Enzymatic regulation of glucose disposal in human skeletal muscle after a high-fat, low-carbohydrate diet

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Ingestion of a high-fat, low-carbohydrate (HF/LC) diet alters the regulation of resting carbohydrate metabolism. Short-term exposure to a HF/LC diet has been shown to reduce the whole body resting respiratory exchange ratio in 1–5 days (19, 30, 36), decrease skeletal muscle PDH in the active form (PDHα) at rest in 3–6 days (34, 38), and increase PDH kinase (PDK) activity after 3 days and again after 6 days, with a corresponding decrease in PDHα at 6 days (31). There have also been reports of decreased glucose disposal during an oral glucose tolerance test (OGTT) after 5 days on a HF/LC diet (1, 10). In a pilot study, we also found decreased glucose disposal during an OGTT in aerobically trained subjects after only 2 days on a HF/LC diet. However, there appear to be no studies that examined the effects of a HF/LC diet on the regulation of GS and PDH activities in human skeletal muscle during an OGTT.

Therefore, the goal of this study was to examine whole body glucose disposal and the enzymatic regulation of skeletal muscle glucose disposal after a short-term HF/LC diet (~2 days) in humans. The first purpose was to determine whether whole body glucose disposal was decreased after a short-term HF/LC diet in aerobically trained men. The second purpose was to examine the regulation of GS and PDH activities during an OGTT after control and HF/LC diets. It was hypothesized that the HF/LC diet would 1) decrease whole body glucose disposal during the OGTT, 2) decrease the active forms of GS and PDH at rest, and 3) decrease the activation of both GS and PDH during the OGTT.

METHODS

Subjects

Six healthy, male university students volunteered for this study (means ± SD: age, 22.8 ± 1.0 yr; weight, 74.1 ± 12.9 kg). All of the subjects were aerobically active on a regular basis (3–6 times/wk). The mean (± SD) relative maximum O2 consumption (V˙O2 max) was 55.4 ± 8.3 ml·min⁻¹·kg⁻¹. Subjects were informed of the study protocol and associated risks before giving their written, informed consent. The study was approved by the ethics committees of the University of Guelph and McMaster University.

Preexperimental Protocol

Subjects provided a 3-day record of their regular diet and the records were analyzed by using Nutripro Diet Analysis Software.
(West Publishing, Salem, OR). Individual eucaloric diets (Con; 51% carbohydrate, 29% fat, 20% protein), and HF/LC diets (5% carbohydrate, 73% fat, 22% protein) were designed and prescribed for each subject. Only slight adjustments to the individual regular diets were needed to achieve the Con diets. Food was provided for each subject with detailed dietary guidelines to ensure compliance. \( V_{O2 \max} \) was also determined before the experiment using a continuous incremental protocol on a cycle ergometer (Excalibur, manufactured by Lode and distributed by Quinton Instruments, Seattle, WA) with a metabolic cart (model 2900, SensorMedics, Yorba Linda, CA).

**Experimental Protocol**

During the experiment, subjects reported to the laboratory on two separate occasions (Fig. 1). Subjects consumed the Con diet for 3 days and restricted physical activity for 24 h before the first visit. They arrived at the laboratory after a 10- to 12-h overnight fast. A catheter was inserted into a peripheral vein of one arm, and a resting blood sample (–30 min) was obtained (Fig. 1). Patency was maintained with a sterile isotonic saline solution. One leg was prepared for muscle biopsies taken from the vastus lateralis under a local anesthetic, as previously described (3). A second resting blood sample and two resting muscle biopsies were taken at 0 min (pre-OGTT). An oral glucose load (1 g/kg body mass, Trutol 75, Custom Laboratory, Baltimore, MD) was then ingested within 5 min, and blood samples were taken every 30 min for a 3-h period (OGTT). An additional muscle biopsy was taken 75 min into the OGTT.

After the OGTT, subjects immediately began their HF/LC diet. Physical activity was restricted to activities of daily living throughout the HF/LC diet. After the 56-h HF/LC diet, subjects fasted overnight (10–12 h), returned to the laboratory in the morning, and the above protocol was repeated with muscle biopsies taken from the other leg.

