Carbohydrate refeeding after a high-fat diet rapidly reverses the adaptive increase in human skeletal muscle PDH kinase activity

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Carbohydrate refeeding after a high-fat diet rapidly reverses the adaptive increase in human skeletal muscle PDH kinase activity. Am J Physiol Regul Integr Comp Physiol 297: R885–R891, 2009. First published July 22, 2009; doi:10.1152/ajpregu.90604.2008.—Pyruvate dehydrogenase (PDH) regulates oxidative carbohydrate disposal in skeletal muscle and is downregulated by reversible phosphorylation catalyzed by PDH kinase (PDK). Previous work has demonstrated increased PDK activity and PDK4 expression in human skeletal muscle following a high-fat low-carbohydrate (HF) diet, which leads to decreased PDH in the active form (PDHa) and carbohydrate oxidation. The purpose of this study was to examine the time course of changes in PDK and PDHa activities with refeeding of carbohydrates after an HF diet in human skeletal muscle. Healthy male volunteers (n = 8) consumed a standardized 3-day Pre-diet with the same energy content as their habitual diet, followed by a eucaloric 6-day HF diet (Pre-diet: 50:30:20%; HF diet: 75:20%; carbohydrate/fat/protein). Muscle biopsies were taken before and after the HF diet and at 45 min and 3 h after carbohydrate refeeding with a single high-glycemic index carbohydrate meal (88.5:7% carbohydrate/fat/protein) representing approximately one third of the individual subjects’ habitual energy intake. PDK activity increased from 0.08 ± 0.01 Pre- to 0.25 ± 0.02 min (P < 0.001) Post-HF diet, and decreased with carbohydrate refeeding to 0.17 ± 0.05 (P = 0.014) and 0.11 ± 0.01 min (P = 0.006) at 45 min and 3 h, respectively. PDHa decreased from 0.89 ± 0.20 to 0.32 ± 0.05 (P = 0.007) mmol·min⁻¹·kg⁻¹ wet wt⁻¹ following the HF diet, and was increased transiently with refeeding at 45 min, but returned to lower values by 3 h (P = 0.025 compared with Pre). The potential mechanism(s) for this attenuation of PDHa activity remains unclear. These data demonstrate that in human skeletal muscle, the adaptive increase in PDK activity following an HF diet is rapidly reversed to Pre-diet activity levels within 45 min to 3 h, and this is accompanied by a short-term increase in PDHa activity.

pyruvate dehydrogenase; carbohydrate oxidation; low-carbohydrate diet; PDK2; PDK4

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R885
METHODS

Subjects

Eight healthy male university students volunteered for this study, and their characteristics are summarized in Table 1. All of the subjects were recreationally active (2–5 times/wk, self-reported moderate physical activity), but none participated in regimented exercise or sports programs. Subjects were informed of the study protocol and associated risks before giving their written informed consent. The study was approved by the research ethics boards of both Brock University and McMaster University.

Preexperimental Protocol

Subjects provided a 3-day record of their regular diet, and the records were analyzed using Diet Analysis Plus 6.0 (Thomson-Nelson, Scarborough, ON). Individual eucaloric Pre- (50% total energy from carbohydrate, 30% fat, and 20% protein) and HF diets (5% carbohydrate, 75% fat, and 20% protein) were designed and prescribed for each subject. Food was provided, and subjects recorded everything consumed so that accurate diet analysis could be performed following the study. Similar protocols have been used previously to induce an adaptive increase in skeletal muscle PDK activity (22, 24, 26, 36).

