity was not observed (12). In contrast, ACCβ-P declines during prolonged glycogen-lowering exercise and returns to resting levels after 1 h of recovery in well-trained individuals (51). In addition, the alteration of circulating FFA levels during postexercise recovery can induce changes in whole-body fat oxidation (45), although whether this metabolic response is related to changes in AMPK, ACC2, and malonyl-CoA remains unknown. On this basis, further research is warranted to elucidate more clearly the mechanisms accounting for changes in skeletal muscle metabolism after exhaustive exercise in humans.

Thus the objectives of this study were to examine mechanisms regulating fat oxidation in humans by modulating plasma FFA availability during recovery from glycogen-lowering exercise. We investigated whether manipulating FFA availability would facilitate changes in whole-body fat oxidation (45), although whether metabolic responses are consistent with the modification of skeletal muscle malonyl-CoA-P, AMPKα-P, and ACCβ-P. Furthermore, we also investigated whether changes in lipid availability would alter IMTG concentration during a 6-h postexercise recovery period.

**METHODS**

Subjects. Eight endurance-trained men [age, 32 ± 1 yr; body mass, 79.8 ± 5.5 kg; peak power output, 5.0 ± 0.04 W/kg; and maximum oxygen uptake (VO2_peak), 61.1 ± 3.0 ml·kg⁻¹·min⁻¹ (mean ± SE)] volunteered to participate in this study. All subjects were healthy, did not smoke or take any medications, and had no evidence of cardiovascular or metabolic disease. The experimental procedures and possible risks of the study were explained verbally and in writing to all participants before obtaining their informed, written consent to participate. The study protocol (Fig. 1) was approved by the Deakin University Human Research Ethics Committee and was performed according to the Declaration of Helsinki.

**Pre-experimental protocol.** Daily energy intake and composition of each subject’s habitual diet were assessed using a 4-day food record (3 weekdays and 1 weekend day) and analyzed for energy intake (14.5 ± 1.4 MJ) and macronutrient composition [57 total energy percent (57E%) carbohydrate (CHO), 27E% fat, 16E% protein], using commercially available software (ESHA Research, Salem, OR). One to 2 wk prior to testing, peak oxygen uptake (VO2_peak) was determined on an electromagnetically braked cycle ergometer (Excalibur Sport; Lode, Groningen, The Netherlands). Expired air was analyzed by O2 and carbon dioxide (CO2) analyzers (AEI Technologies, Pittsburgh, PA). From the VO2_peak test, power outputs, corresponding to submaximal VO2 values to be used during the experimental trial, were determined using linear regression analysis for each subject. All subjects were familiarized with the intensity of the glycogen-lowering protocol, and the experimental power outputs were verified ~1 wk before the experiments. For 2 days prior to experimental trials, subjects abstained from exercise and the consumption of caffeine and alcohol. In addition, on the day before each experimental trial, subjects were provided with a high-CHO diet (13.8 ± 0.6 MJ; 76E% CHO, 11E% fat, 13E% protein) to maximise glycogen stores.

**Experimental protocol.** Subjects participated in three randomized, counterbalanced experimental trials, each separated by at least 1 wk. On each occasion, subjects reported to the laboratory at 0715 after a 10-12-h overnight fast. After voiding, body mass was recorded, and a Teflon catheter was inserted into an antecubital vein for blood sampling. Following 30 min of rest in a supine position, resting VO2 and rate of elimination of CO2 (VCO2) were measured [for determination of the respiratory exchange ratio (RER)], and a 5-ml venous blood sample was drawn. Subjects then consumed a light, high-CHO (1,281 kJ; 82E% CHO, 13E% fat, 5E% protein) breakfast. During a subsequent, 90-min rest period, three incisions were made over the vastus lateralis muscle under local anesthesia (1% xylocaine solution). Expired gas and blood samples were then obtained and exercise initiated on a Lode Excalibur Sport cycle ergometer.
The glycogen-lowering protocol was performed as described previously (21). This consisted of cycling at 75% \( \text{VO}_{\text{max}} \) for 20 min; followed by alternating, 2-min bouts of 90% and 50% of \( \text{VO}_{\text{max}} \) for five to 10 intervals; then decreasing intensity to 80% and 50%, 2-min bouts for another four to five intervals; and finishing with 2-min, 70% and 50% bouts until 90 min of exercise had been completed. During exercise, subjects ingested water ad libitum and after 60 min, were administered a capsule of nicotinic acid (NA; 8 mg/kg) or placebo. A blood sample was also taken ~5 min prior to exhaustion. After cessation of exercise, subjects moved from the cycle ergometer to a bench, where a percutaneous needle muscle biopsy was obtained from the thigh. Approximately 30 s lapsed between the termination of the exercise and the procurement of the muscle sample, using the percutaneous needle biopsy technique with suction. Additional muscle biopsies were taken from separate incisions on the same thigh at 3 h and at completion of the 6-h recovery period.