**Blood Analyses**

One portion of whole blood (200 \( \mu \)l) was added to 0.6 N HClO\(_4\) (800 \( \mu \)l), vortexed, and centrifuged at 10,000 rpm for 1 min. The supernatant was removed for analysis of glucose, \( \beta \)-hydroxybutyrate (\( \beta \)-OH), lactate, and glycerol (2). A second portion of whole blood was centrifuged, and 400 \( \mu \)l of plasma were added to 5 M NaCl (100 \( \mu \)l) and incubated for 30 min at 56°C to inhibit lipoprotein lipase activity. Plasma free fatty acids (FFA) were measured using a Wako NEFA C test kit (Wako Chemicals, Richmond, VA). The additional plasma was analyzed for insulin by using a Coat-a-Count Insulin test kit (Diagnostics Products, Los Angeles, CA).

**Processing of Muscle Samples**

A portion of fresh muscle (50–60 mg) was separated from the first pre-OGTT muscle biopsy in each trial and processed for the extraction of intact mitochondria to measure PDK and citrate synthase (CS) activities. A second biopsy portion was frozen in liquid \( N_2 \) for measurement of total homogenate CS activity (to calculate mitochondrial recovery). A third portion was frozen in liquid \( N_2 \) for the measurement of muscle glycogen.

**Mitochondrial Extraction**

Intact mitochondria were extracted from the muscle homogenate by differential centrifugation, as previously described (18, 25). Briefly, minced muscle was homogenized in 20 volumes of a buffer containing (in mM) 100 KCl, 40 Tris·HCl, 10 Tris base, 5 magnesium sulfate, 1 EDTA, and 1 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 (in mM) 100 KCl, 40 Tris·HCl, 10 Tris base, 1 magnesium sulfate, 0.1 EDTA, and 0.25 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 \( \mu \)l/mg fresh muscle extracted. The final buffer contained (in mM) 220 sucrose, 70 mannitol, 10 Tris·HCl, and 1 EDTA (pH 7.4). All procedures were carried out at 0–4°C. Unless specifically stated, all chemicals were obtained from Sigma Chemical (St. Louis, MO).

**Incubation of Mitochondria for PDK Activity**

The final mitochondrial suspension (50 \( \mu \)l) was incubated for 20 min at 30°C in a buffer containing 10 mM carbonyl cyanide \( m \)-chlorophenylhydrazone, 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, PDHa (6). Mitochondria were pelleted (7,000 g, 10-min) and stored in \( N_2 \) for later analysis of PDK activity.

**PDK Activity**

PDK activity was measured as previously outlined by Peters et al. (31). Briefly, the mitochondrial pellet was resuspended in a phosphate buffer (pH 7.0) and freeze-thawed twice to break all of the mitochondria. Magnesium ATP (3 mM) was added to the remaining suspension at 30°C and timed samples were removed and diluted 1:1 in a buffer containing 200 mM sucrose, 50 mM KCl, 5 mM MgCl\(_2\), 5 mM EGTA, 50\( \mu \)M Tris·HCl, 50 mM NaF, 5 mM dichloroacetate, and 0.1% (wt/vol) Triton X-100 (pH 7.8) for later analysis of PDH activity as previously described (5, 34). PDK activity is reported as the apparent first-order rate constant of the inactivation of PDH (min\(^{-1}\)) or as the slope of \( \ln \% (PDH_a \text{ activity with ATP addition}/\text{total PDH activity}) \) vs. time (6).

**Calculation of Recovery and Quality of Mitochondria**

CS activity in the total muscle homogenate and in the mitochondrial suspension were measured (37), and the values were used to calculate the recovery and quality of the mitochondrial preparations as previously described (31). The average skeletal muscle mitochondrial activity of muscle glycogen.

![Fig. 1. Study design. Subjects consumed a prescribed 3-day prediet (Con; 51% carbohydrate, 29% fat, 20% protein of total energy intake) or 56-h high-fat, low-carbohydrate diet (HF/LC; 5% carbohydrate, 73% fat, 22% protein) followed by an overnight fast (10–12 h) before arriving at the laboratory. OGTT, oral glucose tolerance test; Leg Prep, leg preparation.](http://jap.physiology.org/Downloaded from http://jap.physiology.org/ by 101120.33.6 on April 29, 2017)
recovery for this study was 18.9 ± 4.4% of the total mitochondria, and the quality of the extraction (% of intact mitochondria) was 87 ± 4%.