Experimental Protocol

Throughout the study, subjects were asked to refrain from exertional activities, but still maintain the physical activities of daily living. They were instructed to refrain from alcohol consumption for the duration of the study. During the experiment, subjects reported to the laboratory on two separate occasions (Fig. 1). Subjects consumed the Pre-diet for 3 days prior to the first visit, and arrived at the laboratory after a 10- to 12-h overnight fast. A single venipuncture was made to collect a Pre-diet blood sample. One leg was prepared for two muscle biopsies taken from the vastus lateralis under a local anesthetic, as previously described (2). One biopsy was frozen immediately in liquid nitrogen for PDHa activity and muscle metabolite analysis. The second biopsy was used fresh to extract intact mitochondria for PDK activity as previously described (22, 24, 26, 36). Similar paired samples were taken at all subsequent time points. Subjects immediately began their prescribed HF diet. Following the 6-day HF diet, subjects fasted overnight (10–12 h) and returned to the laboratory in the morning; a catheter was inserted into a peripheral vein of one arm and an initial blood sample (Post-diet or 0 min) was taken. Patency was maintained with a sterile isotonic saline solution. Another pair of muscle biopsies (fresh and frozen) were obtained prior to refeeding (Post-diet or 0 min), and subjects were then refed a standardized high-carbohydrate meal (88:5:7% carbohydrate/fat/protein) consisting of fruits (fresh and frozen) were obtained prior to refeeding (Post-diet or 0 min), and subjects were then refed a standardized high-carbohydrate meal (88:5:7% carbohydrate/fat/protein) consisting of fruits (fresh and frozen) were obtained prior to refeeding (Post-diet or 0 min), and subjects were then refed a standardized high-carbohydrate meal (88:5:7% carbohydrate/fat/protein) consisting of fresh and frozen) were obtained prior to refeeding (Post-diet or 0 min), and subjects were then refed a standardized high-carbohydrate meal (88:5:7% carbohydrate/fat/protein) consisting of carbohydrates (88:5:7% carbohydrate/fat/protein) was given, and muscle biopsies were taken at 45 min and 3 h after refeeding. Blood samples were taken every 15 min for the first hour after refeeding and then every 30 min for the remainder of the 3 h. Muscle biopsies; *blood samples.

In these pilot subjects, muscle biopsy samples were obtained 3 and 6 h following refeeding with an additional carbohydrate meal taken immediately after the 3-h samples were taken. Pilot data demonstrated that PDK activity had returned to baseline by 3 h in both subjects and the 3-h and 6-h values were similar (data not shown). Therefore, the following six subjects in this study had muscle biopsy pairs taken as described above at Pre-diet, Post-0 min, 45 min, and 3 h after the meal (Fig. 1). The data from the two pilot subjects was included in the Pre-diet, Post-0 min, and 3-h time points for muscle measures. Therefore, n = 8 for most time points, but n = 6 at 45 min.

Blood Analyses

One portion of whole blood (200 μl) was added to 0.6 N HClO₄ (800 μl), vortexed, and centrifuged at 10,000 rpm for 1 min. The supernatant was removed for analysis of glucose, β-hydroxybutyrate, lactate, and glycerol (1). A second portion of whole blood was centrifuged and 400 μl of plasma was added to 5 M NaCl (100 μl) and incubated for 30 min at 56°C to inhibit lipoprotein lipase activity. Plasma free fatty acids were measured using a Wako NEFA C test kit (Wako Chemicals, Richmond, VA). The additional plasma was analyzed for insulin using a Coat-a-Count Insulin test kit (Diagnostics Products, Los Angeles, CA).

Mitochondrial Extraction

Intact mitochondria were extracted from the fresh muscle homogenate by differential centrifugation, as previously described (16, 19). Briefly, minced muscle was homogenized in 20 volumes of a buffer containing: 100 mM KCl, 40 mM Tris·HCl, 10 mM Tris base, 5 mM magnesium sulfate, 1 mM EDTA, and 1 mM ATP (pH 7.5). The supernatant was retained after centrifugation (700 g, 10 min), and a crude mitochondrial pellet was extracted with centrifugation (14,000 g, 10 min). The pellet was washed, resuspended, and pelleted twice (7,000 g, 10 min) in 10 volumes of 100 mM KCl, 40 mM Tris·HCl, 10 mM Tris base, 1 mM magnesium sulfate, 0.1 mM EDTA, and 0.25 mM ATP (pH 7.5). The first wash buffer included 1% (wt/vol) bovine serum albumin and the second was protein free. The final mitochondrial pellet was resuspended in a volume corresponding to 1 μl/1 mg fresh muscle extracted. The final pellet contained 220 mM sucrose, 70 mM mannitol, 10 mM Tris·HCl, and 1 mM EDTA (pH 7.4). All procedures were carried out at 0–4°C. Unless specifically stated, all chemicals were obtained from Sigma (St. Louis, MO).

