Effects of increased fat availability on fat-carbohydrate interaction during prolonged exercise in men

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Odland, L. Maureen, George J. F. Heigenhauser, Denis Wong, Melanie G. Hollidge-Horvat, and Lawrence L. Spriet. Effects of increased fat availability on fat-carbohydrate interaction during prolonged exercise in men. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R894–R902, 1998.—The study examined the existence and regulation of fat-carbohydrate interaction during low- and moderate-intensity exercise. Eight males cycled for 10 min at 40% and 60 min at 65% maximal O2 uptake (V̇O2max) while infused with either Intralipid and heparin (Int) or saline (Con). Before exercise, plasma arterial free fatty acid (FFA) was 0.69 ± 0.04 mM (Int) vs. 0.25 ± 0.04 mM (Con). Muscle biopsies were taken at rest and at 10, 20, and 70 min of exercise. Arterial and femoral venous blood samples and expired gases were collected simultaneously throughout exercise, and blood flow was estimated from pulmonary O2 uptake and the leg arterial-venous O2 difference. Respiratory exchange ratio was higher in Con (0.94 ± 0.01) compared with Int (0.91 ± 0.01). Mean net leg FFA uptake was higher in Int (0.16 ± 0.03 vs. 0.04 ± 0.01 mmol/min), and net lactate efflux was reduced (Int, 1.55 ± 0.36 vs. Con, 3.07 ± 0.47 mmol/min). Leg net glucose uptake was unaffected by Int. Muscle glycogen degradation was 23% lower in Int [230 vs. 297 ± 36 mmol glucose units/kg dry muscle (dm)]. Pyruvate dehydrogenase activity in the a form (PDHa) was lower during Int (1.61 ± 0.17 vs. 2.22 ± 0.24 mmol·min⁻¹·kg wet muscle⁻¹), and muscle citrate was higher (0.59 ± 0.04 vs. 0.48 ± 0.04 mmol/kg dm). Muscle lactate, phosphocreatine, ATP, acetyl-CoA, acetyl-carnitine, and P, were unaffected by Int. Calculated free AMP was significantly lower in Int compared with Con at 70 min of exercise (3.3 ± 0.8 vs. 1.5 ± 0.3 μmol/kg dm). The high FFA-induced reduction in glycogenolysis and carbohydrate oxidation at 65% V̇O2max appears to be due to regulation at several sites. The reduced flux through phosphorylase and phosphofructokinase during Int may have been due to reduced free AMP accumulation and increased cytoplasmic citrate. The mechanism for reduced PDH transformation to the a form is unknown but suggests reduced flux through PDH.

glucose-fatty acid cycle; glycogen phosphorylase; pyruvate dehydrogenase; citrate; acetyl-coenzyme A; free adenosine monophosphate; phosphofructokinase

CURRENT CONTROVERSY EXISTS regarding the mechanisms that regulate substrate choice for oxidation in skeletal muscle during aerobic exercise (for review, see Ref. 36). The concept of the glucose-fatty acid (G-FA) cycle was originally introduced by Randle et al. (28, 29) to explain the interaction between carbohydrate (CHO) and fat metabolism. It was proposed that the increased delivery of free fatty acids (FFA) to muscle tissue enhanced the rate of fat oxidation, which led to increased acetyl-CoA and citrate production and resulted in downregulation of the rate-limiting carbohydrate metabolizing enzymes, pyruvate dehydrogenase (PDH) and phosphofructokinase (PFK), respectively. Decreased glycolytic activity also resulted in glucose 6-phosphate (G-6-P) accumulation, which in turn decreased hexokinase activity and reduced glucose uptake (29).

Much of the original support for the G-FA cycle was obtained from perfused contracting heart muscle or resting diaphragm muscle bathed in a high-FFA concentration (ifFA) medium (14, 15, 28, 29). Contracting heart and resting diaphragm muscles perform constant duties, which requires the majority of oxidizable substrate to come from exogenous sources. Conversely, in skeletal muscles other than diaphragm, the high energy demand during exercise dictates that fuel must also be provided from the endogenous glycogen store. Recent studies in humans have shown that CHO use is reduced in the presence of high [FFA], but the classic regulation of the G-FA cycle in skeletal muscle during 15 min of intense aerobic cycling (80–85% maximal O2 uptake [V̇O2max]) does not occur (12, 13). High FFA provision did not alter muscle acetyl-CoA and citrate levels and had no effect on the transformation of PDH to the more active a form. Rather, downregulation appeared to exist at the level of glycogen phosphorylase (Phos), not by altering the transformation to the more active a form but via posttransformational regulation. Enhanced FFA availability and oxidation appeared to provide a better match between energy demand and provision at the onset of exercise, which led to reduced accumulations of freeAMP (allosteric activator of Phos a) and P (substrate for Phos) and a resultant lower flux through Phos (13).