**PDHa Activity**

A small piece of frozen wet muscle (~10–15 mg) was removed from each biopsy under N₂ for the determination of PDHa as previously described (5, 34). Total creatine (Cr) content was measured in the PDHa homogenates (2) and used to correct PDHa activity to the highest Cr content in a set of biopsies from a given subject. PDHa activity was expressed as millimoles of acetyl-CoA per kilogram of wet muscle per minute.

**GS Activity**

A second piece of frozen wet muscle (6–10 mg) was removed from each biopsy under N₂ for the determination of GS activity as previously described (13), with modifications. Briefly, the muscle samples were homogenized by hand in buffer (50 μl/1 mg) containing 50 mM Tris-HCl, 5 mM EDTA, 20 mM NaF, and 5 mM dithiothreitol, pH 7.2–7.4. Homogenates were centrifuged (7,000 g, +4°C) for 5 min and aliquots of the cytosolic fraction (supernatant) were incubated for the determination of active and total GS activity. The incubation media consisted of 50 mM Tris-HCl, 2 mM EDTA, 10 mM NaF, 10 mM glycogen, 0.5 mM dithiothreitol, 0.02% BSA, and either 0.1 mM (active GS) or 10.0 mM G-6-P (total GS), pH 7.2–7.4. Muscle homogenates (100 μl) were incubated with 450 μl each of 0.1 mM and 10.0 mM incubation media for 45 min at 37°C. The reaction was started with the addition of 50 μl of UDP-glucose (8 mM) and stopped by heating at 90°C for 2.5 min. Samples were centrifuged, and the supernatant was removed for fluorometric assay of UDP. Samples, blanks and standards (UDP, 25–150 μM) were added to an assay reagent containing 20 mM Tris-HCl, 30 mM KCl, 4 mM MgCl₂, 0.4 mM phosphoenolpyruvate, 20 μM NADH, and 0.4 U/ml lactate dehydrogenase, pH 7.6. Pyruvate kinase was added (3.0 U/ml) to start the reaction and the samples were incubated for 15 min at room temperature. Last, NADH was fluorometrically determined at 15 min (on the basis of pilot assays to determine the reaction end point). GS activity was calculated as nanomoles of UDP-glucose incorporated into glycogen per minute per milligram of protein, and the value was used to calculate GS fractional velocity (GSfv), defined as the activity of GS at 0.1 mM G-6-P (active GS) or 10.0 mM G-6-P (total GS).

**HK Activity**

A small piece of fresh muscle (3–5 mg) was used to measure maximal HK activity at 22°C as previously described (13) and modified (32). HK activity was determined in muscle homogenates by measuring the fluorescence of NADPH (G-6-P) against a range of G-6-P standards (1.67 to 10.0 mM). HK activity was expressed as moles of D-glucose incorporated into G-6-P per kilogram of protein per hour.

**Protein**

Muscle protein contents for GS and HK homogenates were measured by using a bicinchoninic acid reagent kit containing BSA standard (2 mg/ml) and reagents (Pierce, Rockford, IL).

**Muscle Fuels and Metabolites**

The remainder of the frozen muscle was freeze-dried, powdered, and dissected of all visible blood, connective tissue, and fat. The muscle fuels and metabolites were measured using standard enzymatic methods. Glycogen content (12) was measured in the resting (0 min) samples (2–3 mg) only. The remaining metabolites were measured in resting and 75-min OGTT biopsies on neutralized PCA extracts. Phosphocreatine (PCr), Cr, ATP, lactate, and G-6-P were measured spectrophotometrically (2, 12), pyruvate was measured fluorometrically (29), 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.

**Calculations**

A 90-min OGTT area under the curve (AUC) for blood glucose and plasma insulin concentrations vs. time was calculated for each subject as follows: resting glucose and insulin concentrations (0 min) were used because the baseline value and the total area of deviation from the baseline was calculated between 0 and 90 min (above and below baseline designated positive and negative). A 90-min AUC was calculated to compare the OGTT responses between conditions, because the majority of the glucose and insulin response occurred within this time period.

**Statistical Analyses**

Paired Student’s t-tests were used where results consisted of one data point in each of the Con and HF/LC trials (muscle glycogen content, PDK and HK activities, basal blood and plasma measures, and blood glucose and plasma insulin AUC data). Blood parameters, enzyme activities (PDHa, GS), and muscle metabolite contents over time during the OGTT were analyzed by using a two-way repeated-measures ANOVA (time × diet) with a Tukey’s post hoc test for all pairwise multiple comparisons. All blood and muscle data are presented as means ± SE. Significance was accepted at P < 0.05.