Immediately after the initial muscle sample, a second Teflon catheter was inserted into the opposite antecubital vein. Infusion of an Intralipid 20% solution at 1.5 ml/min (Baxter, Deerfield, IL) and a 200-U bolus of heparin, followed by 0.2 U Kg \(^{-1} \) min \(^{-1} \) (Faulding, Parkville, Australia) or 0.9% saline at 1.5 ml/min, was then commenced for 6 h. During recovery, NA or placebo capsules were administered at 30 min and 1 h (3.7 mg/kg), 2 and 3 h (5 mg/kg), and 4 and 5 h (3.7 mg/kg).

During recovery, blood samples were also obtained at 30-min intervals and breath samples collected every hour. \( \text{VO}_{2} \), \( \text{VCO}_{2} \), and RER were determined at rest and during recovery using a Medgraphics metabolic cart (CardIO2 and CPX/D system; Medical Graphics, St. Paul, MN). Expired air samples were analyzed by breath (reported as 30-s average) and RER calculated by averaging for the final 5 min of a 10-min collection period. The metabolic cart was calibrated manually prior to each test using 0.01% alpha-rated gases. Assuming a nonprotein RER value, whole-body CHO and fat oxidation (g) were calculated from \( \text{VO}_{2} \) and \( \text{VCO}_{2} \) measurements during the last 5 min of each collection period, according to the following equations (33):

\[
\text{CHO oxidation} = 4.585 \cdot \frac{\text{VCO}_{2}}{\text{VO}_{2}} - 3.226 \cdot \text{VCO}_{2} \\
\text{Fat oxidation} = 1.695 \cdot \text{VO}_{2} - 1.701 \cdot \text{VCO}_{2}
\]

Due to difficulties in collecting respiratory measurements for two subjects, RER and substrate oxidation data are presented for six subjects, which is not considered to influence the results. Water ingestion was allowed ad libitum during the postexercise recovery.

Analyses. Blood samples were collected in heparinized collection tubes and placed on ice. A 200-µl aliquot of whole blood was deproteinized in 1.0 ml of 0.6 M perchloric acid (HClO\(_4\)) and spun at 13,000 rpm for 2 min. The supernatant was stored at ~8°C for subsequent fluorometric determination of whole-blood glyceral (4). A second portion of whole blood (1 ml) was added to 20 µl EGTA and reduced glutathione. In addition, 10 µl tetrahydrodipipstan (120 mg/l) from Xenical (orlistat) capsules (Roche, Nutley, NJ) was added to prevent in vitro lipolysis (20). The samples were spun at 13,000 rpm for 2 min and the treated plasma stored at ~20°C prior to analysis for FFA (Non-Ester Fatty Acid C test kit; Wako Chemicals, Richmond, VA). The remaining blood was spun (2 min at 13,000 rpm) and the untreated plasma stored at ~20°C for subsequent analysis of insulin using a radioimmunoassay kit (Phadseph; Pharmacia & Upjohn, Uppsala, Sweden) and glucose and lactate using an automated glucose/lactate analyzer (EML 105; Radiometer, Copenhagen, Denmark).

Muscle biopsies were frozen immediately in liquid nitrogen (N\(_2\)), removed from the needle while frozen, and stored in liquid N\(_2\) until analyzed. A small (50–60 mg) piece of muscle was shipped from each biopsy under liquid N\(_2\) and freeze dried; dissected free of blood, connective tissue, and visible fat; and powdered for subsequent metabolite analyses. One aliquot of powdered muscle (~2 mg) was assayed enzymatically for glycogen, as described previously (13). Total muscle triacylglycerol (IMTG) was determined by extracting a 5- to 7-mg sample of powdered muscle in chloroform-methanol (2:1), saponifying the reconstructed extract with ethanolic potassium hydroxide at 60°C for 1 h, and determining glycerol fluorometrically (11). A coefficient of variation (CV) of 7.1% for the IMTG concentration was obtained when two aliquots from the same muscle were analyzed separately using four muscle samples. The mean intra-assay CV was 2.8% for glycerol from five samples. The remaining powdered muscle was extracted in a solution of 0.5 M HClO\(_4\) and 1 M EDTA and neutralized with 2.2 M potassium bicarbonate. These extracts were assayed spectrophotometrically for ATP, phosphocreatine (PCr), and creatine, as described previously (13). All metabolite measurements were normalized to the highest total creatine content from the nine samples obtained for each subject to correct for nonmuscle contamination.

