Fat is an important source of energy for resting and contracting muscle, and is available exogenously from the circulation (fatty acids, chylomicrons, very low density lipoproteins (VLDL)) and from muscle tissue (intramuscular triacylglycerol; IMTG). IMTG is stored within lipid droplets adjacent to mitochondria (Hoppeler et al. 1999) and is believed to be an energy source during prolonged endurance activities (Havel et al. 1967; Essen, 1977; Staron et al. 1989; Romijn et al. 1993; Klein et al. 1994; Romijn et al. 1995). However, the contribution of the IMTG pool as a metabolic substrate during prolonged submaximal exercise remains controversial (for review, see Watt et al. 2002). Relatively little attention has been given to lipid utilization during the recovery period following exhaustive, glycogen-depleting exercise in humans. The data from several studies indicate an increase in whole-body fat oxidation after exercise as demonstrated by a decrease in the respiratory exchange ratio (RER) (Krzentowski et al. 1982; Bielinski et al. 1985; Kiens & Richter, 1998). Recently, it was also demonstrated that IMTG content decreased significantly during the initial 18 h of recovery in well-trained individuals (Kiens & Richter, 1998). These authors suggested that following glycogen-depleting exercise, muscle glycogen resynthesis is of high metabolic priority, resulting in the preferential utilization of IMTG and circulating lipids by the recovering skeletal muscle. Paradoxically, the reduction in IMTG content occurred during a period of high-CHO feeding when circulating insulin, an antilipolytic hormone, was elevated. However, recent 1H-MRS studies in endurance-trained subjects have
failed to demonstrate a reduction in IMTG content during recovery, with reports of either unchanged (Decombaz et al. 2000, 2001) or increased (Larson-Meyer et al. 2002) IMTG content. It should be noted that there are several differences between the biopsy and MRS studies which may result in disparate findings: (1) sampling site, i.e. soleus/tibialis anterior (Type I fibres) in the MRS studies, vastus lateralis (mixed) in the biopsy studies; (2) differences in muscle recruitment patterns (running, MRS; cycling, biopsy); (3) timing of post-exercise sampling, i.e. IMTG content was not determined until 22 h of recovery in the study by Larson-Meyer et al. (2002) as opposed to within the first 6 h of recovery in the study by Kiens & Richter (1998).

Since the existing MRS data suggest that IMTG are not utilized as an energy source in the post-exercise recovery period, we felt that it was important to verify these findings with direct measurements in human biopsy samples. Furthermore, potential mechanisms underlying altered substrate utilization following glycogen-depleting exercise, when combined with the standard practice of high-CHO feedings to replenish muscle glycogen stores, remain unknown. Thus, the objectives of this study were to: (1) examine the utilization of IMTG as an energy substrate after exhaustive exercise, and (2) provide a more thorough examination of the metabolic changes which occur in skeletal muscle during the recovery period following exhaustive, glycogen-depleting exercise. In order to facilitate comparison to the only previous study to measure IMTG content in muscle biopsies during recovery, we essentially replicated the experimental protocol of Kiens & Richter (1998). There were two major differences in design between these studies. First, in the present study, muscle biopsies were sampled in duplicate at each time point, which should permit a more accurate determination of IMTG content (our coefficient of variation between duplicate biopsies in trained individuals is 12% (Watt et al. 2002a) compared to 24% in untrained individuals (Wendling et al. 1996)). Second, we included additional metabolic measurements, namely pyruvate dehydrogenase activation (PDHa), and changes in muscle pyruvate, acetyl CoA and acetyl carnitine contents during recovery. Based on the premise that elevated insulin levels would be expected to inhibit IMTG lipolysis, and in view of the recent MRS data that do not support the use of IMTG following exercise, we hypothesized that IMTG content would remain unchanged during the recovery phase. Furthermore, we hypothesized that PDHa would decrease during recovery to facilitate the partitioning of glucose towards glycogen resynthesis.

### METHODS

#### Subjects

Eight endurance-trained males, age, 25 ± 3 years; body mass, 72.7 ± 2.1 kg; and maximal oxygen uptake ($\dot{V}_O_2_{max}$), 63.1 ± 2.6 ml kg$^{-1}$ min$^{-1}$ (mean ± S.E.M.) volunteered to participate in this study. Six of the subjects were competitive athletes (three swimmers, two runners, one canoeist) and two were highly active. All subjects were considered to be healthy, did not smoke or take any medication, and had no evidence of cardiovascular or metabolic diseases. The experimental procedures and possible risks of the study were explained orally and in writing to all participants before obtaining their informed, written consent to participate. The study protocol (Fig. 1) was approved by the ethics committees of both institutions, and was performed according to the Declaration of Helsinki.