However, because smaller alterations in the energy status of the cell, and therefore in the requirement for glycogenolysis, would be expected to occur at the onset of lower-intensity exercise, it is possible that the regulation of fat/CHO interaction at lower power outputs (i.e., <85% V̇O2max) may occur as classically proposed. Very few studies have examined both the presence and regulation of the G-FA cycle during low-to-moderate aerobic exercise in humans (20, 21).

The purpose of this study was to enhance FFA provision to human skeletal muscle during low (40% V̇O2max) and moderate (65% V̇O2max) intensity aerobic exercise and determine 1) whether CHO sparing occurred (reduced muscle glycogen use and/or leg glucose uptake) and 2) whether regulation of CHO sparing was as classically proposed. On the basis of our previous findings during intense aerobic exercise (12, 13), it was
hypothesized that elevated FFA before exercise would result in significant sparing of muscle glycogen with no concomitant change in muscle citrate, acetyl-CoA, or PDH activation and no reduction in leg glucose uptake compared with control.

METHODS

Subjects. Eight males volunteered to participate in the study. Three subjects were well trained, three were highly active, and two were untrained (means ± SD: age, 24.5 ± 1.6 yr; height, 175 ± 9 cm; mass, 76.1 ± 9.6 kg). Written consent was obtained after the experimental procedures, and possible risks and benefits were explained. The study was approved by the Human Ethics Committees of both universities.

Preexperimental protocol. All participants initially performed an incremental VO2max test on a cycle ergometer. The mean VO2max 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% VO2max. 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 the subjects’ diets 48 h before each trial [CHO ingestion before the control and Intralipid (Int) trials was 55–60% of total caloric intake].

Experimental protocol. Each subject participated in two experimental trials, separated by 1–2 wk. On the morning of each trial day, subjects reported to the laboratory having eaten a meal high in CHO content 1–2 h before arrival. In addition, all participants consumed 1–2 bagels and 250–500 ml Gatorade −2–3 h before exercise to ensure low resting levels of plasma FFA. Subjects cycled for 10 min at 40% VO2max and 60 min at 65–70% VO2max, 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 the subjects’ diets 48 h before each trial [CHO ingestion before the control and Intralipid (Int) trials was 55–60% of total caloric intake].

Musclesampling. Muscle samples were frozen immediately in liquid N2. A small piece (10–35 mg) was chipped from each biopsy (under liquid N2) for measurement of the fraction of PDH in the more active a form (PDHa) (8, 27). The remainder of the sample was freeze-dried, dissected free of blood and connective tissue, and powdered. A portion of powdered muscle (4–6 mg) from the resting (0 min) and 70-min exercise biopsies was alkaline extracted and used for enzymatic determination of muscle glycogen (18). A further portion of dry muscle (−5 mg) was extracted in 0.5 M perchloric acid and 1 mM EDTA, neutralized to pH 7.0 with 2.2 M KHCO3, and analyzed for acetyl-CoA, acetyl-carnitine (6), phosphocreatine, creatine (Cr), citrate, ATP, and lactate (1, 18). Muscle metabolites were normalized to the highest total Cr content for a given individual [R total Cr = 130.6 ± 4.4 mmol/kg dry muscle (dm)] to correct for nonmuscle contamination. Free contents of ADP and AMP were calculated from the nearequilibrium Cr kinase and adenylate kinase reactions, respectively, as previously described (13), and H+ concentration ([H+]) was determined from the regression equation between lactate accumulation and [H+] for dynamic exercise (34). Free phosphate content (Pit) at rest was assumed to be 10.8 mmol/kg dm (7). This value was added to the difference between rest and exercise phosphocreatine contents to estimate exercise [Pit].
Blood sampling and analysis and calculation of blood flow. Blood samples (−9 ml) were drawn from the radial artery and femoral vein in heparinized plastic syringes and placed on ice. Each blood sample was analyzed for O₂ and CO₂ contents (Cameron Instrument, Port Arkansas, TX), hemoglobin (OSM-3 Hemoximeter; Radiometer, Copenhagen, Denmark), and hematocrit (centrifugation method).

Plasma for FFA determination (Wako NEFA C test kit, Wako Chemical) was obtained by immediate centrifugation of heparinized blood and incubation at 56°C in the presence of NaCl (13). This procedure denatures the lipoprotein lipase released into the blood by heparin injection, and thus avoids falsely elevated FFA measures. 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, lactate, and glycerol (1). Leg blood flow was not measured in this study as it has been demonstrated to be unaffected by Int and heparin infusion (17). However, leg blood flow was estimated from the pulmonary O₂ uptake and the leg arterial-venous (a–v) O₂ difference as described by Jorfeldt and Wahren (22). The leg respiratory quotient (RQ) was calculated from:

\[ \text{RQ} = \frac{V_{\text{CO}_2}}{V_{\text{O}_2}} \]

\[ \text{Leg CO}_2 \text{ production (blood flow × v-a CO}_2 \text{ content difference)} \]

\[ \text{Leg O}_2 \text{ uptake (blood flow × a-v O}_2 \text{ content difference)} \]

Blood substrate uptake and release data were calculated according to the Fick equation: blood flow × a–v substrate concentration difference.