Malonyl-CoA concentration was determined from a small sample of wet muscle tissue (~10 mg), using the previously described radioisotopic method (25) with modifications (37). Briefly, this method is based on the malonyl-CoA-dependent incorporation of labeled acetyl-CoA into palmitic acid, catalyzed by FA synthetase in the presence of NADP. An internal malonyl-CoA standard was used with each sample to correct for differing levels of acetyl-CoA.

For detection of ACC\(_{\beta}\)-P and total AMPK\(_{\alpha}\) (\( \alpha_1 \) and \( \alpha_2 \))-P, ~10–15 mg muscle was homogenized in 20 vol homogenization buffer (50 mM Tris, pH 7.5, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 50 mM sodium fluoride (NaF), 5 mM Na pyrophosphate, 10% glycerol) for ~30 s on ice. The homogenate was then centrifuged (5 min, 1,000 g at 4°C) and stored at ~80°C for subsequent analysis. Protein concentration was determined using the bicinchoninic acid method.

Proteins were separated and identified using SDS-PAGE. Protein (35 µg) from each sample was loaded onto 1.5 mm, 12% polyacrylamide gels before undergoing electrophoresis for 75 min at 150 V. Proteins were wet transferred to a nitrocellulose membrane for 120 min at 100 V. Membranes were then cut at ~100 kDa and the top portion (ACC\(_{\beta}\)-P) blanked for 1 h in blocking buffer (7% skim-milk powder in Tris-buffered saline and 0.25% Tween) and exposed overnight at 4°C to the antiphospho-ACC\(_{\beta}\)-221 polyclonal antibody (1:500) (7). The lower membrane (AMPK\(_{\alpha}\)) was blanked overnight at 4°C in 7% blocking buffer and exposed for 60 min at room temperature to the phospho-specific AMPK\(_{\alpha}\) threonine 172 antibody (1:1,000). Membranes were exposed to anti-rabbit horseradish peroxidase-conjugated secondary antibodies (diluted 1:10,000) in blocking buffer for 45 min at room temperature. Antibody binding was viewed by incubating in enhanced chemiluminescence substrate (Pierce SuperSignal chemiluminescent; Thermo Fisher Scientific, Rockford, IL) and exposing to a Kodak Image Station 440CF (NEN Life Science Products, Boston, MA). Bands were identified and quantified using Kodak 1D Image Analysis Software (Eastman Kodak, Rochester, NY).

Statistics. Results were analyzed using a two-way ANOVA with repeated measures (time \( \times \) trial), and specific differences were located using a Tukey post hoc test (Sigmastat, version 3). Statistical significance was accepted at \( P \leq 0.05 \). Values are presented as the means ± SE.

RESULTS

Blood responses. Plasma FFA levels were similar at rest and prior to and after exercise for all three trials (Fig. 2B). During recovery, fat infusion elevated FFA concentrations significantly above rest (\( P < 0.001 \)) and above the infusion of the saline [control (CON)] trial after 1.5 h (\( P < 0.01 \)). NA ingestion lowered FFA levels to baseline values that were significantly lower than CON (\( P < 0.01 \)) throughout recovery, except at 4 h. Plasma glycerol responses at the end of exercise were significantly higher than rest for CON (\( P < 0.001 \)) and saline + NA [low FFA (LFA; \( P < 0.05 \))] trials only (Fig. 2A). NA, during recovery, reduced plasma glycerol compared with
Significantly different from the resting value of the same trial, *P<0.05 and †P<0.01; significantly different from HFA trial, §P<0.01; significantly different from CON trial, *P<0.01; significantly different from the LFA trial, ‡P<0.01.

Intralipid and heparin [high FFA (HFA; P<0.01)] and CON (P<0.01), except at 1 and 4 h. Plasma glucose did not change during the three trials, except for a lower (P<0.05) postexercise value compared with the pre-exercise value during CON and higher (P<0.05), 1-h value during LFA compared with the 1-h CON value (Table 1). Pre-exercise plasma insulin was significantly higher (P<0.01) than rest for CON and HFA trials as a result of the high-CHO breakfast. During recovery, plasma insulin was higher in HFA than during CON (P<0.05; 2–6 h) and LFA (P<0.001; 1–5 h). Plasma lactate increased significantly (P<0.01) above rest in response to the high-intensity exercise and returned to baseline levels throughout recovery for all trials (Table 1).