**RESULTS**

**Diet Analysis**

Dietary compliance during Con and HF/LC diets was monitored. The subjects consumed 51.4 ± 0.5% carbohydrate, 28.3 ± 6.0% fat, and 19.9 ± 0.8% protein in the Con diet and 4.7 ± 0.2% carbohydrate, 73.4 ± 6.0% fat, and 21.8 ± 5.0% protein in the HF/LC diet (Table 1). Table 1 contains the fatty acid composition of the diets. The fat content increased by −141 g in the HF/LC diet compared with Con, and this reflected increased dietary content from the full range of fatty acids (saturated, monounsaturated, and polyunsaturated fatty acids). Both diets were eucaloric with the subject’s normal diet and not different between trials. Saturated, monounsaturated, and polyunsaturated fat was similar (%total fat) in Con and HF/LC conditions (Table 1).

**Blood Analyses**

**Glucose and plasma insulin.** Pre-OGTT (0 min) whole blood glucose and plasma insulin concentrations were significantly increased by 10.2 ± 3.6 on April 29, 2017 from http://jap.physiology.org/ Downloaded by 10:20:33 on April 29, 2017

| Table 1. Dietary analysis for Con and high-fat, low-carbohydrate diets |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Total kcal      | 2,646 ± 100     | 2,736 ± 57      |
| %Carbohydrate   | 65.1 ± 0.7      | 47.9 ± 2.9      |
| %Fat            | 28.2 ± 0.5      | 73.4 ± 6.0      |
| %Protein        | 16.7 ± 0.4      | 18.7 ± 0.5      |
| Carbohydrate, g | 343.9 ± 11.5    | 346.1 ± 6.6     |
| Protein, g      | 134.7 ± 5.8     | 151.8 ± 6.4     |
| Total fat, g    | 86.2 ± 3.0      | 227.4 ± 3.9     |
| Saturated fat, g| 35.1 ± 2.2      | 78.3 ± 8.8      |
| Monounsaturated fat, g | 29.6 ± 1.5 | 82.9 ± 6.4 |
| Polyunsaturated fat, g | 14.5 ± 1.3 | 44.1 ± 2.5 |

Values are means ± SE for 6 subjects. Values are given in amount per day and represent the average over the 3-day prediet (Con) or 56-h high-fat/low-carbohydrate (HF/LC) diet. *Significantly different from Con, P < 0.05.
lower after HF/LC vs. Con (glucose: 3.39 ± 0.19 vs. 4.22 ± 0.19 mM and insulin: 5.64 ± 0.31 vs. 8.54 ± 0.67 μU/ml) (Fig. 2). The calculated 90-min blood glucose AUC was significantly higher (136.4 ± 48.2 vs. 61.9 ± 43.9 mmol·min⁻¹·l⁻¹) after HF/LC vs. Con diet, and the 90-min plasma insulin AUC was also significantly higher after the HF/LC diet (5,056 ± 670 vs. 4,047 ± 416 μIU·min⁻¹·ml⁻¹). Similar AUC calculations for 120-and 180-min OGTT periods were also significantly higher after HF/LC for both parameters.

Lactate, glycerol, β-OH, and plasma FFA. β-OH and plasma FFA concentrations pre-OGTT were significantly elevated after the HF/LC diet (Table 2). However, FFA and β-OH levels decreased 30 min after glucose ingestion and remained low throughout the OGTT. There were also no significant differences between trials. Whole blood glycerol levels were not affected by the HF/LC diet before the OGTT and decreased similarly in both trials during the OGTT (Table 2). Whole blood lactate was unaffected by the HF/LC diet before the OGTT and during the OGTT in both trials (Table 2).

Muscle Analyses

HK and GS activities. Maximal HK activity was not altered by the 56 h HF/LC diet (Con; 0.42 ± 0.04 vs. HF/LC; 0.36 ± 0.04 mol·kg protein⁻¹·h⁻¹).