Statistical analyses. Experimental data are presented as means ± SE and were analyzed by two-way analysis of variance with repeated measures over time. When a significant F ratio was obtained, the Tukey post hoc test was used to compare means. Pre- and postexercise glycogen contents and glycogen utilization during exercise were compared between conditions using a paired-samples t-test. Significance was accepted at \( P < 0.05 \).

**RESULTS**

Respiratory gas exchange, blood gases, and blood flow. Whole body VO₂ was similar in the two conditions (Table 1). However, RER was lower throughout the elevated FFA condition: 0.92 ± 0.02 vs. 0.89 ± 0.01 at 40% VO₂max and 0.94 ± 0.01 vs. 0.91 ± 0.01 at 65% VO₂max in Con and Int, respectively. The blood a–v O₂ content difference and leg blood flow data were not different between trials. Calculated RQ across the working leg muscles was consistently lower during Int, but this difference was not statistically significant (Table 1).

Blood metabolites. Int infusion led to an elevation in plasma arterial FFA from 0.25 ± 0.03 to 0.69 ± 0.04 mM at rest (Fig. 2A). During exercise, FFA was significantly higher during Int at all time points and peaked at 0.89 ± 0.05 mM at 50 min. In the Con trial, FFA was significantly elevated above rest only at 68 min (Fig. 2A). Changes in plasma FFA during Int were reflected by significant increases in whole blood glycerol (Fig. 2B). Arterial glycerol levels during Con were significantly less than Int at all time points and increased over time such that values were greater than rest by 50 min exercise (Fig. 2B). No significant differences occurred in whole blood arterial glucose or lactate between conditions (Table 2). Blood glucose decreased at all exercise time points, and blood lactate increased above rest levels at both power outputs during both conditions (Table 2). Blood data were not corrected for fluid shifts during exercise, as no differences in hemoglobin concentration or hematocrit occurred between conditions (data not shown).

**Blood metabolite exchange.** Elevated arterial plasma FFA resulted in an approximately fourfold increase in FFA uptake during Int over Con (Table 3). Interestingly, the increased arterial glycerol levels during Int appeared to reverse glycerol exchange from net release during Con to net uptake during Int. Int infusion resulted in a significant reduction in the rate of lactate efflux at 18 and 34 min of exercise compared with Con (Fig. 3A) but had no effect on glucose uptake (Fig. 3B).

Muscle metabolites. Resting glycogen levels were not different between conditions (Table 4). During exercise, muscle glycogen degradation was 297 ± 36 mmol.

### Table 1. Respiratory variables, blood a–v O₂ content difference, leg blood flow, and leg RQ during 70-min cycle exercise at 40 and 65% VO₂max following Intralipid infusion or control