**RER and substrate oxidation.** Resting and pre-exercise RER were not different among trials (Table 2). During the initial 1 h of recovery, RER was significantly lower (P<0.01) than rest for CON and HFA trials only. Between 4 and 6 h of recovery, during the LFA trial, RER was significantly higher than CON (P<0.01; 4 and 5 h) and HFA (P<0.01; 5 and 6 h). Consistent with the RER data, fat oxidation during the entire recovery period was significantly higher for the HFA trial compared with the LFA trial (P<0.05) and greater for HFA at 1 h (P<0.01) and 6 h (P<0.05) compared with the corresponding time in the LFA trial (Fig. 3).

**Muscle metabolites.** No difference in muscle glycogen content among all three trials was observed upon completion of the exercise bout (Table 3). During the LFA trial, muscle glycogen increased significantly (P<0.001) after 3 h, with no further change at 6 h. A significant increase (P<0.01) in glycogen was observed after 6 h for the HFA trial. No change in IMTG concentration was detected during recovery for all three trials (Table 3). Muscle ATP, PCr, and creatine also remained unchanged throughout the recovery period (Table 3).

**Malonyl-CoA.** After glycogen-depleting exercise (0 h), no difference in malonyl-CoA concentration was observed among trials (Fig. 4). During recovery, a significant increase in malonyl-CoA occurred at 3 and 6 h for the CON trial only (P<0.001). Compared with CON, malonyl-CoA was significantly lower at 6 h during the LFA (P<0.01) and at 3 h (P<0.05) and 6 h (P<0.01) during the HFA trial.

**ACCβ-P and AMPK-P.** Immediately after exercise (0 h), ACCβ-P was approximately fourfold higher than 3 or 6 h during CON (P<0.01) and LFA and HFA (P<0.001; Fig. 5A). No treatment effect for ACCβ-P was observed during recovery. An exercise or treatment effect was not observed for AMPKα-P throughout the recovery period (Fig. 5B).

**Table 1. Plasma glucose, insulin, and lactate at rest, before exercise, end of exercise, and during a 6-h postexercise recovery period**

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Glucose, mmol/l</th>
<th>Insulin, µU/ml</th>
<th>Lactate, mmol/l</th>
<th>Glucose, mmol/l</th>
<th>Insulin, µU/ml</th>
<th>Lactate, mmol/l</th>
<th>Glucose, mmol/l</th>
<th>Insulin, µU/ml</th>
<th>Lactate, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>4.9 ± 0.2</td>
<td>5.8 ± 0.8</td>
<td>1.4 ± 0.1</td>
<td>4.9 ± 0.2</td>
<td>5.3 ± 1.2</td>
<td>1.4 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>5.8 ± 0.6</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>Pre-exercise</td>
<td>4.4 ± 0.3</td>
<td>8.8 ± 1.1*</td>
<td>1.7 ± 0.2</td>
<td>4.4 ± 0.3</td>
<td>10.1 ± 3.2</td>
<td>1.8 ± 0.2</td>
<td>4.6 ± 0.3</td>
<td>10.4 ± 1.4*</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>End-exercise</td>
<td>4.3 ± 0.1†</td>
<td>4.3 ± 0.3</td>
<td>5.9 ± 0.9*</td>
<td>4.5 ± 0.4</td>
<td>3.8 ± 0.8</td>
<td>5.2 ± 0.6*</td>
<td>4.6 ± 0.4</td>
<td>4.6 ± 0.5</td>
<td>5.6 ± 0.6*</td>
</tr>
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<td>1</td>
<td>4.5 ± 0.1</td>
<td>3.8 ± 0.3</td>
<td>1.9 ± 0.1</td>
<td>5.0 ± 0.1‡</td>
<td>2.8 ± 0.3‡</td>
<td>1.6 ± 0.1</td>
<td>4.7 ± 0.1</td>
<td>5.4 ± 0.4‡</td>
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<td>3.7 ± 0.4</td>
<td>1.3 ± 0.1</td>
<td>4.7 ± 0.1</td>
<td>3.0 ± 0.5‡</td>
<td>1.4 ± 0.1</td>
<td>4.8 ± 0.1</td>
<td>5.4 ± 0.4‡</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>4.6 ± 0.2</td>
<td>4.0 ± 0.5</td>
<td>1.2 ± 0.1</td>
<td>4.8 ± 0.1</td>
<td>2.9 ± 0.5‡</td>
<td>1.3 ± 0.2</td>
<td>4.8 ± 0.1</td>
<td>6.3 ± 0.5‡</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>4</td>
<td>4.8 ± 0.1</td>
<td>3.8 ± 0.6</td>
<td>1.2 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>2.9 ± 0.5‡</td>
<td>1.3 ± 0.2</td>
<td>4.8 ± 0.1</td>
<td>6.4 ± 0.4‡</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>5</td>
<td>4.8 ± 0.1</td>
<td>3.9 ± 0.3</td>
<td>1.2 ± 0.2</td>
<td>4.8 ± 0.1</td>
<td>2.8 ± 0.6‡</td>
<td>1.3 ± 0.2</td>
<td>4.8 ± 0.1</td>
<td>7.5 ± 1.0‡</td>
<td>1.3 ± 0.1</td>
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<tr>
<td>6</td>
<td>4.7 ± 0.1</td>
<td>3.7 ± 0.4</td>
<td>1.2 ± 0.1</td>
<td>4.8 ± 0.2</td>
<td>3.8 ± 0.9</td>
<td>1.4 ± 0.2</td>
<td>4.7 ± 0.1</td>
<td>6.1 ± 0.5‡</td>
<td>1.4 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n=8. CON, saline (control); LFA, nicotinic acid ingestion [low free fatty acid (FFA)]; HFA, Intralipid infusion (high FFA). Significantly different from the resting value of the same trial, *P<0.01 and †P<0.05; significantly different from CON trial, ‡P<0.05; significantly different from HFA trial, §P<0.001.
of plasma FFAs induced a significant difference \((P < 0.05)\) during postexercise recovery. The alteration of the availability of FFAs levels \((P < 0.05)\) difference in malonyl-CoA concentration at 0, 3, or 6 h of recovery compared with NA ingestion was not associated with a significant increase in fat oxidation observed during Intralipid infusion during the 6-h recovery period (Fig. 3). However, the significant increase in fat oxidation observed during Intralipid infusion compared with NA ingestion was not associated with a difference in malonyl-CoA concentration at 0, 3, or 6 h of recovery. Furthermore, the significant difference in plasma FFA levels \((P < 0.001)\) between the LFA and HFA trials was not associated with a treatment effect for ACC\(_{\beta}\)-P or AMPK\(\alpha\)-P, despite changes in whole-body fat oxidation, and IMTG levels were not altered significantly in any trial during recovery.