Incubation of Mitochondria for PDK Activity

The final mitochondrial suspension (50 μl) was incubated for 20 min at 30°C in a buffer containing 10 μM carbonyl cyanide m-

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Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>20.7 ± 0.3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>83 ± 6</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.80 ± 0.02</td>
</tr>
<tr>
<td>Habitual dietary intake, kcal</td>
<td>2,722 ± 85</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8.

Fig. 1. Experimental protocol. Subjects consumed a standardized Pre-diet for 3 days (~50–60% of the energy from carbohydrate/fat/protein) followed by a 6-day high-fat-low-carbohydrate (HF/LC) diet (~5:75:20% carbohydrate/fat/protein). Blood samples and muscle biopsies were taken prior to and following the HF diet. A single meal of high-glycemic index mixed carbohydrates (88:5:7% carbohydrate/fat/protein) was given, and muscle biopsies were taken at 45 min and 3 h after refeeding. Blood samples were taken every 15 min for the first hour after refeeding and then every 30 min for the remainder of the 3 h.
chlorophenyl-hydrazone, 20 mM Tris·HCl, 120 mM KCl, 2 mM EGTA, and 5 mM potassium (pH 7.4). This incubation drives ATP concentration to zero, thereby causing complete conversion of PDH to the active form, PDHα (7). Mitochondria were pelleted (7,000 g, 10-min) and stored in N₂ for later analysis of PDH activity.

**PDHα Activity**

A small piece of frozen wet muscle (∼10–15 mg) was removed from each biopsy under N₂ for the determination of PDHα as previously described (6, 29). Total creatine (Cr) content was measured in the PDHα homogenates (1) and used to correct PDHα activity to the highest Cr content in a set of biopsies from a given subject. PDHα activity was expressed as millimoles acetyl-CoA per kilogram wet muscle per minute.

**Muscle Metabolites**

The remainder of the frozen muscle was freeze-dried, powdered, and dissected of all visible blood, connective tissue, and fat. Muscle metabolites were measured in neutralized PCA extracts. Phosphocreatine (PCr), Cr, ATP, and lactate were measured spectrophotometrically (1, 10), pyruvate was measured fluorometrically (21), and acetyl-CoA and acetylcarnitine were determined radioisotopically (4). All metabolites were corrected to the highest total Cr content from a given subject’s biopsies.

**Table 2. Comparison of habitual and experimental diets**

<table>
<thead>
<tr>
<th></th>
<th>Habitual Diet</th>
<th>Pre-Diet</th>
<th>HF-Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total energy, kcal</strong></td>
<td>2786±103</td>
<td>2604±100</td>
<td>2807±122</td>
</tr>
<tr>
<td>%Fat</td>
<td>33.6±2.3</td>
<td>30.0±0.7</td>
<td>75.6±0.8†</td>
</tr>
<tr>
<td>%CHO</td>
<td>46.2±4.0</td>
<td>49.1±1.3</td>
<td>4.5±0.2†</td>
</tr>
<tr>
<td>%Protein</td>
<td>16.7±1.2</td>
<td>20.0±0.1*</td>
<td>20.1±0.7*</td>
</tr>
<tr>
<td>Total fat, g</td>
<td>106±10</td>
<td>86±4</td>
<td>235±14†</td>
</tr>
<tr>
<td>Saturated, g</td>
<td>35±4</td>
<td>31±1</td>
<td>72±2*†</td>
</tr>
<tr>
<td>Monounsaturated, g</td>
<td>21±4</td>
<td>26±2</td>
<td>100±12*†</td>
</tr>
<tr>
<td>Polyunsaturated, g</td>
<td>11±2</td>
<td>15±3</td>
<td>39±5*†</td>
</tr>
<tr>
<td>Protein, g</td>
<td>116±8</td>
<td>137±3*</td>
<td>141±3*</td>
</tr>
<tr>
<td>CHO, g</td>
<td>348±14</td>
<td>321±18</td>
<td>32±2*†</td>
</tr>
</tbody>
</table>

Values are means ± SE; CHO, carbohydrate; HF-diet, high-fat diet. *Significantly different from habitual diet; †significantly different from Pre-diet, P < 0.05.