<table>
<thead>
<tr>
<th></th>
<th>40% VO₂max 8 min</th>
<th>65% VO₂max 8 min</th>
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<tbody>
<tr>
<td>VO₂ max, l/min</td>
<td></td>
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<tr>
<td>Con</td>
<td>1.56 ± 0.09</td>
<td>1.58 ± 0.09</td>
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<tr>
<td>Int</td>
<td>1.58 ± 0.09</td>
<td>1.58 ± 0.09</td>
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<tr>
<td>RER</td>
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<tr>
<td>Con</td>
<td>0.92 ± 0.02</td>
<td>0.99 ± 0.01</td>
</tr>
<tr>
<td>Int</td>
<td>0.89 ± 0.01</td>
<td>0.95 ± 0.01</td>
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<tr>
<td>a–v O₂ diff, ml/100 ml</td>
<td></td>
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<tr>
<td>Con</td>
<td>14.51 ± 0.6</td>
<td>15.43 ± 0.5</td>
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<tr>
<td>Int</td>
<td>15.43 ± 0.5</td>
<td>15.43 ± 0.5</td>
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<tr>
<td>LBF, l/min</td>
<td></td>
<td></td>
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<tr>
<td>Con</td>
<td>3.48 ± 0.23</td>
<td>3.28 ± 0.15</td>
</tr>
<tr>
<td>Int</td>
<td>3.28 ± 0.15</td>
<td>3.28 ± 0.15</td>
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<tr>
<td>Leg RQ</td>
<td></td>
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<tr>
<td>Con</td>
<td>0.91 ± 0.05</td>
<td>0.87 ± 0.05</td>
</tr>
<tr>
<td>Int</td>
<td>0.87 ± 0.05</td>
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<table>
<thead>
<tr>
<th></th>
<th>18 min</th>
<th>34 min</th>
<th>50 min</th>
<th>68 min</th>
<th>( \pi )</th>
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</thead>
<tbody>
<tr>
<td>VO₂ max, l/min</td>
<td></td>
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<tr>
<td>Con</td>
<td>2.36 ± 0.11</td>
<td>2.55 ± 0.12</td>
<td>2.60 ± 0.13</td>
<td>2.67 ± 0.13</td>
<td>2.54 ± 0.06</td>
</tr>
<tr>
<td>Int</td>
<td>2.67 ± 0.09</td>
<td>2.57 ± 0.09</td>
<td>2.66 ± 0.10</td>
<td>2.70 ± 0.12</td>
<td>2.57 ± 0.06</td>
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<tr>
<td>RER</td>
<td></td>
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<tr>
<td>Con</td>
<td>0.99 ± 0.01</td>
<td>0.94 ± 0.01</td>
<td>0.93 ± 0.01</td>
<td>0.92 ± 0.01</td>
<td>0.94 ± 0.01</td>
</tr>
<tr>
<td>Int</td>
<td>0.95 ± 0.01</td>
<td>0.90 ± 0.01</td>
<td>0.89 ± 0.01</td>
<td>0.90 ± 0.01</td>
<td>0.91 ± 0.01*</td>
</tr>
<tr>
<td>a–v O₂ diff, ml/100 ml</td>
<td></td>
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<td></td>
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<tr>
<td>Con</td>
<td>16.62 ± 0.41</td>
<td>17.2 ± 0.37</td>
<td>17.93 ± 0.36</td>
<td>17.36 ± 0.40</td>
<td>17.2 ± 0.21</td>
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<tr>
<td>Int</td>
<td>17.00 ± 0.78</td>
<td>17.35 ± 0.68</td>
<td>17.37 ± 0.67</td>
<td>16.64 ± 0.71</td>
<td>17.09 ± 0.4</td>
</tr>
<tr>
<td>LBF, l/min</td>
<td></td>
<td></td>
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<tr>
<td>Con</td>
<td>4.76 ± 0.23</td>
<td>5.01 ± 0.26</td>
<td>4.92 ± 0.31</td>
<td>5.20 ± 0.29</td>
<td>4.97 ± 0.14</td>
</tr>
<tr>
<td>Int</td>
<td>4.69 ± 0.11</td>
<td>4.99 ± 0.13</td>
<td>5.19 ± 0.18</td>
<td>5.52 ± 0.31</td>
<td>5.10 ± 0.12</td>
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<tr>
<td>Leg RQ</td>
<td></td>
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<tr>
<td>Con</td>
<td>1.03 ± 0.03</td>
<td>0.99 ± 0.03</td>
<td>0.97 ± 0.03</td>
<td>0.99 ± 0.03</td>
<td>1.00 ± 0.02</td>
</tr>
<tr>
<td>Int</td>
<td>0.93 ± 0.04</td>
<td>0.93 ± 0.03</td>
<td>0.95 ± 0.02</td>
<td>0.97 ± 0.02</td>
<td>0.94 ± 0.01</td>
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</table>

Data are means ± SE; \( n = 7 \). RER, respiratory exchange ratio; a–v O₂ diff, arterial-venous O₂ content difference; LBF, leg blood flow; RQ, respiratory quotient. VO₂ max, maximal O₂ uptake; Int, Intralipid infusion; Con, control. *Significantly different from Con.
glucosyl units·min⁻¹·kg dm⁻¹ in the Con trial vs. 230 ± 66 mmol·min⁻¹·kg dm⁻¹ following Int infusion. This corresponded to a 22.6% sparing during Int.

Muscle ATP remained constant throughout exercise in both conditions (Table 5). Phosphocreatine decreased significantly from rest during exercise at 65% \( \dot{V}O_{2\text{max}} \), but was not affected by Int. Similarly, muscle lactate increased above rest levels at 65% \( \dot{V}O_{2\text{max}} \) and revealed no differences between conditions. Muscle acetyl-CoA and acetyl-carnitine both increased from rest to 40% \( \dot{V}O_{2\text{max}} \) and from 40 to 65% \( \dot{V}O_{2\text{max}} \) but were unaffected by Int infusion (Table 5). Elevation of FFA levels with Int infusion resulted in a concomitant increase in muscle citrate at rest, which remained elevated throughout exercise (Fig. 4).

Calculated values for free ADP, AMP, and P, were similar between conditions at rest and at 10 and 20 min exercise, but were significantly reduced at 68 min during exercise in the presence of elevated FFA (Fig. 5, A and B). P, accumulation was also reduced at 68 min during Int, but this difference failed to reach significance (Fig. 5C).