Malonyl-CoA is a well-known inhibitor of CPTI activity in a variety of tissues (24), and it is previously demonstrated to be a key regulator of fat oxidation in rodent skeletal muscle during muscle contraction (49). However, malonyl-CoA is unchanged in human skeletal muscle during moderate-intensity exercise when there are large increases in the rate of fat oxidation (9, 28, 29), suggesting that the regulation of CPTI activity is more complex than simple changes in malonyl-CoA levels and/or that malonyl-CoA inhibition is over-ridden during exercise (5). Indeed, CPTI-mediated mitochondrial transport of FAs can occur in the presence of constant or elevated malonyl-CoA levels (16), suggesting that factors other than malonyl-CoA may also regulate CPTI activity during rest and exercise (16, 18). After a 4-h postexercise recovery period, a reduction in malonyl-CoA content relative to resting levels in human skeletal muscle has been observed; however, rates of fat oxidation were not reported (12). In the present study, malonyl-CoA content is unrelated to circulating FFA levels and rates of whole-body fat oxidation, providing further evidence that malonyl-CoA is unlikely to be a primary regulator of human skeletal muscle fat metabolism. It should be noted, however, that we did not measure pre-exercise skeletal muscle malonyl-CoA content, which prevents any interpretation of postexercise malonyl-CoA changes compared with resting values. On the basis of this limitation, we are unable to discount a potential role for malonyl-CoA in the regulation of postexercise fat oxidation, particularly in the first hour during acute recovery when RER values indicate high rates of whole-body fat oxidation.Interestingly, we observed low concentrations of malonyl-CoA immediately after glycogen-lowering exercise compared with values reported after high-intensity, knee-extensor exercise to exhaustion (9) and 60 min of cycling at 65\% \(\text{VO}_2\)peak (35). Endurance training also appears to reduce malonyl-CoA significantly (22), which may have also contributed to the lower malonyl-CoA concentrations in the present study. Therefore, the observed increase in malonyl-CoA during CON may reflect a shift toward resting values that was blunted during the LFA and HFA trials. The reason for an increase in malonyl-CoA during recovery with only saline infusion remains unknown, although may be related to a ketone body-mediated effect (39).