Before the OGTT, the activity of GS in the active form (0.1 mM G-6-P) was similar in the Con and HF/LC trials (1.66 ± 0.36 and 1.43 ± 0.18 nmol·min⁻¹·mg⁻¹). The OGTT significantly increased the active GS to 3.04 ± 0.46 and to 2.69 ± 0.53 nmol·min⁻¹·mg⁻¹ in the Con and HF/LC condition, respectively, with no difference between diets. Total GS activity (10.0 mM G-6-P) was unchanged throughout the experiment (Con 0; 9.82 ± 1.02, Con 75; 10.80 ± 1.90, HF/LC 0; 10.41 ± 1.51, HF/LC 75; 10.90 ± 1.80 nmol·min⁻¹·mg⁻¹). Therefore, the GSfv increased significantly in response to the OGTT in both Con and HF/LC trials (Fig. 3).

PDK and PDHₐ activities. Resting PDK activity was significantly increased by the HF/LC diet (0.19 ± 0.05 vs. 0.08 ± 0.02 min⁻¹) (Fig. 4). The PDHₐ activity pre-OGTT was sig-

Fig. 2. Whole blood glucose (A and B) and plasma insulin concentrations (C and D) over time during an oral glucose tolerance test after a Con diet (A and C: ○) or a HF/LC diet (B and D: □). Values are means ± SE. Dotted lines, mean fasting glucose and insulin concentrations. Insets, area under the glucose (top) and insulin (bottom) curves after Con and HF/LC diets, respectively. *Significantly different from control, P < 0.05.
significantly lower after the HF/LC diet vs. Con (0.38 ± 0.08 vs. 0.79 ± 0.10 mmol acetyl-CoA·kg⁻¹·min⁻¹) (Fig. 5). During the OGTT, PDHₐ increased to a similar extent in both diets, but it remained lower after HF/LC (0.60 ± 0.11 vs. 1.04 ± 0.09 mmol acetyl-CoA·kg⁻¹·min⁻¹).

Muscle fuels and muscle metabolites. Resting muscle glycogen was not different between trials (Table 3). Generally, the diet and the OGTT had little effect on the muscle metabolite contents (Table 3). However, there were significant decreases in acetyl-CoA and acetyl carnitine contents during the initial 75 min of the OGTT in both trials (Table 3).

**DISCUSSION**

The 56-h HF/LC diet had no effect on the maximal activity of HK and the fraction of GS in the active form. However, the HF/LC diet did produce a stable increase in resting muscle PDK activity, which was associated with decreased PDH activation. This was associated with reduced whole body glucose disposal in aerobically trained men. During the OGTT, the absolute increase in the fractional activities of both GS and PDH were similar after the Con and HF/LC diets, 75 min after ingestion of the glucose load. This left the absolute PDHₐ activity lower after the HF/LC diet. These data suggest that the decreased skeletal muscle glucose disposal in response to the OGTT after a very short-term HF/LC diet was, in part, related to PDK-induced decreases in oxidative carbohydrate disposal and not to decreases in glycogen storage.

**Whole Body Glucose Disposal**

The AUC for blood glucose and plasma insulin increased during the OGTT after the HF/LC diet. The majority of an oral glucose load is taken up by skeletal muscle (20), suggesting that the decrease in glucose disposal after the HF/LC diet was mainly accounted for by decreased insulin-stimulated skeletal muscle glucose uptake. In this study, we were interested in the changes in skeletal muscle glucose uptake in response to the HF/LC diet, but the OGTT is a measure of whole body glucose disposal. As such, it includes a variable portion that results from hepatic glucose output and uptake. In normal subjects, hepatic glucose uptake accounted for 25% of the disposal of an oral glucose load (20), whereas hepatic glucose output was suppressed by 50% (7). However, the liver glycogen stores would be lower after the 56 h HF/LC diet vs. Con (17), and we expected lower liver glucose output and higher hepatic glucose uptake during the HF/LC OGTT. With less glucose released and more glucose taken up by the liver, it seems likely that the present results underestimated the severity of the attenuated glucose disposal by skeletal muscle after the HF/LC diet.