The transformation of PDH into the more active \( PDH_a \) was significantly reduced at all exercise time points during the Int trial (Fig. 6).

**DISCUSSION**

The present study demonstrated that elevated plasma FFA levels produced a significant reduction in glycogenolysis (23%) during 68 min of moderate cycle exercise. This result is consistent with previous investigations (9, 38) in which subjects exercised at 70% \( \dot{V}O_{2\text{max}} \) for 30 and 60 min and used 40 and 28% less muscle glycogen compared with low-fat control trials. In the present study, the RER (pulmonary) and RQ data (measured across the working leg muscles) also indicated reduced CHO use and increased fat oxidation in the Int trial.

Classic regulation of the G-FA cycle is based on the premise that increased availability of FFA will enhance the rate of fat oxidation and lead to increased acetyl-CoA and citrate concentrations (28). These fat-induced increases in citrate and acetyl-CoA are proposed to downregulate PFK and PDH, respectively, and consequently lead to a decline in the amount of CHO oxidized. Decreased activity of PFK should result in accumulation of G-6-P, which in turn is proposed to decrease hexokinase activity and lead to reduced glucose uptake (29).

In addition to glycogen sparing, Int infusion in the present study resulted in an elevated muscle citrate content and reduced transformation of PDH to the more active a form. These results are consistent with the classic description of the G-FA cycle (29). However, there was no concomitant increase in muscle acetyl-CoA content and no reduction in glucose uptake associated with increased FFA availability. Calculated free

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**Table 2. Arterial glucose and lactate concentrations during 70-min cycle exercise at 40 and 65% \( \dot{V}O_{2\text{max}} \) following Intralipid infusion or control**

<table>
<thead>
<tr>
<th></th>
<th>Rest 0 min</th>
<th>40% ( \dot{V}O_{2\text{max}} ) 8 min</th>
<th>65% ( \dot{V}O_{2\text{max}} )</th>
<th>18 min</th>
<th>34 min</th>
<th>50 min</th>
<th>68 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>4.62±0.30</td>
<td>5.05±0.14</td>
<td>3.96±0.20 *</td>
<td>4.06±0.22 *</td>
<td>4.01±0.14 *</td>
<td>3.91±0.18 *</td>
<td>3.80±0.19 *</td>
</tr>
<tr>
<td>Int</td>
<td>5.28±0.17</td>
<td>4.87±0.26</td>
<td>4.28±0.16 *</td>
<td>4.01±0.14 *</td>
<td>4.18±0.15 *</td>
<td>4.25±0.21 *</td>
<td>4.36±0.14 *</td>
</tr>
<tr>
<td>Lactate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>0.45±0.12</td>
<td>0.20±0.03</td>
<td>1.08±0.21 *</td>
<td>2.98±0.53 *</td>
<td>3.17±0.62 *</td>
<td>2.95±0.52 *</td>
<td>2.31±0.37 *</td>
</tr>
<tr>
<td>Int</td>
<td>0.42±0.14</td>
<td>0.20±0.10</td>
<td>0.66±0.18 *</td>
<td>2.65±0.29 *</td>
<td>3.00±0.44 *</td>
<td>3.01±0.46 *</td>
<td>3.12±0.62 *</td>
</tr>
</tbody>
</table>

Data are means ± SE in mM; n = 7. *Significantly different from 0 min.
ADP and AMP contents were also reduced in Int compared with Con at 68 min of exercise. The reduced flux through Phos during Int may have been due to reduced free AMP and Pi contents during exercise at 65% \( \dot{V}O_{2\text{max}} \), and PFK may have been inhibited by increased cytoplasmic citrate. The mechanism for reduced transformation of PDH to the more active \( \alpha \) form (which was not related to acetyl-CoA) is unknown, but suggests reduced flux through PDH. Therefore, although downregulation of CHO metabolism in the presence of high [FFA] is partially regulated as classically proposed, other regulatory factors appear to contribute.

Glucose uptake. Elevation of plasma FFA did not reduce glucose uptake at rest or during exercise (Fig. 3B). Previous studies have demonstrated reduced whole body glucose disposal (4), leg muscle glucose uptake (17, 23), and forearm glucose oxidation (39) at rest following Int infusion. Insulin was not measured in this study, although a similar protocol reported no change in resting insulin levels with Int (17). Also, some of the studies demonstrating a reduction in glucose disposal were done under conditions of hyperinsulinemia/high FFA for a much longer time than the 30 min in the present study (4, 23). Last, although the glucose uptake values were not statistically different between trials (Con, 0.09 ± 0.07 mmol·min\(^{-1} \cdot \text{leg}^{-1} \); Int, 0.03 ± 0.11 mmol·min\(^{-1} \cdot \text{leg}^{-1} \)), they were similar to the resting data reported by Hargreaves et al. (17) (Con, 0.11 ± 0.02 mmol·min\(^{-1} \cdot \text{leg}^{-1} \); Int, 0.04 ± 0.01 mmol·min\(^{-1} \cdot \text{leg}^{-1} \)).