**Calculating Recovery and Quality of Mitochondria**

Citrate synthase activity in the total muscle homogenate and in the mitochondrial suspension were measured (30) and used to calculate the recovery and percentage of intact mitochondria in the mitochondrial preparations as previously described (26). The average skeletal muscle mitochondrial recovery was comparable to our previous studies (31 ± 5% of the total mitochondria were recovered, and % intact mitochondria was 83 ± 4%) (22–24, 26).

**Statistical Analyses**

Muscle and blood data were analyzed using a one-way (time) repeated-measures ANOVA using SigmaStat 3.1.1 (Point Richmond, CA). When significance was detected, a Fisher protected post hoc test was used to determine differences in individual means. All blood and muscle data are presented as means ± SE. Significance was accepted at P < 0.05.

**RESULTS**

**Diet Analysis**

Diet analysis for the recorded dietary intake during the habitual and experimental diets is summarized in Table 2. The Pre-diet and HF diet intake were successfully isonenergetic with the subject’s habitual diet. Only minor modifications in protein intake were made in the subjects’ habitual diet to design the Pre-diet, and this was maintained with the HF diet intervention. Therefore, only fat and carbohydrate content differed between the Pre-diet and the HF diet intervention. Fat intake increased almost threefold with the HF diet, and carbohydrate intake was restricted to < 5%. The increase in total fat was reflected by increases in all of the fatty acid subtypes in the HF intervention: saturated (2.3-fold higher), poly- (2.6-fold higher) and mono-unsaturated fat (3.8-fold higher).
Blood Analyses

Blood glucose, lactate, and plasma insulin. Fasting blood glucose concentrations were unchanged following the HF diet (Pre, 3.8 ± 0.2 vs. Post, 3.7 ± 0.4 mM). However, both blood lactate (Pre, 1.4 ± 0.1 vs. Post, 1.1 ± 0.1 mM), and fasting insulin levels (Pre 3.7 ± 0.6 vs. Post 2.4 ± 0.4 μIU/ml) were lower following the HF diet (P < 0.05).

Immediately after refeeding (15 min), blood glucose concentrations were ~1.5-fold elevated and remained higher for 45 min (P < 0.05), returning to normal levels by 90 min (Fig. 2A). Plasma insulin concentrations increased to peak values within 15 min (P < 0.05), but remained elevated for a longer period until 120 min (Fig. 2B). Plasma lactate concentration increased almost threefold 30 min after refeeding and remained approximately twofold elevated throughout the experiment (Fig. 3, P < 0.05).

Blood glycerol, β-hydroxybutyrate, and plasma free fatty acids. Plasma free fatty acids and β-hydroxybutyrate levels were higher following the HF diet (P < 0.05), but blood glycerol was unaltered by the diet (Table 3).

With carbohydrate refeeding, plasma free fatty acids dropped immediately after the carbohydrate meal (15 min, P < 0.05) and fell to levels lower than fasting Pre-diet levels from 45–180 min (Table 3, P < 0.05). Blood concentrations of β-hydroxybutyrate and glycerol were significantly reduced by 45 and 60 min after refeeding, respectively (P < 0.05), and remained similar to Pre-diet levels for the duration of the study (Table 3).

Muscle Analyses

PDK and PDHa activities. PDK activity was threefold higher following the 6-day HF diet compared with control Pre-diet (Fig. 4, P < 0.001). After refeeding, PDK activity decreased progressively throughout the first 3 h. At both 45 min and 3 h, PDK activity was significantly lower than Post-0 min (P = 0.014 and 0.006, respectively) and was not significantly different from Pre-diet levels (Fig. 4, P = 0.06 and 0.19, respectively, for 45 min and 3 h).

PDHa activity was significantly decreased after the HF diet (P = 0.007), and with carbohydrate refeeding PDHa activity increased compared with Post-HF diet/0 min refeeding, and was not significantly different from Pre-diet values by 45 min (P = 0.38). However, at 3 h after refeeding PDHa activity had decreased significantly again compared with Pre-diet values (P = 0.025; Fig. 5).