**Skeletal Muscle Glucose Phosphorylation**

Other studies have demonstrated that high-fat diets decrease glucose uptake and GLUT-4 translocation to the membrane (11). Our findings are consistent with these data, because more insulin was required to clear the glucose load after the 56-h

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**Table 2. Plasma FFA and whole blood β-hydroxybutyrate, glycerol, and lactate responses during an OGTT after a Con or HF/LC diet**

<table>
<thead>
<tr>
<th>Time, min (OGTT)</th>
<th>FFA</th>
<th>β-Hydroxybutyrate</th>
<th>Glycerol</th>
<th>Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Con</td>
<td>HF/LC</td>
<td>Con</td>
<td>HF/LC</td>
</tr>
<tr>
<td>−30</td>
<td>0.30±0.08</td>
<td>0.52±0.08*</td>
<td>0.10±0.02</td>
<td>0.31±0.09*</td>
</tr>
<tr>
<td>0</td>
<td>0.31±0.07</td>
<td>0.45±0.06</td>
<td>0.13±0.04</td>
<td>0.28±0.08*</td>
</tr>
<tr>
<td>30</td>
<td>0.21±0.06</td>
<td>0.27±0.05</td>
<td>0.07±0.02</td>
<td>0.16±0.05</td>
</tr>
<tr>
<td>60</td>
<td>0.12±0.05†</td>
<td>0.12±0.04†</td>
<td>0.04±0.01†</td>
<td>0.09±0.04†</td>
</tr>
<tr>
<td>90</td>
<td>0.11±0.05†</td>
<td>0.07±0.03†</td>
<td>0.04±0.01†</td>
<td>0.05±0.01†</td>
</tr>
<tr>
<td>120</td>
<td>0.09±0.03‡</td>
<td>0.06±0.04‡</td>
<td>0.04±0.01‡</td>
<td>0.04±0.01‡</td>
</tr>
<tr>
<td>150</td>
<td>0.22±0.05</td>
<td>0.27±0.14</td>
<td>0.06±0.02†</td>
<td>0.07±0.02‡</td>
</tr>
<tr>
<td>180</td>
<td>0.40±0.14</td>
<td>0.47±0.10</td>
<td>0.11±0.04</td>
<td>0.22±0.08</td>
</tr>
</tbody>
</table>

Values are means ± SE given in mM. FFA, free fatty acid; OGTT, oral glucose tolerance test. *Significantly different from Con, P < 0.05. †Significantly different from basal concentration (−30 and 0 min), P < 0.05.
HF/LC diet. This suggests that the diet caused either a disruption in signaling process and/or decreased GLUT-4 translocation. However, the present study did not measure muscle glucose uptake or GLUT-4 translocation to the muscle membrane during the OGTT, because of the amount of muscle required to measure GLUT-4 translocation in humans (9). Glucose uptake may also be depressed through decreased phosphorylation (through HK), which effectively decreases the glucose concentration gradient across the membrane (35). However, maximal HK activity was not altered after the HF/LC diet in the present study, suggesting that acute changes in glucose transport and phosphorylation in response to increased plasma FFA metabolism result from mechanisms unrelated to increased maximal activity (35). Whereas skeletal muscle HKII expression can adapt rapidly to increased insulin levels (4–6 h insulin infusion; Refs. 26, 33), rodent studies reported no changes in HK II activity after 3–4 wk of a high-fat diet (22, 43), and a human study (8) reported decreased maximal HK activity after a 4-wk very-low-carbohydrate diet (<20 g/day). This suggests a much slower adaptation to diet manipulations.

Glucose Oxidation

An important finding of this study was the rapid increase in PDK activity and the associated decrease in PDH activation after a 56-h HF/LC diet and before the OGTT. Our laboratory previously reported increased PDK activity after 3 and 6 days of a HF/LC diet (31), and a recent study demonstrated that PDK activity was already increased after only 1 day on this diet when a dramatic shift toward almost exclusively fat oxidation had occurred (30). The present results extend these findings, because decreased PDHa accompanied the rapid increase in PDK activity on this diet, suggesting that the increased PDK activity was responsible for the decrease in PDH. It is unlikely that diet-induced changes in the acute regulators of PDK can explain the decrease, because there were no changes in these regulators (ATP, acetyl-CoA, and pyruvate) after the HF/LC diet, although NADH was not measured.