Few researchers have investigated glucose uptake during exercise in humans. Romijn et al. (33) reported that blood glucose disappearance (stable-isotope tracer) was unaffected by elevated plasma FFA during cycling at 85% \( \dot{V}O_{2\text{max}} \). A significant reduction in glucose uptake was, however, reported during 60 min of leg extension exercise at 80% work capacity in response to elevated FFA (17). The discrepancy in results is difficult to explain but may be due to differences in experimental protocols.

In the Hargreaves et al. (17) study, all Con trials were performed immediately before Int trials (opposite legs) and subjects were fasted overnight, producing high

| Table 3. Net leg FFA and glycerol uptake/release during 70-min cycle exercise at 40 and 65% \( \dot{V}O_{2\text{max}} \) following Intralipid infusion or control |
|---------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|
|                                | 40% \( \dot{V}O_{2\text{max}} \) | 65% \( \dot{V}O_{2\text{max}} \) |
|                                | 8 min       | 18 min      | 34 min      | 50 min      | 68 min      | \( \bar{x} \) |
| FFA, mmol·min\(^{-1} \cdot \text{leg}^{-1} \) |
| Con                        | 0.04 ± 0.01 | 0.03 ± 0.02 | 0.04 ± 0.03 | 0.03 ± 0.02 | 0.10 ± 0.02 | 0.05 ± 0.01 |
| Int                        | 0.18 ± 0.05*| 0.16 ± 0.04*| 0.31 ± 0.09*| 0.20 ± 0.09*| 0.24 ± 0.08*| 0.23 ± 0.04* |
| Glycerol, µmol·min\(^{-1} \cdot \text{leg}^{-1} \) |
| Con                        | −1.66 ± 5.29 | −6.23 ± 4.0  | −10.13 ± 7.6 | −7.42 ± 6.9  | −6.69 ± 10.3 | −7.62 ± 3.85 |
| Int                        | 28.9 ± 14.5 | 30.8 ± 23.3  | 13.7 ± 35.7  | −11.3 ± 19.7 | 47.7 ± 20.0  | 19.2 ± 14.0  |

Data are means ± SE; \( n = 7 \). FFA, plasma free fatty acids. Negative values indicate net release. *Significantly different from Con.

Fig. 3. Net leg lactate efflux (A) and glucose uptake (B) during 70 min of cycling at 40 and 65% \( \dot{V}O_{2\text{max}} \) with Intralipid infusion or control. Values are means ± SE. *Significantly different from Intralipid.
resting control levels of plasma FFA (0.60 ± 0.07 vs. 0.25 ± 0.04 mM, present study). Interestingly, no increase in FFA uptake and no reduction in muscle glycogen use occurred during knee-extensor exercise in response to elevated FFA (17). The knee-extensor model has increased muscle blood flow relative to power output compared with conventional dynamic exercise (i.e., cycling or running). This, combined with the relatively high control FFA levels, may have accounted for the inability of Int to increase FFA uptake, as the delivery of FFA to the muscles was already high in the Con trial. The Int infusion had no independent effect on leg blood flow because measured values were the same in the Con and Int trials (17).

Hargreaves et al. (17) did not measure muscle citrate but found similar G-6-P and glucose accumulations and leg citrate release between trials. They suggested that reduced glucose uptake was due to direct inhibition of glucose transport rather than by the classical G-FA cycle. If elevated plasma FFA exert a direct effect on glucose transport, this effect was not observed in the present study or a similar protocol at a higher power output (85% VO_{2max}) (33).

Control of glycogen Phos. In the present study, glycogen Phos activity was reduced during exercise in the high-fat condition (less glycogen use), independent of inhibition of PFK or PDH and the regulatory scheme proposed in the classic G-FA cycle. Phos exists in two interconvertible forms: a more active a and a less active b form. Transformation from Phos b to a is thought to occur at the onset of exercise, primarily due to stimulation of Phos kinase by increased cytoplasmic [Ca^{2+}] and to a minor degree by epinephrine (7). Phos a is then regulated posttransformationally by freeAMP, an allosteric modulator, and substrate availability (P_i and glycogen) (32).