Muscle metabolites. Muscle lactate decreased slightly following the HF diet, and increased with refeeding to concentrations similar to Pre-diet levels (Table 3, P < 0.05). However, muscle acetyl-CoA, pyruvate, ATP, phosphocreatine, and creatine were unaffected by either the HF diet or refeeding.

DISCUSSION

The primary purpose of this study was to examine the time course for the reversal of the chronic upregulation in PDK activity in resting skeletal muscle following a 6-day HF diet through administration of a single standardized, high-carbohydrate meal. To the best of our knowledge, this is the first study to examine the time course as early as 45 min of refeeding carbohydrate in human skeletal muscle and to combine measurements of PDK and PDHa activity and muscle metabolic measurements. The novel finding was that total maximal PDK activity was decreased by 10.2 ± 0.3 nmol/min. The decrease was significant at 15 min (P = 0.001) and remained significant at 3 h (P = 0.006). The decrease in PDK activity was not associated with a decrease in PDHa activity. The decrease in PDHa activity was significant at 45 min (P = 0.007) and returned to Pre-diet levels by 3 h (P = 0.38). The reason for the difference in the time course of PDK and PDHa activity is not clear, but it may be related to the different mechanisms of regulation of these enzymes.

Table 3. Blood glycerol, β-hydroxybutyrate (β-HB), and plasma free fatty acids (FFA). Lipid-related blood parameters Pre- and Post-HF diet (0 min) and 180 min after refeeding a high-carbohydrate meal

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post, 0 min</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>150 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>0.05±0.01</td>
<td>0.05±0.01</td>
<td>0.05±0.01</td>
<td>0.05±0.01</td>
<td>0.05±0.005</td>
<td>0.04±0.01*</td>
<td>0.04±0.01*</td>
<td>0.04±0.01*</td>
<td>0.04±0.01*</td>
<td>0.04±0.01*</td>
</tr>
<tr>
<td>β-HB</td>
<td>0.10±0.05</td>
<td>0.10±0.05</td>
<td>0.10±0.05</td>
<td>0.10±0.05</td>
<td>0.10±0.05</td>
<td>0.09±0.03*</td>
<td>0.05±0.02*</td>
<td>0.05±0.02*</td>
<td>0.04±0.01*</td>
<td>0.04±0.01*</td>
</tr>
<tr>
<td>FFA</td>
<td>0.52±0.11</td>
<td>0.52±0.11</td>
<td>0.52±0.11</td>
<td>0.52±0.11</td>
<td>0.52±0.11</td>
<td>0.27±0.05†</td>
<td>0.24±0.04†</td>
<td>0.18±0.03†</td>
<td>0.23±0.05†</td>
<td>0.25±0.06†</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmol/l (mM). Lipid-related blood parameters Pre- and Post-HF diet (0 min) and 180 min after refeeding a high-carbohydrate meal. *Significantly different from 0 min during refeeding; †significantly different from Pre-diet value.
activity decreased rapidly and progressively during the first 3 h following a single high-carbohydrate meal. PDHa activity increased significantly within 45 min of meal consumption, but in contrast to our hypothesis, it had returned to lower values by 3 h, the inverse of what was expected in light of the changes in total PDK activity. Muscle concentrations of known effectors of PDHa activity (acetyl-CoA and pyruvate) were unaltered throughout the treatments.

PDK activity. Measurement of PDK activity in this study reflects the total activity of the kinases and is independent of dynamic changes in mitochondrial effectors (such as acetyl-CoA, NADH, and pyruvate) that regulate kinase activity in vivo (15). We completed two pilot subjects for muscle enzymes activity and determined that PDK activity was back to Pre-diet values at 3 h after refeeding, and therefore the remaining subjects had biopsies taken at 45 min, which was expected to follow closely the elevation of plasma insulin and blood glucose with refeeding. Our results demonstrate that the fat-adapted increase in PDK activity is rapidly and progressively reversed in human skeletal muscle following feeding of a single high carbohydrate meal, suggesting that the fat-induced increase in fat oxidation and decreased carbohydrate oxidation would not be expected to persist once refeeding of carbohydrates begins. This agrees with earlier studies on rat skeletal muscle, which observed rapid reversion of starvation-induced PDK activity with refeeding (12, 32).