#### Pre-experimental protocol

Daily energy intake and composition of each subject’s habitual diet was assessed using a 4-day food record (3 weekdays and 1 weekend day). Dietary data (Table 1) were analysed for energy intake and macronutrient composition (Food Processor Nutrition Analysis Software, version 7, ESHA Research, Salem OH, USA). Subjects’ $\dot{V}_O_2_{max}$ was determined on an electromagnetically braked cycle ergometer (Lode Excalibur, Quinton Instruments, Seattle, WA, USA). $\dot{V}_O_2_{max}$ was determined from a plateau in $\dot{V}_O_2$ (increase < 2 ml kg$^{-1}$ min$^{-1}$) with a further increase in power output. From the $\dot{V}_O_2_{max}$ test, power outputs corresponding to submaximal $\dot{V}_O_2$ values to be used during the experimental trial (see below) were determined using linear regression analysis for each subject. All subjects were familiarized with the intensity of the glycogen-depleting protocol and the experimental power outputs verified ~1 week before the experiments. For 2 days prior to the experimental trials, all subjects consumed a high-CHO diet

### Table 1. Daily energy intake, dietary composition and glycaemic index before and during post-exercise recovery

<table>
<thead>
<tr>
<th></th>
<th>Pre-exercise</th>
<th></th>
<th></th>
<th>Recovery</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Habitual</td>
<td>2 days</td>
<td>Breakfast</td>
<td>Meal 1 1 h</td>
<td>Meal 2 4 h</td>
<td>Meal 3 7 h</td>
<td>Average</td>
<td></td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>2987 ± 229</td>
<td>3005 ± 300</td>
<td>205 ± 0</td>
<td>1446 ± 94</td>
<td>690 ± 33</td>
<td>815 ± 57</td>
<td>984 ± 78</td>
<td></td>
</tr>
<tr>
<td>CHO (%)</td>
<td>54 ± 1.6</td>
<td>60 ± 1.8</td>
<td>84 ± 0</td>
<td>70 ± 0.5</td>
<td>64 ± 0.8</td>
<td>65 ± 1.7</td>
<td>66 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Fat (%)</td>
<td>32 ± 1.2</td>
<td>26 ± 1.6</td>
<td>12 ± 0</td>
<td>19 ± 0.3</td>
<td>22 ± 0.4</td>
<td>22 ± 1.9</td>
<td>21 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Protein (%)</td>
<td>14 ± 0.7</td>
<td>14 ± 0.9</td>
<td>4 ± 0</td>
<td>11 ± 0.2</td>
<td>14 ± 0.4</td>
<td>13 ± 0.3</td>
<td>13 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>CHO (g (kg body wt)$^{-1}$)</td>
<td>ND</td>
<td>ND</td>
<td>0.63 ± 0.02</td>
<td>3.5 ± 0.2</td>
<td>1.5 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td>2.3 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Glycaemic index (GI)</td>
<td>ND</td>
<td>ND</td>
<td>68.7 ± 0.0</td>
<td>64.1 ± 0.3</td>
<td>62.3 ± 0.3</td>
<td>55.8 ± 0.5</td>
<td>64.5 ± 0.6</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± s.e.m., n = 8. CHO, carbohydrate; ND, not determined. *Significantly different (P < 0.05) from habitual.
to maximize glycogen stores (Table 1) and abstained from exercise, and consumption of caffeine and alcohol.