Table 5. Muscle metabolites during cycle exercise (10 min at 40% and 60 min at 65% VO_{2max}) in control and Intralipid trials

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Preexercise</th>
<th>40% VO_{2max} 10 min</th>
<th>65% VO_{2max} 20 min</th>
<th>65% VO_{2max} 70 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>26.6 ± 1.2</td>
<td>26.1 ± 3.0</td>
<td>24.3 ± 2.9</td>
<td>24.6 ± 1.3</td>
</tr>
<tr>
<td>Int</td>
<td>24.1 ± 2.9</td>
<td>25.4 ± 3.1</td>
<td>25.9 ± 0.6</td>
<td>24.6 ± 0.9</td>
</tr>
<tr>
<td>PCr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>83.4 ± 1.4</td>
<td>76.6 ± 8.8</td>
<td>54.4 ± 6.5†</td>
<td>43.5 ± 4.6†</td>
</tr>
<tr>
<td>Int</td>
<td>81.7 ± 9.4</td>
<td>74.3 ± 9.2</td>
<td>50.1 ± 3.4†</td>
<td>55.5 ± 4.6†</td>
</tr>
<tr>
<td>Lactate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>6.2 ± 1.7</td>
<td>7.2 ± 2.4</td>
<td>29.0 ± 5.7†</td>
<td>25.6 ± 6.1†</td>
</tr>
<tr>
<td>Int</td>
<td>8.2 ± 1.6</td>
<td>10.1 ± 2.4</td>
<td>30.2 ± 6.2†</td>
<td>25.2 ± 4.3†</td>
</tr>
<tr>
<td>Acetyl-CoA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>8.8 ± 0.9</td>
<td>14.8 ± 1.8*</td>
<td>23.2 ± 3.3†</td>
<td>26.7 ± 1.9†</td>
</tr>
<tr>
<td>Int</td>
<td>8.6 ± 1.2</td>
<td>16.8 ± 2.8*</td>
<td>27.0 ± 2.1†</td>
<td>26.6 ± 2.3†</td>
</tr>
<tr>
<td>Acetyl-carn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>1.2 ± 0.2</td>
<td>5.4 ± 0.9*</td>
<td>11.4 ± 1.7†</td>
<td>13.3 ± 1.0†</td>
</tr>
<tr>
<td>Int</td>
<td>1.1 ± 0.2</td>
<td>5.4 ± 0.9*</td>
<td>12.2 ± 0.8†</td>
<td>13.6 ± 1.0†</td>
</tr>
</tbody>
</table>

Data are means ± SE in mmol/kg dry muscle (acetyl-CoA in µmol/kg dry muscle). Acetyl-carn, acetyl-carnitine; PCr, phosphocreatine. *Significantly different from preexercise; †significantly different from 10 min.

Fig. 4. Muscle citrate content at rest and accumulation during 70 min of cycling at 40 and 65% VO_{2max} with Intralipid infusion or control. Values are means ± SE. dm, Dry muscle. *Significantly different from control.

Fig. 5. Muscle freeADP (A), freeAMP (B), and P_i (C) contents during 70 min of cycling at 40 and 65% VO_{2max} with Intralipid infusion or control. Values are means ± SE. *Significantly different from control.
transformation despite a 45% sparing of muscle glycogen contents and epinephrine concentrations (13). In addition, previous investigations have suggested that the reduction of Phos activity early in exercise are unknown. The mechanisms that may have reduced glycogenolysis and CHO use occurred, both early in exercise and beyond 20 min during Int. The mechanisms that may have reduced Phos activity early in exercise are unknown.

Muscle citrate and PFK activity. The classic G-FA cycle suggests that increased fat oxidation results in citrate-mediated inhibition of PFK, accumulation of G-6-P, and subsequent inhibition of glucose uptake (28, 29). In this study, glucose uptake was unaffected by Int, but muscle citrate was slightly (but significantly) elevated at rest and during exercise. Because muscle G-6-P and glucose levels were not measured, it is difficult to assess whether PFK activity was downregulated by the accumulation of muscle citrate or lower due to the reduced Phos activity higher up in the pathway. However, the unaffected glucose uptake suggests that either PFK/hexokinase activities were not inhibited, or, if enzyme activities were reduced by citrate, this reduction had no effect on glucose uptake.

Previous investigations of muscle citrate accumulation in response to increased FFA availability during exercise have produced variable results. Studies involving high-intensity exercise (75–85% VO_{2max}) have consistently reported no effect of FFA elevation on muscle citrate levels, despite the occurrence of significant glycogen sparing (12, 13, 27, 37). Two earlier studies reported increased muscle citrate and decreased muscle glycogenolysis during cycle exercise at 65% VO_{2max} in response to 5 days of high-fat diet and aerobic training, respectively (20, 21). Studies at lower power outputs (<65% VO_{2max}) have not measured muscle citrate (24, 30), while others have reported increased resting muscle citrate with elevated FFAs (13, 20, 27, 37).

Citrate is produced in the mitochondria and must move to the cytoplasm to affect PFK. Whole muscle measurements of citrate do not allow for partitioning of citrate to the different cellular compartments. Therefore, an increase in total muscle citrate may not reflect increased cytoplasmic citrate.