PDK activity with carbohydrate refeeding has not been previously measured in human skeletal muscle. To date, only one other paper has addressed the question of changes in PDK4 mRNA with refeeding following starvation in resting human skeletal muscle, and they did not measure enzyme activity (28). In that paper, Pilegaard et al. (28) paradoxically observed increased PDK4 gene transcription and mRNA content following starvation and refeeding of either carbohydrate or fat meals. Our data demonstrates that either the effect of starvation is markedly different than the HF-diet treatment, or this increased PDK4 mRNA content would not be expected to translate into new PDK4 protein and increased PDK activity with carbohydrate refeeding. More recently, Chokkalingam et al. (5) observed decreased PDK4 mRNA with a hyperinsulinemic/euglycemic clamp in human skeletal muscle following a similar high-fat diet, which would support our work. However, this was not accompanied by decreased PDK4 protein content (5). It is not clear what was the mechanism for the rapid reversion of PDK activity observed in our study with refeeding, because PDK isoform content was not determined. However, this dissociation between PDK activity and isoform content has been observed previously in short-term situations. For example, during 4 h of prolonged exercise, a steady increase in PDK activity was observed, although this was not accompanied by changes in PDK2 or PDK4 isoform content (38). This suggests that there may be another mechanism to chronically alter total PDK activity by changing the activity of the existing isoforms in an effector-independent manner in short-term situations, potentially through binding of existing PKDs to the complex core (37).

PDHa activity. Muscle PDHa activity was lower in our subjects after the HF diet, and increased rapidly within 45 min of refeeding, coinciding with peak plasma glucose and insulin levels following the high carbohydrate meal. However, by 3 h, PDHa activity had decreased again and was not different from Pre-HF-diet levels. Blood lactate concentration also increased ~2.5-fold within 30 min of carbohydrate refeeding and remained elevated for 180 min, which is consistent with the idea that with refeeding of carbohydrate, skeletal muscle glycolytic flux was greater than could be matched by pyruvate oxidation through the PDH complex, in spite of the transient increase in PDHa activity observed at 45 min. This lower level of PDHa activity and decreased carbohydrate oxidation was also observed during a hyperinsulinemic clamp after a high-fat diet (5). Therefore, although total maximal PDK activity decreased rapidly and was significantly lower 3 h after the carbohydrate meal, PDHa activity did not increase and was still lower than Pre-diet levels at 3 h of carbohydrate refeeding. This suggests that after the early transient increase in PDHa activity, there is a longer-term attenuation that is independent of alterations in total PDK activity. Similar to this, Turvey et al. (36) reported that PDHa activity was dependent on the composition of the diet, and observed lower PDK activity following an HF diet rich in n-3 fatty acids, but this was not accompanied by any alterations in PDHa activity. Although it was not the purpose of this study to measure the fate of the ingested carbohydrate, the increase in plasma lactate and the persistently lower PDHa activity at 3 h suggest that a proportion of the ingested carbohydrate load with refeeding was used for muscle glycogen and oxidation. The decrease in PDHa activity causes the

Table 4. Muscle metabolites

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post, 0 min</th>
<th>45 min</th>
<th>3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetyl-CoA</td>
<td>7.6 ±1.4</td>
<td>13.5 ±2.5</td>
<td>11.9 ±2.8</td>
<td>10.3 ±2.0</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.12 ±0.02</td>
<td>0.10 ±0.01</td>
<td>0.09 ±0.02</td>
<td>0.12 ±0.03</td>
</tr>
<tr>
<td>Lactate</td>
<td>5.6 ±0.5</td>
<td>3.9 ±0.5*</td>
<td>5.3 ±0.4</td>
<td>5.6 ±1.0</td>
</tr>
<tr>
<td>ATP</td>
<td>27.0 ±0.6</td>
<td>27.3 ±0.6</td>
<td>27.2 ±1.1</td>
<td>27.2 ±0.9</td>
</tr>
<tr>
<td>Phosphocreatine</td>
<td>83.6 ±4.1</td>
<td>83.6 ±4.4</td>
<td>79.1 ±4.3</td>
<td>85.1 ±5.5</td>
</tr>
<tr>
<td>Creatine</td>
<td>42.0 ±2.7</td>
<td>42.4 ±2.8</td>
<td>47.1 ±2.0</td>
<td>41.5 ±3.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. Lactate, ATP, phosphocreatine, and creatine are in mmol/kg dry wt; acetyl-CoA and pyruvate are μmol/kg dry wt. *Significantly different from Pre; P < 0.05.
diversion of pyruvate toward lactate production and into the circulation to preserve three-carbon intermediates when there is little energetic need for oxidation.