During postexercise recovery, decreased malonyl-CoA content in rodent skeletal muscle is associated with a coordinate reduction in ACC and increase in MCD activities, mediated by the activation of AMPK (31). However, recent evidence from human skeletal muscle demonstrates an exercise-induced increase in ACC\(_{\beta}\)-P after 4 h of recovery that was associated with reduced malonyl-CoA content but not an increase in AMPK-P or activity (12). Evidence from rodent muscle also demonstrates a mismatch between AMPK activation and ACC2 Ser221-P, which suggests that AMPK is not mandatory for exercise-induced increases in FA oxidation (41). For example, AMPK\(\alpha\)-kinase dead mice do not show an increase in AMPK activity, despite the maintenance of ACC2 Ser221-P and FA oxidation ex vivo in isolated, contracting muscles and in vivo during treadmill running (10). Furthermore, only a modest decline in ACC2-P is observed in LKB1-deficient mice, with no activation of AMPK\(\alpha\)2 during muscle contraction (44). In this study, ACC\(_{\beta}\)-P, which is inversely related to ACC activity in rat muscle (32), declined rapidly after 3 h and remained unchanged after 6 h of recovery, despite a significant

### Table 2. Respiratory exchange ratio before exercise and during a 6-h postexercise recovery period

<table>
<thead>
<tr>
<th>Trial</th>
<th>Rest</th>
<th>Pre-exercise</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>5 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>0.81 ± 0.02</td>
<td>0.84 ± 0.02</td>
<td>0.69 ± 0.04*</td>
<td>0.79 ± 0.02</td>
<td>0.81 ± 0.02</td>
<td>0.77 ± 0.01</td>
<td>0.75 ± 0.01</td>
<td>0.78 ± 0.02</td>
</tr>
<tr>
<td>LFA</td>
<td>0.79 ± 0.02</td>
<td>0.82 ± 0.03</td>
<td>0.77 ± 0.03*</td>
<td>0.85 ± 0.02</td>
<td>0.82 ± 0.02</td>
<td>0.80 ± 0.02†</td>
<td>0.85 ± 0.02†</td>
<td>0.79 ± 0.01†</td>
</tr>
<tr>
<td>HFA</td>
<td>0.79 ± 0.01</td>
<td>0.80 ± 0.03</td>
<td>0.69 ± 0.02*</td>
<td>0.78 ± 0.03</td>
<td>0.79 ± 0.02</td>
<td>0.73 ± 0.01</td>
<td>0.73 ± 0.01</td>
<td>0.74 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n = 6\). Significantly different from the resting value of the same trial, *\(P < 0.01\); significantly different from CON trial, †\(P < 0.01\); significantly different from HFA trial, ‡\(P < 0.05\).

### DISCUSSION

Recovery from prolonged, strenuous exercise is characterized by an increase in FAs as an oxidative energy source; however, the cellular and molecular mechanisms that facilitate enhanced fat oxidation remain poorly defined. In this context, we examined the effect of plasma-derived fat availability on whole-body substrate use—factors associated with the regulation of human skeletal muscle fat oxidation (malonyl-CoA content, ACC\(_{\beta}\)-P, and AMPK\(\alpha\)-P) and IMTG concentration during postexercise recovery. The alteration of the availability of plasma FFAs induced a significant difference \((P < 0.05)\) in whole-body fat metabolism between the HFA and LFA trials during the 6-h recovery period (Fig. 3). However, the significant increase in fat oxidation observed during Intralipid infusion compared with NA ingestion was not associated with a difference in malonyl-CoA concentration at 0, 3, or 6 h of recovery. Furthermore, the significant difference in plasma FFA levels \((P < 0.001)\) between the LFA and HFA trials was not associated with a treatment effect for ACC\(_{\beta}\)-P or AMPK\(\alpha\)-P, despite changes in whole-body fat oxidation, and IMTG levels were not altered significantly in any trial during recovery.