Early in vitro investigations overestimated the potency of citrate to inhibit PFK activity during exercise, because increases in positive regulators override its inhibitory effect (5, 25). Recent in vitro work reported that the citrate-mediated inhibition of PFK appears to be most powerful at rest and that additional increases that occur in response to increased FFA availability and exercise would not increase PFK inhibition at rest or during exercise (25). In the present study, we cannot conclusively state whether the small increases in muscle citrate with high [FFA] decreased CHO metabolism but predict that they would have little effect on PFK.

Control of PDH activity. In response to Int infusion, the transformation of PDH to the more active a form was reduced at all exercise time points, while acetyl-CoA increased to the same level in both conditions. PDH activity is regulated by a complex phosphorylation cycle. It is dephosphorylated and activated (to PDHa) by PDH phosphatase, and phosphorylated and inactivated by PDH kinase. Several metabolites are purported to be regulators of the PDH complex (see Refs. 19 and 31 for review). Ca^{2+} is a potent activator of PDH phosphatase, whereas PDH kinase can be inhibited by pyruvate and a high NAD^{+}-to-NADH ratio and activated by high ATP-to-ADP and acetyl-CoA-to-CoA ratios.

The concept of the G-FA cycle is partially based on the premise that increased fat oxidation will result in the accumulation of acetyl-CoA, which in turn will activate PDH kinase and downregulate PDH (28). Acetyl-CoA control of PDH activity appears to exist in humans at rest (4, 27), but several recent investigations have suggested that this potential regulatory effect is overridden by other factors during exercise (8, 12, 13, 27). Because acetyl-CoA increased at a time when PDH was transformed to the a form during exercise in both trials, our results support this concept.

There are other potential regulators of PDH that may account for the lower transformation of PDH to the
more active a form during Int. Ca\(^{2+}\) activates PDH phosphatase (10), but Ca\(^{2+}\) was assumed to be similar between trials because power output was constant. PDH kinase activity can be inhibited by ADP, which may accumulate in the mitochondria during exercise. The calculated accumulation of free ADP was reduced during Int only at 68 min of exercise and therefore was unlikely to contribute early in exercise. Also, the intra-mitochondrial [ADP] is unknown, making it difficult to assess the potential effect of ADP on PDH transformation.

PDH kinase can also be inhibited by pyruvate, and, although it was not measured, the reduced glycogenolytic flux during Int suggests that less pyruvate per unit time was available to PDH. A feed-forward mechanism could exist whereby reduced glycogenolytic flux is directly linked to downregulation of PDHs. Support for this suggestion was reported in a study that utilized dietary manipulations to alter plasma FFA levels and found reduced glycogenolysis, pyruvate content, and PDH during cycle exercise at 75% \(V_{\text{O2max}}\) following a low-CHO diet (27).

Measurements of muscle pyruvate in control and high-FFA conditions are needed to examine this possibility. Evidence from in vitro studies suggests that a decrease in the NAD\(^+\)-to-NADH ratio decreases the transformation of PDH to the more active a form (26). Direct measurements of the mitochondrial reduction-oxidation (redox) state of the NAD\(^{+}\)/NADH couple in skeletal muscle during contraction have produced inconsistent results. Using the glutamate dehydrogenase reaction, a substantial increase in estimated mitochondrial redox state (increased NAD\(^+\)-to-NADH ratio) was reported during cycle exercise in humans (75 and 100% \(V_{\text{O2max}}\)) (16). In contrast, Duhaeylongsd et al. (11) monitored redox state in stimulated canine gracilis muscle using near-infrared spectroscopy and reported a decreased redox state at all power outputs. Furthermore, NADH measured in human muscle has been shown to decrease at 45% \(V_{\text{O2max}}\) and to increase at higher power outputs (35). No attempt was made to estimate the mitochondrial redox state in the present study, but it is possible that NADH accumulation was increased by elevated FFA availability during Int. This would reduce PDH transformation if NADH exerts a significant regulatory effect on PDH kinase.

In summary, the present study confirms previous findings that elevated FFA levels result in significant sparing of muscle glycogen during moderate-intensity exercise. Although certain aspects of the classical regulation of fat/CHO interaction were present, other regulatory factors were involved. No difference in glucose uptake occurred between trials; thus regulation of CHO use occurred at the level of glycogen Phos, PK, and/or PDH. Reduced flux through Phos with high [FFA] may have been due to reduced free AMP and P, accumulation, which occurred late in exercise, and PKF may have been inhibited by increased cytoplasmic citrate. PDH transformation to the more active a form during exercise was reduced following Int infusion, but the decrease was not due to increased acetyl-CoA accumulation. The mechanisms for the reduced transformation of PDH are unknown but may be due to reduced pyruvate and/or NADH accumulation during exercise with Int infusion.

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