When the oral glucose was ingested, PDH activation increased in both the Con and HF/LC diets but remained lower after HF/LC. Assuming that the measured PDHa was representative of in vivo PDH flux and glucose oxidation (27, 34) and the 75-min biopsy was representative of the 90-min period of glucose disposal, the reduced PDHa decreased the oxidative disposal of glucose after the HF/LC diet. To explain the increased PDHa during the OGTT in both trials, we again examined the acute regulators of PDK and PDH phosphatase. Of the PDK regulators, the acetyl-CoA content decreased very slightly in both trials, whereas the ATP and pyruvate contents were unchanged, suggesting little or no acute regulation of the kinase. On the phosphatase side, PDH phosphatase (PDP) 1 would be not be expected to be affected by the OGTT, because Ca2+ concentration would not change in response to a glucose load. However, the high insulin levels during the OGTT would be expected to activate PDHa through PDP2 (15). There is some evidence in rat heart and kidney that PDP2 protein expression is decreased after starvation and diabetes, and although this may contribute to the reduced activation of the complex after the HF/LC diet, there is no evidence that PDP2 is similarly downregulated in either rat or human skeletal muscle (16). In addition, another difference between the two conditions was that the influence of insulin on the phosphatase side was against a lower basal PDHa observed after the HF/LC diet before the OGTT.

The downregulation of PDHa by PDK appears to account for the initial adaptation of skeletal muscle to a HF/LC diet. Whereas PDHa increased in the face of the glucose challenge, the lower final PDHa and potential for carbohydrate oxidation was associated with the reduced muscle glucose uptake after the HF/LC diet. In human skeletal muscle, an increase in PDK activity has been seen as early as 1 day on the HF/LC diet and it increased linearly during the first 6 days (30, 31). Increased fat utilization, decreased insulin concentrations, or decreased

Table 3. Muscle fuels and metabolites before (0 min) and 75 min after the ingestion of an oral glucose load after a Con and HF/LC diet

<table>
<thead>
<tr>
<th></th>
<th>Con 0 min</th>
<th>Con 75 min</th>
<th>HF/LC 0 min</th>
<th>HF/LC 75 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR, mmol/kg dry mass</td>
<td>89.5±1.5</td>
<td>83.3±2.0</td>
<td>82.6±4.1</td>
<td>82.9±2.6</td>
</tr>
<tr>
<td>ATP, mmol/kg dry mass</td>
<td>26.64±0.83</td>
<td>26.40±0.84</td>
<td>23.65±0.65*</td>
<td>25.65±1.25</td>
</tr>
<tr>
<td>Glycogen, mmol/kg dry mass</td>
<td>346±22.3</td>
<td>320±14.7 *</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>G-6-P, mmol/kg dry mass</td>
<td>0.71±0.17</td>
<td>0.40±0.14</td>
<td>0.72±0.21</td>
<td>0.55±0.09</td>
</tr>
<tr>
<td>Pyruvate, mmol/kg dry mass</td>
<td>0.14±0.06</td>
<td>0.15±0.04</td>
<td>0.11±0.03</td>
<td>0.11±0.03</td>
</tr>
<tr>
<td>Lactate, mmol/kg dry mass</td>
<td>3.9±0.9</td>
<td>5.4±2.15</td>
<td>7.8±1.4</td>
<td>6.3±1.7</td>
</tr>
<tr>
<td>Acetyl-CoA, μmol/kg dry mass</td>
<td>8.70±1.46</td>
<td>4.89±0.44‡</td>
<td>8.64±0.46</td>
<td>6.39±0.75‡</td>
</tr>
<tr>
<td>Acetyl carnitine, mmol/kg dry mass</td>
<td>3.7±0.9</td>
<td>3.0±0.9 †</td>
<td>3.8±0.8</td>
<td>1.7±0.6 †</td>
</tr>
</tbody>
</table>

Values are means ± SE. PCR, phosphocreatine; G-6-P, glucose 6-phosphate; ND, not determined. *Significantly different from Con, P < 0.05. †Significantly different from 0 min, P < 0.05.
sensitivity to insulin have been implicated as the potential mechanisms for the increased PDK expression (24, 39, 42).

The increase in PDK activity during the first 3 days of a HF/LC diet was accompanied by an increase in only the PDK-4 isoform (30). Increased PDK-4 isoform is associated with decreased pyruvate sensitivity of the PDH complex in rat skeletal muscle mitochondria (14, 39, 42). This ensures that, after starvation or a high-fat diet, increasing pyruvate production in the glycolytic pathway is less able to inhibit PDK activity and activate the complex, effectively suppressing carbohydrate oxidation. It is suggested that this regenerates cytosolic NAD\(^+\) through increased lactate production and conserves three-carbon intermediates of glucose for use as gluconeogenic precursors in the liver (40). Therefore, both at rest and in the face of a similar glucose load, the PDH complex would be more resistant to increases in pyruvate concentration after a HF/LC diet compared with a normal diet, causing decreased PDH activation and oxidative glucose disposal (14, 39). This mechanism may have been at work in response to the OGTT in the present study, although muscle pyruvate was not increased in the HF/LC condition.