The mechanism for this perpetuated decrease in PDHa activity with the HF diet 3 h after carbohydrate refeeding is not clear. However, potential mechanisms might include 1) an adaptive and persistent decrease in the total PDP activity; 2) acute regulation of either PDK or PDP activities through persistent changes in intramitochondrial effectors; or 3) adaptive increases in maximal activities of key enzymes responsible for fatty acid oxidation. Although it is generally believed that adaptive increases in PDK activity and PDK4 protein account for the fat adaptation in response to dietary perturbations, little is known about adaptive changes in PDP activity in response to diet. Early studies demonstrated that PDP activity (measured as $^{32}$P release from $^{32}$P-labeled phosphorylated PDH complex) was unchanged in rat skeletal muscle (8) and heart mitochondria (33) following starvation and diabetes. However, recent studies using a different assay have demonstrated that total PDP activity is decreased in response to starvation in rat heart, liver (14), and skeletal muscle (18), and this is associated with decreased PDP2 protein. Therefore, it is possible that the persistent decrease in PDHa activity may be due to an adaptive change in PDP activity. However, further work would be necessary to see whether these changes are measurable in human skeletal muscle mitochondria.

Transformation of PDHs is not only dependent on the total maximal PDK and PDP activities, but on the more acute regulation by changes in mitochondrial effectors that modulate these enzymes. On the phosphatase side, PDP1 activity increases in response to higher Ca$^{2+}$ levels and PDP2 activity is increased as insulin levels increase (13). In this study, although Ca$^{2+}$ concentrations would be unaltered in the resting muscle, insulin increased rapidly to peak at 20–40 min, and this coincided with higher PDHa activities at 45 min. However, at 3 h, insulin concentrations had returned to baseline and would not be expected to exert any further influence to maintain transformation to PDHs. On the kinase side, PDK activity is inhibited by higher intramitochondrial pyruvate concentrations and is activated by higher energy charge, acetyl-CoA, and increased NADH. If skeletal muscle fat oxidation was adaptively increased with the HF diet through an unknown mechanism, the resultant reliance on fat metabolism could decrease PDHa activity in an acute manner through alterations in [acetyl-coenzyme A] and higher ATP-to-ADP and NADH-to-NAD$^+$ ratios. However, no alterations in whole muscle levels of pyruvate, energy status or acetyl-CoA were observed in this study. It remains possible that changes in intramitochondrial NADH concentration with increased fat oxidation may have affected PDH activity through activation of PDK, but measurement of mitochondrial concentrations of this regulator are elusive.

These acute changes in mitochondrial effectors might be due to persistent adaptive changes in enzymes that regulate fatty acid uptake and oxidation. In rodents, increased lipid availability has been shown to adaptively increase mitochondrial fat oxidation in isolated mitochondria, and this is accompanied by increases in the mRNA and protein of mitochondrial proteins (20, 35). However, in humans the data is not as clear. A couple of studies have documented increased mRNA concentrations in human skeletal muscle of several regulatory enzymes of fat metabolism (3, 34). However, short-term diets of 5–6 days have not documented increased protein concentration or activity of many key regulatory steps in muscle fatty acid uptake and oxidation (for a review see Ref. 25), but it is still possible that such a fat adaptation would persist after carbohydrate refeeding.

Perspectives and Significance

It would seem that the adjustment for PDK activity to increase in response to increased dietary fat is rapidly reversed with the reintroduction of carbohydrates, although suppression of PDHa activity and impaired oxidative carbohydrate disposal persists for at least 3 h. However, the mechanism for this separation is still unclear, and future work should focus not only on the changes in PDH isoform content, but also on other mechanisms that may be responsible for altering PDK activity and changing the phosphorylation and activity of the PDH complex.

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