Malonyl-CoA is a well-known inhibitor of CPTI activity in a variety of tissues (24), and it is previously demonstrated to be a key regulator of fat oxidation in rodent skeletal muscle during muscle contraction (49). However, malonyl-CoA is unchanged in human skeletal muscle during moderate-intensity exercise when there are large increases in the rate of fat oxidation (9, 28, 29), suggesting that the regulation of CPTI activity is more complex than simple changes in malonyl-CoA levels and/or that malonyl-CoA inhibition is over-ridden during exercise (5). Indeed, CPTI-mediated mitochondrial transport of FAs can occur in the presence of constant or elevated malonyl-CoA levels (16), suggesting that factors other than malonyl-CoA may also regulate CPTI activity during rest and exercise (16, 18). After a 4-h postexercise recovery period, a reduction in malonyl-CoA content relative to resting levels in human skeletal muscle has been observed; however, rates of fat oxidation were not reported (12). In the present study, malonyl-CoA content is unrelated to circulating FFA levels and rates of whole-body fat oxidation, providing further evidence that malonyl-CoA is unlikely to be a primary regulator of human skeletal muscle fat metabolism. It should be noted, however, that we did not measure pre-exercise skeletal muscle malonyl-CoA content, which prevents any interpretation of postexercise malonyl-CoA changes compared with resting values. On the basis of this limitation, we are unable to discount a potential role for malonyl-CoA in the regulation of postexercise fat oxidation, particularly in the first hour during acute recovery when RER values indicate high rates of whole-body fat oxidation. Interestingly, we observed low concentrations of malonyl-CoA immediately after glycogen-lowering exercise compared with values reported after high-intensity, knee-extensor exercise to exhaustion (9) and 60 min of cycling at 65\% \(\text{VO}_2\)peak (35). Endurance training also appears to reduce malonyl-CoA significantly (22), which may have also contributed to the lower malonyl-CoA concentrations in the present study. Therefore, the observed increase in malonyl-CoA during CON may reflect a shift toward resting values that was blunted during the LFA and HFA trials. The reason for an increase in malonyl-CoA during recovery with only saline infusion remains unknown, although may be related to a ketone body-mediated effect (39).
change in whole-body fat oxidation between the HFA and LFA trials. A substantial increase in ACC-P, followed by a rapid decline after 1 h of moderate-intensity exercise in well-trained individuals, has also been reported, indicating that ACC-P is particularly sensitive to exercise when muscle glycogen is reduced (50). Furthermore, the decline in postexercise ACC-P, an accurate measure of in vivo AMPK signaling (43), also suggests that AMPK activity decreased during recovery. In support of this, total AMPK-P tended to decline into recovery in the present study, as has been observed previously (51, 52). Although no pre-exercise biopsy was taken, the possibility exists that AMPK activity at 3 and 6 h during recovery was similar to resting values, and this may account for the lack of increase in ACC-P during the CON and HFA trials. In support of this hypothesis, 5 h of Intralipid infusion has no effect on AMPK activity (50). Furthermore, the low AMP/ATP ratio and stable ATP demand under resting conditions during recovery would be expected to keep AMPK in its inactive (dephosphorylated) form. Together, these data suggest that AMPK regulation of ACC is not important in determining human skeletal muscle metabolism following exercise and that alternative ACC kinase(s) exist for activating ACC, or other enzymes, such as MCD, are more critical for controlling malonyl-CoA production (41). In support of the latter hypothesis, endurance training is associated with a reduction in resting malonyl-CoA that is most likely explained by a peroxisome proliferator-activated receptor-γ coactivator 1α-mediated increase in MCD expression and activity (22).

Consistent with the absence of any change in AMPK-P during recovery, no difference in ACC-P was observed, despite significantly higher rates of whole-body fat oxidation during Intralipid infusion compared with the NA trial (Fig. 5). These data suggest that there is dissociation between skeletal muscle ACC-P and whole-body fat oxidation in humans during a 6-h postexercise recovery period. A similar dissociation between ACC-P and fat oxidation has been reported in human skeletal muscle during exercise (8, 51). The significant decline in postexercise ACC-P should also suggest an increase in FA synthesis. Indeed, a trend toward an increase in

![Fig. 4. Malonyl-CoA concentration immediately after exercise (0 h) and during a 6-h recovery period with CON and HFA or LFA. Values are means ± SE for 8 subjects. Significantly different from 0 h, †P < 0.05 and ‡P < 0.001; significantly different from 3 h, *P < 0.01; significantly different from CON trial, #P < 0.05.](http://jap.physiology.org/)

![Fig. 5. Acetyl-CoA carboxylase-β (ACCβ; A) and 5′AMP-activated protein kinase or threonine 172 (AMPKα th172) phosphorylation (B) immediately after exercise (0 h) and during a 6-h recovery period with CON and HFA or LFA. Values are means ± SE for 8 subjects. Significantly different from 0 h, †P < 0.01 and ‡P < 0.05; significantly different from CON trial, #P < 0.05.](http://jap.physiology.org/)
IMTG was apparent in the LFA and HFA trials after 6 h of recovery (Table 3). Dissociation between ACCβ-P and malonyl-CoA at 3 and 6 h in all trials suggests that factors other than the ACCβ-P are regulating ACC activity and thus malonyl-CoA in human skeletal muscle. Although not determined in this study, these factors could include the cytosolic concentration of citrate, an allosteric activator of ACC (3, 38, 39), or LCFA-CoA, an allosteric inhibitor of ACC (37). Alternatively, differences in MCD activity could account for the differing malonyl-CoA values in the three trials. Insufficient FA flux in exercised skeletal muscle during recovery may also contribute to absence of an AMPK-ACCβ-malonyl-CoA signaling pathway in this study. In support of this postulate, the contribution from leg skeletal muscle to whole-body FA and glycerol turnover and FA re-esterification is small (47) relative to the substantial splanchnic re-esterification during postexercise recovery (46).