**OGTT and Muscle Acetylcarnitine Content**

The muscle acetylcarnitine content decreased during the OGTT in both trials. We expected that the disposal of glucose in this situation would lead to an increase in acetylcarnitine, as seen in other situations of increased glycolytic flux (i.e., onset of exercise). The reason for this decrease is unknown, although our laboratory previously reported a similar acetylcarnitine decrease during an OGTT (41). Acetylcarnitine acts as a buffer of acetyl units in the muscle, storing excess acetyl-CoA when the provision is greater than its use and providing acetyl units when the supply is reduced. The decrease in acetylcarnitine during the OGTT suggests that the muscle was not providing adequate acetyl units and therefore reducing equivalents (NADH), from carbohydrate and fat sources to meet the energy needs of the electron transport chain in resting muscle. On the carbohydrate side, even though PDH activation increased during the OGTT in both trials, insufficient pyruvate provision may have limited the production of acetyl-CoA from carbohydrate. The competition for G-6-P higher in the glycolytic pathway may be dominated by GS, leaving little substrate for the lower portion of the pathway. One might have expected a decreased pyruvate level during the OGTT if it were limiting PDH flux, but this did not occur. On the fat side, the high insulin concentration during the OGTT had a profound inhibitory effect on adipose tissue lipolysis, as evidenced by the low FFA concentration during the OGTT in both trials. It is also possible that the high insulin concentration inhibited hormone sensitive lipase activity, the enzyme that regulates the breakdown of muscle triacylglycerol. Limitations in both sources of FFA may have resulted in a shortage of substrate for fatty-derived acetyl-CoA in the muscle during the OGTT.

**Glucose Storage**

Contrary to our second hypothesis, the HF/LC diet did not blunt the change in GS\(_{\text{f}}\) (fraction of GS in active form) before the OGTT, and GS\(_{\text{f}}\) increased to the same extent during the OGTT in both trials. However, it is important to note that the present GS results were obtained in biopsies taken at 75 min after the ingestion of glucose, and they may not reflect GS activity over the entire 90-min glucose disposal period.

A recent study in rodents found no decrease in insulin-stimulated GS activity after 2 wk on a high-fat diet, suggesting that this is not an early adaptation in the development of insulin resistance (22). However, the same group previously demonstrated decreased GS activity and accumulation of muscle glycogen during a 2-h euglycemic hyperinsulinemic clamp after a 3-wk high-fat diet (23). The present results demonstrated that insulin-stimulated GS activity was not inhibited during an OGTT after 56 h of HF/LC exposure in human skeletal muscle. However, with our short-term perturbation, muscle glycogen was replete and G-6-P content was unaltered in response to the HF/LC diet, and therefore it is possible that GS would not have been stimulated to increase glycogen storage during the OGTT. However, we are unaware of GS measurements after longer-term exposure to HF/LC diets in humans to determine whether an adaptation in the ability of insulin to stimulate GS would occur.

**Summary**

Short-term exposure (56 h) to a HF/LC diet reduced whole body glucose disposal during an OGTT in aerobically trained men. In skeletal muscle, the maximal activity of HK and the fraction of GS in the active form before the OGTT were unaltered by the HF/LC diet. However, the HF/LC diet increased muscle PDK activity, which was associated with decreased PDH activation before the OGTT. The active fractions of GS and PDH increased similarly in the Con and HF/LC conditions, 75 min after the ingestion of glucose, resulting in lower absolute PDH activation after the HF/LC diet. The results imply that muscle glucose transport was decreased during the OGTT after the HF/LC diet, presumably due to reduced GLUT-4 translocation to the muscle membrane. These results also suggest that the decreased skeletal muscle glucose disposal during the OGTT after the HF/LC diet was related to decreased oxidative carbohydrate disposal in skeletal muscle and not to decreased glycogen storage. The rapid increase in PDK activity during the HF/LC diet appeared to account for the reduced potential for oxidative carbohydrate disposal during the oral glucose challenge.

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