**Experimental protocol**

Subjects reported to the laboratory at 09.30 h on the experiment day after a 10–12 h overnight fast. After voiding, body mass was recorded and a teflon catheter inserted into an antecubital vein for blood sampling. Following 30 min of rest in a supine position, resting \( \dot{V}_O_2 \) and \( \dot{V}_C_O_2 \) were measured (for determination of the respiratory exchange ratio (RER)), and a 5 ml venous blood sample was drawn prior to exercise. Subjects then consumed a light breakfast consisting of CHO-rich, high glycaemic index (GI) foods (Table 1). During a subsequent 2 h rest period, two incisions were made over the vastus lateralis muscle under local anaesthesia (2% lignocaine (lidocaine) without adrenaline (epinephrine)). Respiratory and blood samples were then obtained and exercise initiated on a Lode Excalibur cycle ergometer. The glycogen-depleting protocol was performed as previously described (Kuipers *et al.* 1987; Kiens & Richter, 1998). This consisted of cycling at 75% \( \dot{V}_O_2_{max} \) for 20 min, followed by alternating 2 min bouts of 90% and 50% of \( \dot{V}_O_2_{max} \) for 4–5 intervals, then decreasing intensity to 80% and 50% \( \dot{V}_O_2_{max} \) 2 min bouts for another 4–5 intervals, and finishing with 2 min 70% and 50% \( \dot{V}_O_2_{max} \) bouts until exhaustion. The mean exercise duration was 85.8 ± 3.2 min. During exercise, subjects ingested water *ad libitum* and a blood sample was taken just prior to exhaustion. After cessation of exercise (exhaustion), subjects were moved from the cycle ergometer to an examination table, at which point two percutaneous needle muscle biopsies were obtained from the same thigh. Approximately 3–4 min lapsed between the termination of the exercise and the procurement of the muscle samples. During the recovery period, duplicate muscle biopsy samples were obtained at 3, 6 and 18 h by alternating between left and right thighs through different incisions spaced 2–3 cm apart. Blood and breath samples were collected in heparinized collection tubes and placed on ice. A 200 µl aliquot was deproteinized in 1.0 ml of 0.6 M HClO₄ and the supernatant stored at −80 °C for subsequent fluorometric determination of whole blood glycerol, lactate and glucose (Bergmeyer, 1974). A second portion of whole blood was centrifuged, and 800 µl of plasma was added to 200 µl of 5 M NaCl at 56 °C for 30 min, to inactivate lipoprotein lipase. This treated plasma was analysed for fatty acids (Wako NEFA C test kit, Wako Chemicals, Richmond, VA, USA). The remaining untreated plasma was assayed in duplicate for insulin using an RIA specific kit (LINCO Research, St Charles, MO, USA). Muscle biopsies were immediately frozen in liquid N₂, removed from the needle while frozen, and stored in liquid N₂ until analysed. A small (10–20 mg) piece of muscle was chipped from one of the duplicate biopsies under liquid N₂ and analysed for PDH activation (PDHa), as previously described (Putman *et al.* 1993). The remaining muscle was freeze-dried, dissected free of blood, connective tissue and visible fat, and powdered for subsequent metabolite analyses. One aliquot of powdered muscle

**Recovery diet**

Energy and macronutrient intake for the three meals consumed during the recovery period (Table 1) were calculated for each subject and were similar to those used previously (Kiens & Richter, 1998). All subjects ate the same type of common food items and each meal was carefully prepared and weighed to the nearest gram prior to each trial. The first meal was ingested after 1 h of recovery and provided sufficient energy to replace that used during the exercise bout (~1450 kcal; 1 kcal = 4.184 kJ). A much lighter second meal was eaten after 4 h of recovery, followed by a third meal 7 h into the recovery period. After the final meal, subjects were only permitted to drink water until their final two muscle biopsies the next morning at 18 h of recovery. Total energy intake during recovery was matched closely to the average energy intake reported from individual 4-day food records (Table 1). The composition of the post-exercise recovery diet was calculated to provide 65–70% of energy from CHO, 20% from fat, and 10–15% from protein, with an average glycaemic index (GI) of 60–65 using glucose as a reference (Table 1).

**Analyses**

Blood samples were collected in heparinized collection tubes and placed on ice. A 200 µl aliquot was deproteinized in 1.0 ml of 0.6 M HClO₄ and the supernatant stored at −80 °C for subsequent fluorometric determination of whole blood glycerol, lactate and glucose (Bergmeyer, 1974). A second portion of whole blood was centrifuged, and 800 µl of plasma was added to 200 µl of 5 M NaCl at 56 °C for 30 min, to inactivate lipoprotein lipase. This treated plasma was analysed for fatty acids (Wako NEFA C test kit, Wako Chemicals, Richmond, VA, USA). The remaining untreated plasma was assayed in duplicate for insulin using an RIA specific kit (LINCO Research, St Charles, MO, USA).

Muscle biopsies were immediately frozen in liquid N₂, removed from the needle while frozen, and stored in liquid N₂ until analysed. A small (10–20 mg) piece of muscle was chipped from one of the duplicate biopsies under liquid N₂ and analysed for PDH activation (PDHa), as previously described (Putman *et al.* 1993). The remaining muscle was freeze-dried, dissected free of blood, connective tissue and visible fat, and powdered for subsequent metabolite analyses. One aliquot of powdered muscle

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**Figure 1. Schematic representation of protocol**

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(2–3 mg) was assayed enzymatically for glycogen as previously described (Harris et al. 1974). Total muscle triacylglycerol (IMTG) was extracted from a 4–8 mg sample of powdered muscle using chloroform–methanol (Frayn & Maycock, 1980). After phospholipid removal using silicic acid, the extracts were saponified with ethanolic KOH at 60 °C for 1 h, and neutralized with MgSO4. Triacylglycerol concentration was then determined by assaying the liberated glycerol fluorometrically. IMTG content was calculated from the mean of the paired biopsies at each time point. The remaining powdered muscle was extracted in a solution of 0.5M HClO4 and 1 M EDTA and neutralized with 2.2 M KHCO3. These extracts were assayed by spectrophotometry for ATP, phosphocreatine (PCr) and creatine as previously described (Bergmeyer, 1974; Harris et al. 1974). Pyruvate was determined fluorometrically (Passoneau & Lowry, 1993) and acetyl CoA and acetylcarnitine were determined by radiometric assays (Cederblad et al. 1990).

Statistics

Results were analysed using a one-way ANOVA with repeated measures for the time factor to test for changes in each variable measured during the recovery period. A Newman-Keuls post hoc test was used if differences between time points were detected. Significance was accepted at $P \leq 0.05$. Values are presented as means ± S.E.M.

RESULTS

Diet analysis

Total energy intake (Table 1) during the trial day (breakfast + recovery meals) averaged 3156 ± 164 kcal and was closely matched to daily habitual caloric intake (2987 ± 229 kcal). Dietary composition during recovery consisted of an average of 67 ± 0.9% of kcal from CHO, 21 ± 0.8% from fat and 13 ± 0.3% from protein, and an average GI of 64.5 ± 0.6. Total CHO intake during the recovery period was 491 ± 28 g or 6.8 ± 0.3 g kg⁻¹. Total fat intake was 73 ± 3 g during the recovery period. The first meal consumed during recovery provided an average of 46 ± 0.9 and 52 ± 0.5%, respectively, of the total energy and CHO intake for the trial day.

Respiratory and blood responses

Resting RER was 0.77 ± 0.02 at 2 h prior to exercise, and 0.80 ± 0.02 immediately prior to exercise. These were not statistically significant (Table 2). During the hour following exhaustive exercise, RER was significantly lower than baseline ($P < 0.001$), and returned to basal levels 2 h after exercise. Resting whole blood glucose was 4.1 ± 0.12 mmol l⁻¹ (Table 2) immediately prior to the commencement of exercise. After 2 h of recovery (1 h after the first high-CHO meal), blood glucose concentrations rose significantly above baseline and remained elevated for another 3 h ($P < 0.001$). Plasma insulin concentrations increased markedly above baseline after meal 1 to reach a peak of 68.3 ± 13.3 μU ml⁻¹ ($P < 0.001$) at 2 h of recovery (Table 2). For the following 4 h, plasma insulin concentration remained significantly elevated and returned to baseline levels by the following morning at 18 h after exercise. Plasma fatty acid levels were 0.22 ± 0.03 mmol l⁻¹ immediately prior to exercise and increased

### Table 2. RER, blood glucose, plasma insulin, fatty acids, glycerol and lactate before exercise, near exhaustion and during an 18 h post-exercise recovery period

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>RER</th>
<th>Glucose (mmol l⁻¹)</th>
<th>Insulin (μU ml⁻¹)</th>
<th>Fatty acids (mmol l⁻¹)</th>
<th>Glycerol (mmol l⁻¹)</th>
<th>Lactate (mmol l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>0.77 ± 0.02</td>
<td>4.2 ± 0.12</td>
<td>8.9 ± 0.9</td>
<td>0.26 ± 0.04</td>
<td>0.08 ± 0.01</td>
<td>0.3 ± 0.05</td>
</tr>
<tr>
<td>Pre-exercise</td>
<td>0.80 ± 0.02</td>
<td>4.1 ± 0.10</td>
<td>14.3 ± 2.0</td>
<td>0.22 ± 0.03</td>
<td>0.09 ± 0.003</td>
<td>0.5 ± 0.04</td>
</tr>
<tr>
<td>End-exercise</td>
<td>ND</td>
<td>3.6 ± 0.15</td>
<td>4.5 ± 0.6</td>
<td>0.40 ± 0.06</td>
<td>0.23 ± 0.02</td>
<td>5.2 ± 0.6</td>
</tr>
<tr>
<td>0.5</td>
<td>0.62 ± 0.03 ‡</td>
<td>3.6 ± 0.10</td>
<td>5.5 ± 0.4</td>
<td>1.27 ± 0.16 ‡</td>
<td>0.20 ± 0.02 ‡</td>
<td>1.7 ± 0.3 ‡</td>
</tr>
<tr>
<td>1</td>
<td>0.66 ± 0.03 ‡</td>
<td>3.7 ± 0.18</td>
<td>5.5 ± 1.3</td>
<td>1.54 ± 0.17 ‡</td>
<td>0.19 ± 0.02 ‡</td>
<td>0.8 ± 0.15</td>
</tr>
<tr>
<td>2</td>
<td>0.77 ± 0.02</td>
<td>6.3 ± 0.30 ‡</td>
<td>68.3 ± 13.3 ‡</td>
<td>0.40 ± 0.07</td>
<td>0.11 ± 0.01</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>0.77 ± 0.02</td>
<td>5.7 ± 0.31 ‡</td>
<td>65.7 ± 1.6 ‡</td>
<td>0.19 ± 0.03</td>
<td>0.09 ± 0.01</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>4</td>
<td>0.80 ± 0.02</td>
<td>5.3 ± 0.30 ‡</td>
<td>61.9 ± 9.4 ‡</td>
<td>0.18 ± 0.03</td>
<td>0.09 ± 0.01</td>
<td>0.9 ± 0.08</td>
</tr>
<tr>
<td>5</td>
<td>0.84 ± 0.02</td>
<td>4.8 ± 0.25 ‡</td>
<td>66.6 ± 8.3 ‡</td>
<td>0.18 ± 0.02</td>
<td>0.09 ± 0.01</td>
<td>0.9 ± 0.10</td>
</tr>
<tr>
<td>6</td>
<td>0.79 ± 0.02</td>
<td>4.2 ± 0.21 ‡</td>
<td>41.7 ± 3.1 ‡</td>
<td>0.22 ± 0.07</td>
<td>0.08 ± 0.01</td>
<td>0.8 ± 0.09</td>
</tr>
<tr>
<td>18</td>
<td>0.76 ± 0.02</td>
<td>4.3 ± 0.11 ‡</td>
<td>10.2 ± 1.0</td>
<td>0.44 ± 0.06</td>
<td>0.10 ± 0.04</td>
<td>0.4 ± 0.05</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M., n = 8. RER, respiratory exchange ratio; ND, not determined. Fatty acids are free fatty acids. Significantly different from before exercise, *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$. 

Figure 2. Muscle triacylglycerol (IMTG) contents in the vastus lateralis muscle immediately after exercise and during an 18 h recovery period

Values are means ± S.E.M. for 8 subjects.
significantly ($P < 0.001$) during the first hour of recovery to $1.54 \pm 0.17 \text{mm}$ (Table 2). After ingestion of the first meal, fatty acid concentration rapidly declined at 2 h ($0.40 \pm 0.07 \text{mm}$) and remained near baseline throughout the recovery period. Plasma glycerol concentrations were highest near exhaustion ($0.23 \pm 0.02 \text{mm}$), and similar to fatty acid responses, showed a marked reduction at 2 h and throughout recovery (Table 2). The exercise protocol produced a significant elevation in plasma lactate concentration ($5.2 \pm 0.6 \text{mm}$, $P < 0.001$), although levels quickly returned to baseline after 1 h and remained unchanged during the recovery (Table 2).

### Intramuscular triacylglycerol (IMTG)

At the termination of exercise, IMTG content was $23.5 \pm 3.5 \text{mmol (kg dry wt)}^{-1}$ (Fig. 2), and remained constant at $24.6 \pm 2.6$, $25.7 \pm 2.8$ and $28.4 \pm 3.0 \text{mmol (kg dry wt)}^{-1}$ after 3, 6 and 18 h of recovery, respectively.

### Muscle glycogen and metabolites

Muscle glycogen content was $37 \pm 11 \text{mmol (kg dry wt)}^{-1}$ upon completion of the exercise bout and increased significantly throughout the recovery period to reach a maximal content of $424 \pm 22 \text{mmol (kg dry wt)}^{-1}$ ($P < 0.001$) after 18 h of recovery (Fig. 3). Muscle ATP, PCr and creatine were generally unchanged throughout the recovery period (Table 3). Acetyl CoA and acetylcarnitine content decreased significantly during the first 3 h of recovery ($P < 0.001$) and remained significantly below post-exercise concentrations throughout the recovery period (Table 3). Pyruvate ($P < 0.01$) was also significantly reduced compared to levels at exhaustion and remained low throughout the post-exercise period (Table 3).

### Pyruvate dehydrogenase (PDHa)

PDHa was $1.37 \pm 0.17 \text{mmol min}^{-1} (\text{kg wet wt})^{-1}$ after exhaustive exercise (Fig. 4), and decreased during recovery, being significantly different from exhaustion at 6 h ($0.66 \pm 0.10 \text{mmol min}^{-1} (\text{kg wet wt})^{-1}$; $P < 0.01$) and 18 h ($0.71 \pm 0.15 \text{mmol min}^{-1} (\text{kg wet wt})^{-1}$; $P < 0.05$) of recovery (Fig. 4).

### DISCUSSION

Consistent with previous studies (Krzentowski et al. 1982; Bielinski et al. 1985; Kiens & Richter, 1998), our results indicate a greater contribution from fat oxidation during recovery in the presence of reduced muscle glycogen. This is in support of the hypothesis that a metabolic priority of recovering muscle is to resynthesize glycogen stores, resulting in a relative shift from CHO to fat oxidation. Indeed, activation of glycogen synthase in exercised human muscle has been recently demonstrated (Wojtaszewski et al. 2001), supporting the contention that glycogen resynthesis is of high priority early in recovery. However, our data do not support the previous findings of Kiens & Richter (1998) that IMTG are a significant source of lipid to be utilized during recovery. Rather, our data support the more recent findings of $^1$H-MRS studies that IMTG

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**Table 3. Muscle metabolite concentrations immediately after exercise and during an 18 h post-exercise recovery period**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>0 h</th>
<th>3 h</th>
<th>6 h</th>
<th>18 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>23.5 ± 0.9</td>
<td>25.0 ± 1.0</td>
<td>26.2 ± 1.1</td>
<td>27.0 ± 1.6†</td>
</tr>
<tr>
<td>PCr</td>
<td>64.4 ± 3.0</td>
<td>68.0 ± 3.0</td>
<td>69.2 ± 3.1</td>
<td>69.1 ± 4.8</td>
</tr>
<tr>
<td>Creatine</td>
<td>48.4 ± 3.1</td>
<td>44.8 ± 2.5</td>
<td>43.7 ± 2.0</td>
<td>43.8 ± 2.6</td>
</tr>
<tr>
<td>Acetyl CoA</td>
<td>22.0 ± 1.8</td>
<td>3.7 ± 1.0‡</td>
<td>9.5 ± 2.0†</td>
<td>7.3 ± 0.8‡</td>
</tr>
<tr>
<td>Acetyl-carnitine</td>
<td>17.5 ± 2.7</td>
<td>1.9 ± 0.4‡</td>
<td>2.3 ± 0.6‡</td>
<td>3.0 ± 0.5‡</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.34 ± 0.07</td>
<td>0.15 ± 0.03‡</td>
<td>0.14 ± 0.05‡</td>
<td>0.10 ± 0.01†</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M., $n = 8$. All measurements are given in $\text{mmol (kg dry wt)}^{-1}$, except for $\text{acetyl CoA (\mu mol (kg dry wt)}^{-1}$. Significantly different from 0 h, †$P < 0.01$, ‡$P < 0.001$. **Downloaded from J Physiol (jp.physoc.org) at Karolinska Institutet on February 22, 2011**
content does not decrease during recovery from exhaustive exercise in well-trained individuals even when low fat meals are ingested after exercise (Decombaz et al. 2000, 2001; Larson-Meyer et al. 2002). This suggests that the primary lipid source during recovery may be derived from the circulation (i.e. plasma fatty acids and triacylglycerol (TG)), which is in agreement with our observation of a rapid reduction of plasma fatty acids during the first 2 h of recovery. However, this reduction may also be due in part to reduced lipolysis as indicated by the low glycerol. Furthermore, the large reduction in muscle acetyl carnitine stores early in recovery may have provided additional acetyl units for oxidation, and therefore have contributed to energy provision during this period. Despite the significant increase of insulin in response to the high-CHO feedings during recovery, muscle PDHa decreased. This reduction in activity is probably due, at least in part, to reduced substrate (pyruvate) availability as a result of the preferential partitioning of glucose towards glycogen resynthesis.

**Metabolic response during recovery**

**Responses during the first hour of recovery.** During the first hour of recovery, we observed very low RER values (i.e. below 0.70). The explanation for this is uncertain, but is likely to be due to a shift towards predominantly fat oxidation, as well as the replenishment of the body’s CO₂ stores within the bicarbonate pool (Jones & Heigenhauser, 1996). This would lead to a reduction in CO₂ release at the mouth and consequently, an underestimation of the true RER. The rapid increase in plasma fatty acids post-exercise, subsequent to the release of fatty acid trapped within adipose tissue (Hodgetts et al. 1991; Romijn et al. 1993) would have provided a large source of energy for the recovering muscle. In addition, the reduced insulin and glucose levels, i.e. factors that are anti-lipolytic and inhibit fat oxidation, are likely to contribute to the increase in fat utilization during the immediate recovery period. Muscle acetyl-CoA and acetyl carnitine accumulation was also evident post-exercise as previously reported (Howlett et al. 1998; Watt et al. 2002a).

**Responses during 1–6 h of recovery.** After ingestion of the first high CHO meal at 1 h, insulin and glucose levels were increased markedly while fatty acids and glycerol concentrations were rapidly reduced. Despite these responses, RER values remained low (0.77–0.80) until the following meal at 4 h, indicating predominantly fat oxidation, as previously observed during the early phase of recovery from prolonged (Krzentowski et al. 1982; Bielinski et al. 1985; Horton et al. 1998) or exhaustive exercise (Kiens & Richter, 1998). The source of increased fat oxidation after glycogen-depleting exercise has previously been attributed to the utilization of IMTG (Kiens & Richter, 1998). However, in this study, IMTG content remained unchanged during the first 6 h of recovery, suggesting that circulating sources such as plasma fatty acids, and possibly plasma TG, are major sources of fat oxidation during recovery. Indeed, previous studies have found an increase in the activity of the muscle lipoprotein lipase (LPL) activity after exhaustive exercise (Lithell et al. 1981; Kiens et al. 1989; Kiens & Richter, 1998), as well as increased muscle LPL mRNA at 4 h and protein content at 8 h post-exercise (Seip et al. 1997).

Glycogen content increased rapidly after 3 and 6 h of recovery (4.5- and ~7-fold respectively) in the presence of high circulating insulin and glucose. The average rate of glycogen resynthesis was ~40 mmol (kg dry wt)⁻¹, which is very similar to that reported previously by Kiens & Richter (1998) using a nearly identical protocol to ours. This rapid rate of glycogen resynthesis was probably influenced by the extremely low glycerol content immediately following exercise, the well-trained status of the subjects, and the large amount of CHO consumed during the first meal.

In spite of the elevated insulin, PDHa declined after 3 h and showed a further downward trend at 6 h to a value significantly (P < 0.01) below that observed immediately post-exercise (Fig. 4). Coincident with this reduction in PDHa was a reduction in muscle acetyl carnitine stores, indicating that this pool may have been a significant source of acetyl units for oxidation early in recovery. Taken together, these data indicate a relative partitioning of glucose uptake during recovery towards glycogen resynthesis and an increased reliance on fatty acids as an oxidative fuel. Approximately 365 g of glucose were consumed during the first 6 h of recovery. During this period, muscle glycogen content increased by ~200 mmol (kg dry wt)⁻¹, which corresponds to a total of ~100 g of glucosyl units, assuming that ~12 kg of muscle mass was utilized during the exhausting exercise and that the muscle was actively resynthesizing glycogen during recovery. Thus, assuming complete absorption, ~40% of the ingested glucose was directed towards muscle glycogen resynthesis during the first 6 h of recovery. Of the remaining glucose absorbed (365 − 100 = 265 g), ~210 g can be accounted for by (1) whole-body glucose oxidation (~30 g) based on an average RER and VO₂ of 0.78 and 0.31 min⁻¹, respectively, and (2) liver glycogen resynthesis (~180 g), assuming a liver mass of ~2 kg, and near repletion of its glycogen stores. The remaining unaccounted glucose (55 g) may be due to a combination of factors, including an underestimation of the working muscle mass, and glucose oxidation if our measured RER values are slightly low.

**Responses during 6–18 h of recovery.** The main finding during this period of recovery was that there was no change in IMTG content. Glycogen content continued to increase significantly during this period, increasing from 250.2 ± 17.7 to 423.8 ± 22.2 mmol (kg dry wt)⁻¹. The RER remained low, as expected, following the overnight fast.
(0.76 ± 0.02), indicating a continued reliance on fat. In addition, plasma fatty acid levels were slightly elevated in response to low levels of circulating insulin. In further support of a shift in metabolic priority towards muscle glycogen resynthesis and away from CHO oxidation during recovery, PDHa activity, muscle pyruvate and acetyl-CoA concentrations were similar to levels found at 6 h of recovery.

Mechanisms for increased lipid metabolism during recovery

The mechanism for increased fat oxidation during recovery, in the presence of elevated insulin and glucose, is not readily apparent. IMTG content was unchanged during recovery, suggesting muscle lipid stores are not an appreciable source of fatty acids for increasing fat oxidation. Therefore, it is likely that elevated plasma fatty acids during the first hour of recovery resulted in an increased uptake and oxidation of fatty acids within glycogen-depleted muscle. This is supported by the rapid decline in plasma fatty acid levels early in recovery, which can probably be attributed to both the rapid clearance by peripheral tissues, including skeletal muscle, as well as an insulin-mediated reduction in peripheral lipolysis (Horowitz et al. 1997; Mittendorfer et al. 2002) following the first high-CHO meal during recovery. It is paradoxical, though, that fat oxidation remained elevated in the presence of elevated insulin levels. It should be noted, however, that resistance to insulin’s inhibition of fatty acid oxidation during recovery from a marathon has previously been reported (Tuominen et al. 1996).

Regulation of IMTG content during post-exercise recovery

Enzymatic regulation of muscle TG esterification and hydrolysis during resting and contracting conditions is poorly understood. Recently, a neutral hormone-sensitive lipase (HSL) was identified in skeletal muscle (Langfort et al. 1998) and appears to be under dual control by adrenaline and contraction-related mechanisms (Langfort et al. 2000). Plasma adrenaline concentrations were not determined in the present study, but would be expected to decrease rapidly (i.e. within 30 min) during recovery. Regulation of HSL activity may also result from allosteric feedback inhibition by long chain fatty acyl-CoA (Jepson & Yeaman, 1992). Thus, the large increase in plasma fatty acids observed early in recovery may have elevated cytosolic long chain fatty acid- (LCFA)-CoA concentrations and reduced IMTG hydrolysis by inhibiting HSL. For example, during a resting hyperinsulinaemic glucose clamp with elevated fatty acid levels, a rapid increase in the IMTG pool was found during a 5 h time period (Brechtel et al. 2001). It is therefore probably not surprising that IMTG content did not decrease during the early stages of recovery, given the marked elevation of insulin, and the possible accumulation of cytosolic LCFA-CoA in the recovering muscle. However, in fairness, it should be pointed out that during the first hour of recovery in the study by Kiens & Richter (1998), plasma fatty acids were also significantly elevated (~2 mM), at a time when IMTG stores were being utilized.

In spite of our conclusions regarding IMTG use, the possibility that we were simply unable to detect a small, but physiologically meaningful contribution from IMTG hydrolysis and/or oxidation must be acknowledged. Estimation of IMTG utilization, using biochemical analysis or other established techniques such as isotope tracer kinetics and 1H-MRS, is limited by the inability to account for triacylglycerol (TG) and/or fatty acid cycling. Using the dual label pulse-chase technique in isolated rat soleus muscle, it has been demonstrated that the IMTG pool undergoes simultaneous esterification and hydrolysis during both rest and contraction (Dyck & Bonen, 1998). This raises the possibility that TG resynthesis during recovery may have masked the utilization of IMTG as an oxidative fuel during recovery from exhaustive exercise. A recent pulse-chase study in exercising humans (Guo et al. 2000) indicated that fatty acid incorporation into IMTG was relatively small compared to the rates of IMTG oxidation i.e. ~8%, making this possibility unlikely. However, it is possible that the incorporation of fatty acids into TG might be greater during recovery. Furthermore, determining the specific contribution of IMTG as an oxidative fuel during post-exercise recovery is complicated by the methodological limitations associated with the muscle biopsy technique. While the use of trained subjects reduces the coefficient of variation between biopsies to 12.3 ± 9.4% (Watt et al. 2002a), compared to 23.5 ± 14.6% in untrained subjects (Wendling et al. 1996), this does not explain the discrepancy between the present findings and those of Kiens & Richter (1998), who used equally well-trained subjects in their study.

Regulation of PDHa during post-exercise recovery

While the regulation of PDH activity during acute and prolonged exercise has been widely examined, to our knowledge this study is the first to report PDH activation in human skeletal muscle during CHO feeding after exhaustive exercise. PDH activity is controlled by the relative activities of PDH kinase (PDK), which phosphorylates and deactivates PDH, and PDH phosphatase (PDP), which dephosphorylates and activates PDH. PDK is stimulated by high ATP/ADP, acetyl-CoA/CoA-SH and NADH/NAD⁺ ratios and is inhibited by elevated pyruvate concentrations, whereas PDP is stimulated by Ca²⁺ and increased by pyruvate. Muscle ATP, acetyl-CoA and pyruvate concentrations were unchanged during recovery, while Ca²⁺ and the NADH/NAD⁺ ratio were not measured in this study. However, since pyruvate is a substrate for PDH and an inhibitor of PDK, the possibility exists that a decline in pyruvate availability contributed to the trend in PDHa reduction between 3 and 6 h of recovery. In
addition, it is also well documented that PDK4 expression is increased during recovery from prolonged, exhaustive exercise (Pilegaard et al. 2000, 2002), which may also account for the observed decrease in PDHs.

Conclusions

In summary, we report that IMTG content remains unchanged during recovery from glycogen-depleting exercise in the presence of elevated glucose and insulin levels. It appears that the partitioning of exogenous glucose towards glycogen resynthesis is of high metabolic priority during immediate post-exercise recovery, and is supported by the trend towards reduced PDH activity and increased fat oxidation. Plasma fatty acids (and possibly plasma TG), as well as intramuscular acylcarnitine stores are likely to be important fuels for muscle metabolism in the immediate recovery period. However, IMTG appears to have a negligible role in contributing to the enhanced fat oxidation during recovery from exhaustive exercise.

REFERENCES


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