In addition to plasma-derived FFAs, hydrolysis of IMTG may be an important source of LCFA, supporting the increase in postexercise fat metabolism (17). With the use of chemical extraction of muscle samples, no change in IMTG concentration was observed during recovery, despite the marked reduction in circulating FFAs with NA ingestion, supporting the previous hypothesis that plasma-derived FFAs are the preferential fuel source for skeletal muscle fat metabolism during recovery from glycogen-lowering exercise (19). Although NA ingestion reduced FA oxidation significantly during the entire 6-h recovery period compared with Intralipid infusion (Fig. 3), FA oxidation remained at a similar rate to that observed during CON, and IMTG concentration tended to increase during the NA trial. These results indicate that a NA-induced reduction in postexercise lipolysis did not limit fat oxidation significantly, relative to control conditions, consistent with the observation that only a small fraction of the substantial adipose tissue lipolysis during postexercise recovery is taken up by active muscle in the lower extremities (26). Indeed, up to 90% of the FFAs released during recovery are re-esterified (53), and leg net FA uptake decreases immediately upon cessation of exercise to near-resting levels (47). Furthermore, the lack of a significant decline in whole-body lipid oxidation during the NA trial compared with CON, despite markedly reduced plasma FFA levels throughout recovery, suggests that circulating FFAs are not a key factor regulating postexercise fat oxidation. Although total leg fat oxidation (47) and whole-body lipid oxidation (14) remain significantly higher during a 3-h postexercise recovery period compared with resting conditions, the percentage of plasma FA and “other FA” (nontracer-derived measurement) oxidized is not different from a resting control (14). Therefore, the absence of an increased reliance on nonplasma FA oxidation after strenuous exercise compared with rest appears to account for the negligible effect of FA availability on fat oxidation and IMTG concentration during LFA and HFA compared with CON. Surprisingly, IMTG levels were not increased significantly in response to high FFA concentrations during Intralipid and heparin infusion, although an average increase of 35% was observed after 6 h (Table 3). With the use of the chemical extraction technique, Schenk et al. (40) have demonstrated that overnight postexercise lipid and heparin infusion can increase IMTG concentration significantly, by ~30% in healthy women. In resting skeletal muscle, increased IMTG content has been observed after 4 h of elevated plasma FFAs (6); however, this difference is most likely attributable to the reduced sensitivity of the muscle biopsy technique compared with the noninvasive method of hydrogen-1-NMR, used by Boden et al. (6).

In conclusion, the alteration of the availability of plasma FFA during recovery induces changes in whole-body fat oxidation that are unrelated to differences in skeletal muscle malonyl-CoA, although malonyl-CoA-mediated regulation cannot be discounted completely in the acute recovery period. The absence of any change in AMPKα-P during recovery and dissociation between ACCβ-P and malonyl-CoA at 3 and 6 h in all trials suggest that factors other than the AMPK-ACCβ-malonyl-CoA signaling pathway have a primary role in regulating fat metabolism during long-term postexercise recovery. Despite marked changes in plasma FFA availability, no significant change in IMTG concentration was detected, providing further evidence that plasma-derived FFAs are the major fuel source contributing to the enhanced fat oxidation during recovery from glycogen-lowering exercise.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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Intramyocellular lipids form an important substrate source for skeletal muscle metabolism during aerobic and anaerobic exercise. The mitochondrial carnitine palmitoyltransferase system is essential for the efflux of long-chain fatty acids from the mitochondrial matrix to the cytosol, where they can be further metabolized to acetyl-CoA. This process is critical for the regulation of fat oxidation in human skeletal muscle during exercise.

The mitochondrial carnitine palmitoyltransferase system is regulated by several factors, including the activity of acetyl-CoA carboxylase, the concentration of malonyl-CoA, and the intramyocellular levels of fatty acids. Malonyl-CoA, a product of fatty acid oxidation, inhibits acetyl-CoA carboxylase, leading to a decrease in the production of malonyl-CoA. This in turn allows for the increased oxidation of fatty acids via beta-oxidation.

The regulation of fat oxidation is critical for the maintenance of energy homeostasis during exercise. The coordinated regulation of fatty acid oxidation and glycogenolysis ensures that both substrates are available for energy production, thereby allowing for optimal exercise performance.

In conclusion, the mitochondrial carnitine palmitoyltransferase system plays a central role in the regulation of fat oxidation in human skeletal muscle during aerobic exercise, and its optimal function is essential for the maintenance of energy homeostasis during prolonged exercise.

Reference:


