Elevated n-3 fatty acids in a high-fat diet attenuate the increase in PDH kinase activity but not PDH activity in human skeletal muscle

Erik A. Turvey, George J. F. Heigenhauser, Michelle Parolin, and Sandra J. Peters. Elevated n-3 fatty acids in a high-fat diet attenuate the increase in PDH kinase activity but not PDH activity in human skeletal muscle. J Appl Physiol 98: 350–355, 2005. First published September 17, 2004; doi:10.1152/japplphysiol.00604.2004.—We tested the hypothesis that a high-fat diet (75% fat; 5% carbohydrates; 20% protein), for which 15% of the fat content was substituted with n-3 fatty acids, would not exhibit the diet-induced increase in pyruvate dehydrogenase kinase (PDK) activity, which is normally observed in human skeletal muscle. The fat content was the same in both the regular high-fat diet (HF) and in the n-3-substituted diet (N3). PDK activity increased after both high-fat diets, but the increase was attenuated after the N3 diet (0.051 ± 0.007 and 0.218 ± 0.047 min⁻¹ for pre- and post-HF, respectively; vs. 0.073 ± 0.016 and 0.133 ± 0.032 min⁻¹ for pre- and post-N3, respectively). However, the active form of pyruvate dehydrogenase (PDHa) activity decreased to a similar extent in both conditions (0.93 ± 0.17 and 0.43 ± 0.09 mmol/kg wet wt pre- and post-HF; vs. 0.87 ± 0.19 and 0.39 ± 0.05 mmol/kg wet wt pre- and post-N3, respectively). This suggested that the difference in PDK activity did not affect PDHa activation in the basal state, and it was regulated by intramitochondrial effectors, primarily muscle pyruvate concentration. Muscle glycogen content was consistent throughout the study, before and after both diet conditions, whereas muscle glucose-6-phosphate, glycerol-3-phosphate, lactate, and pyruvate were decreased after the high-fat diets. Plasma triglycerides decreased after both high-fat diets but decreased to a greater extent after the N3 diet, whereas plasma free fatty acids increased after both diets, but to a lesser extent after the N3. In summary, PDK activity is decreased after a high-fat diet that is rich in n-3 fatty acids, although PDHa activity was unaltered. In addition, our data demonstrated that the hypolipidemic effect of n-3 fatty acids occurs earlier (3 days) than previously reported and is evident even when the diet has 75% of its total energy derived from fat.

Eucaloric high-fat, low-carbohydrate (CHO) diets, such as those used in this study, have demonstrated decreased insulin sensitivity and glucose disposal by skeletal muscle in both animals and humans (1, 9, 28). The mechanism(s) behind the diet-induced defect is multifaceted and not well understood, particularly in human skeletal muscle. Decreased skeletal muscle uptake and disposal of glucose may be due to defective insulin signaling, decreased translocation of the glucose transporters, reduced muscle glycogen synthesis and oxidative disposal of glucose, or any combination of these factors.

Oxidative disposal of CHO is regulated by the pyruvate dehydrogenase (PDH) complex, which exists in both an active (PDHα) and inactive form (as reviewed in Ref. 33). Conversion between the two forms is regulated by reversible phosphorylation catalyzed by a family of four intrinsic PDH kinases (PDK1–4) and a pair of PDH phosphatases such that the inactive form of the complex is phosphorylated at one of three possible sites (as reviewed in Ref. 30). The kinases and phosphatases are acutely regulated by concentrations of intramitochondrial effectors. Calcium ions and insulin stimulate PDH phosphatases 1 and 2, respectively, to activate the complex (15). PDH activity is enhanced by a high-energy charge or accumulation of the products NADH and acetyl-CoA and suppressed allosterically with high pyruvate concentrations (33). The PDK isoforms differ in their intrinsic activities and responses to effectors (30). Separate from acute regulation, adaptive “effector-independent” increased human skeletal muscle PDH activity and PDK4 isoform protein has been observed in response to a low-CHO, high-fat diet, accompanied by decreased CHO oxidation and PDHα activity (24). This same response is elicited in rat skeletal muscle in response to a high-fat diet, but the increase in PDH activity and PDK4 protein required 28 days of dietary intervention compared with as little as 1 day in humans (14, 23).

Fryer et al. (8) observed that the diet-induced increase in PDK activity in response to a high-fat diet is dependent on fatty acid composition in animal models, such that the substitution of n-3 fatty acids into the high-fat diet completely eliminated the increase in PDK activity normally observed with the high-fat diet. However, there are no studies in human skeletal muscle examining PDK activity and the effect of n-3 fatty acids.

N-3 fatty acids (also known as omega-3 fatty acids or fish oils) have generated a great deal of interest because of their cardio-protective and anti-hyperlipidemic effects (29). The fish oils, primarily eicosapentaenoic acid and docosahexaenoic acid, prevent the development of insulin resistance, although the mechanism for this is not clearly understood (29). Using stable isotopes, Jucker and coworkers (17) examined the effect of safflower oil compared with fish oil in rats and reported decreased glycolytic and oxidative disposal of glucose in the safflower oil group compared with the fish oil group. However, their study did not directly measure PDH activity in response to the diets, and therefore the mechanism responsible was still undetermined.

The purpose of this study was to examine the effect of a high-fat diet that is high in n-3 fatty acids on PDK and PDHα.
activity in human skeletal muscle. The hypothesis was that a high-fat diet rich in n-3 fatty acids would not exhibit the diet-induced increase in PDK activity that is normally observed. This would allow PDH activity to remain more active after the high-fat diet.

METHODS

Subjects. Eight male university students volunteered for participation in this study. All subjects were in good health with a mean age, height, and mass of 24.7 ± 1.6 yr, 180.0 ± 2.7 cm, and 80.5 ± 2.8 kg, respectively. Subjects were moderately active and regularly engaged in at least 20 min of aerobic activity three times per week. Their mean relative maximum oxygen consumption was 42.1 ± 2.2 ml·min⁻¹·kg⁻¹ (range 36.1–50.6 ml·min⁻¹·kg⁻¹). This study received ethical approval from the McMaster University Ethics Committee, and written consent was obtained from the subjects.

Study design. Subjects were asked to provide typical, carefully detailed, 3-day dietary records that were used to calculate normal daily caloric intake, dietary composition, and caffeine intake for each subject, and they were then asked to report to the laboratory four times for testing. Each subject consumed an individualized prediet designed to be isocaloric with their habitual diet and composed of ~50% CHO, 30% fat, and 20% protein for 3 days and then reported to the laboratory for muscle biopsies and blood sampling. Subjects immediately began to consume either an isocaloric regular high-fat diet (HF) or an n-3 fatty acid-enriched high-fat diet (N3) for 3 days according to a diet plan. Compliance was monitored by daily verbal contact with the subjects, and any deviation in intake from the diet plan was recorded and analyzed in the dietary composition. These diets are not difficult to tolerate for 3 days and have been used successfully in previous studies (23–25). No changes were made in daily caffeine intake, and subjects were asked to abstain from alcohol and exercise (beyond that required for normal daily living) during the prediet and high-fat diet periods. After 3 days on the high-fat diet, subjects again reported to the laboratory for biopsies and blood. Each subject was tested with both high-fat diets, and the two different diets were separated by a 4-wk washout period, with the order of the diets chosen randomly for each subject (Fig. 1). When reporting to the laboratory for sampling, subjects were asked to consume breakfast 1 h before their arrival at the laboratory. The composition of this breakfast varied according to the dietary condition they were presently completing (prediet, HF, or N3).

High-fat diets. The dietary composition goal of HF was 75% fat, 20% protein, and 5% CHO. The presence of n-3 fatty acids in the HF was negligible, as no natural sources were provided in the diet during this period.

The composition of N3 was identical to HF except that ~15% of the energy derived from fat or ~12% of the total calories in N3 was substituted with n-3 fatty acids. These fatty acids were consumed in the form of soft gelatin capsules filled with 1,000 mg of fish oil (36.8% eicosapentaenoic acid and 26.6% docosahexaenoic acid) (In Cromega TG3525, Lot no. 8906025, Croda Canada, Toronto, ON, Canada). The energy removed from HF to allow n-3 fatty acid substitution was evenly taken from the dietary saturated and unsaturated fat consumed.

Blood analyses. A single resting blood sample of ~6–7 ml was drawn from the antecubital vein of the forearm before and after each diet. A portion of the plasma supernatant was deproteinized with 6% perchloric acid (in a ratio of 1:2, respectively) and centrifuged at 900 g for 2 min, and the supernatant was removed and stored for subsequent analysis of glucose, glycerol, and lactate. The remaining plasma from the blood sample was aliquoted appropriately for later analysis of β-hydroxybutyrate, free fatty acids (FFA), insulin, triglyceride, mean cholesterol, high-density cholesterol (HDL), and low-density lipoprotein (LDL). All samples were stored at ~20°C until the day of analysis.

Perchloric acid plasma extract was utilized for determination of glucose, glycerol, and lactate as described by Bergmeyer (2). Untreated plasma was utilized for the remainder of the blood analysis. Insulin was measured with a Coat-a-Count Insulin test kit (Diagnostic Products, Los Angeles, CA), and a Wako NEFA C kit (Wako Chemicals, Richmond, VA) was used to analyze plasma FFA. The β-hydroxybutyrate assay was carried out as described previously (2). Triglyceride, cholesterol, and HDL levels were determined using the test methodologies of Vitros Chemistry Products, and LDL values were calculated from the results of the cholesterol, triglyceride, and LDL results (Vitros Chemistry Products, Raritan, NJ).

Muscle sampling. Two resting muscle biopsies were taken from the vastus lateralis as previously described (3). The first biopsy was immediately frozen and stored in liquid nitrogen. A 5- to 15-mg piece was chipped from this biopsy sample for determination of PDHs. The remaining portion of this first biopsy was freeze-dried and stored in a dessicator at ~80°C. The second biopsy was prepared immediately for mitochondrial extraction and was utilized to determine PDK activity.

Mitochondrial extraction. Differential centrifugation was utilized to obtain intact mitochondria from fresh muscle, as previously described (4, 20, 24). Quality and recovery of the mitochondrial preparations were determined by citrate synthase activity in extra-mitochondrial and total mitochondrial fractions and total homogenate as described previously (4, 24, 27). In this study, the quality and recovery of the mitochondrial preparations were 86.3 ± 0.8 and 16 ± 1%, respectively.

PDK activity. The final mitochondrial suspension (50 μl) was diluted with 250 μl of buffer containing 10 μM carbonyl cyanide m-chlorophenyl-hydrazone, 20 mM Tris·HCl, 120 mM KCl, 2 mM EGTA, and 5 mM potassium phosphate (monobasic) (pH 7.4) and incubated for 20 min at 30°C. This incubation reduced the ATP concentration to zero, fully deactivating PDK, thus completely converting all of the PDH into its active form (7). The suspension was then centrifuged at 7,000 g for 10 min and stored in liquid nitrogen until incubated for total PDH and PDK activities.

Determination of total PDH and PDK activities were carried out as described by Peters et al. (24). Mitochondria were suspended in

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RESULTS

Diets. Total caloric intake was maintained throughout the diets (2,757 ± 78 kcal/day) and was designed to be isonergic with each subject’s habitual energy intake. Compliance to the high-fat diet was monitored daily and was good, as evidenced by how closely the actual diets matched the target intake ratios. There were no significant differences between HF and N3 with respect to the percentage of the daily energy intake from fat (~75% of the total energy) or the contributions of fat, CHO, and protein to the daily intake (Table 1). In N3, 51.7 ± 0.8 g of n-3 fatty acids were added to the diets, accounting for 11.8 ± 0.2% of the total daily energy intake or 15.9 ± 0.3% of the fat in N3.

Table 1. High-fat diet analysis

<table>
<thead>
<tr>
<th></th>
<th>Pre-HF Diet</th>
<th>HF Diet</th>
<th>Pre-N3 Diet</th>
<th>N3 Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>%Total fat</td>
<td>29.2 ± 1.5</td>
<td>74.7 ± 0.2</td>
<td>29.7 ± 3.5</td>
<td>74.5 ± 0.5</td>
</tr>
<tr>
<td>Total fat, g</td>
<td>92.9 ± 9.7</td>
<td>240.4 ± 8.5</td>
<td>90.7 ± 13.3</td>
<td>229.8 ± 9.9</td>
</tr>
<tr>
<td>Saturated fat, g</td>
<td>30.2 ± 3.9</td>
<td>85.9 ± 4.6</td>
<td>30.5 ± 5.2</td>
<td>75.7 ± 5.1</td>
</tr>
<tr>
<td>%Unsaturated fat</td>
<td>62.7 ± 6.9</td>
<td>150.9 ± 4.2</td>
<td>57.0 ± 9.2</td>
<td>154.0 ± 5.1</td>
</tr>
<tr>
<td>%Protein</td>
<td>17.3 ± 1.1</td>
<td>19.8 ± 0.3</td>
<td>17.7 ± 1.6</td>
<td>20.6 ± 0.3</td>
</tr>
<tr>
<td>%Carbohydrate</td>
<td>52.7 ± 1.6</td>
<td>5.2 ± 0.2</td>
<td>510.2 ± 2.5</td>
<td>5.2 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. Percentages of the total energy intake and weight of fats, protein, and carbohydrate of prediets, and regular high-fat diet (HF) compared with n-3 fatty acid-substituted high-fat diet (N3).

−300 μl of buffer [30 mM KH2PO4, 5 mM EGTA, 5 mM dithiothreitol, 25 μg/ml oligomycin B, 1.0 mM tosyl-lysyl-chloro-methyl-ketone, 0.1% Triton, and 1% bovine serum albumin (pH 7.0)] and freeze-thawed twice to ensure that all mitochondria were broken. The suspension was warmed to 30°C, and two aliquots were diluted in a ratio of 1:1 in a buffer containing 200 mM sucrose, 50 mM potassium chloride, 5 mM magnesium chloride, 5 mM EGTA, 50 mM Tris·HCl, 50 mM sodium fluoride, 5 mM dichloroacetate, and 0.1% Triton (pH 7.8) for later analysis of total PDH activity or zero time. Magnesium ATP (0.3 mM) was added, and timed samples were removed at timed intervals for 3–5 min and stopped in buffer. The samples were stored on ice for subsequent analysis of PDHa activity. PDK activity is reported as the apparent first-order rate constant of the inactivation of PDH (min−1) or as the natural log of [(PDHa activity/total PDH activity)×100%] vs. time (7, 24, 32). The slope of the decrease in PDHs with respect to time was determined by regression analysis.

PDHa. A 5- to 15-mg piece of muscle was chipped from the first biopsy of the trial (immediately frozen and stored in liquid nitrogen). This sample was homogenized and subsequently analyzed for PDHa activity according to the method described previously (5, 21). PDHa activity was normalized to the highest total creatine content from the four samples obtained for each subject to correct for nonmuscle contamination.

Muscle metabolites. The remaining analyses were carried out on freeze-dried muscle. Muscle samples were powdered and dissected free of connective tissue and blood (as much as possible). These samples were aliquoted for later analysis and stored at −80°C in a dessicator until the day of extraction.

An alkaline extraction was performed on 2–4 mg of dry muscle to determine muscle glycogen, as described by Harris et al. (10). Muscle acetylcarminite, free carnitine, acetyl-CoA, and free CoA (CoASH) were determined by radioisotopic methods described by Cederblad et al. (5). Glucose, glucose-6-phosphate, pyruvate, lactate, creatine, phosphocreatine, and glycerol-3-phosphate were analyzed using enzymatic assays (10). Concentrations of muscle metabolites were normalized to the highest total creatine content from the four samples obtained for each subject to correct for nonmuscle contamination.

Diet analysis and statistics. The dietary records, prediets, and high-fat diets were recorded and analyzed using Diet and Fitness Software (Expert Software, San Bruno, CA). Results were calculated and reported as the daily means of the diet periods with respect to caloric intake and composition.

Data were analyzed using a two-way ANOVA with repeated measures over time. When a significant F ratio was found, Fisher’s protected post hoc test was used to compare means. Significance was accepted at P < 0.05.

Table 2. Blood lipid profiles

<table>
<thead>
<tr>
<th></th>
<th>Pre-HF</th>
<th>Post-HF</th>
<th>Pre-N3</th>
<th>Post-N3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides, mM</td>
<td>1.6 ± 0.3</td>
<td>0.8 ± 0.1*</td>
<td>1.1 ± 0.2</td>
<td>0.43 ± 0.07†</td>
</tr>
<tr>
<td>Free fatty acids, mM</td>
<td>0.15 ± 0.03</td>
<td>0.47 ± 0.09*</td>
<td>0.16 ± 0.06</td>
<td>0.38 ± 0.08**</td>
</tr>
<tr>
<td>Glycerol, μM</td>
<td>22.7 ± 0.4</td>
<td>31.5 ± 0.6</td>
<td>28.3 ± 0.7</td>
<td>35.7 ± 0.6</td>
</tr>
<tr>
<td>LDL, mM</td>
<td>2.6 ± 0.4</td>
<td>3.1 ± 0.5</td>
<td>2.8 ± 0.5</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>HDL, mM</td>
<td>1.08 ± 0.06</td>
<td>1.17 ± 0.07</td>
<td>1.11 ± 0.11</td>
<td>1.08 ± 0.11</td>
</tr>
</tbody>
</table>

Data are means ± SE. LDL, low-density lipoprotein; HDL, high-density lipoprotein. *Significantly different from prediet value (P < 0.05). †Significantly different from HF value (P < 0.05).

Table 3. Plasma β-hydroxybutyrate, glucose, lactate, and insulin concentrations

<table>
<thead>
<tr>
<th></th>
<th>Pre-HF</th>
<th>Post-HF</th>
<th>Pre-N3</th>
<th>Post-N3</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Hydroxybutyrate, mM</td>
<td>0.21 ± 0.03</td>
<td>0.73 ± 0.19*</td>
<td>0.19 ± 0.02</td>
<td>0.63 ± 0.19*</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>5.5 ± 0.4</td>
<td>5.3 ± 0.3</td>
<td>5.3 ± 0.3</td>
<td>5.3 ± 0.2</td>
</tr>
<tr>
<td>Lactate, mM</td>
<td>1.3 ± 0.1</td>
<td>0.9 ± 0.2*</td>
<td>1.4 ± 0.2</td>
<td>0.7 ± 0.1†</td>
</tr>
<tr>
<td>Insulin, μU/ml</td>
<td>12 ± 5</td>
<td>6.2 ± 1*</td>
<td>13 ± 3</td>
<td>4.8 ± 0.5*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significantly different from prediet value (P < 0.05). †Significantly different from HF value (P < 0.05).
solely the high-fat content or restricted levels of CHO, or protein. Thus the different outcomes of these two dietary fat, with no difference in the proportions of total fat, experimental diets varied only in terms of the composition of CHO restriction (5% of the total energy). Here, the two feeding was due to an excess supply of fat or due to severe increase in skeletal muscle PDK activity (23, 24). In these has also been utilized in previous studies examining the adap-
tivity was in mmol·min⁻¹·kg wet wt. *Significantly different from prediet value (P < 0.05).

![Fig. 2. Pyruvate dehydrogenase kinase (PDK) activity. PDK activity was measured as the first-order rate constant before and after the high-fat diets.](Image)

![Fig. 3. Active form of pyruvate dehydrogenase (PDHa) activity. PDHa activity was in mmol·min⁻¹·kg wet wt. *Significantly different from prediet (P < 0.05).](Image)

**DISCUSSION**

This is the first study to examine the regulation of human skeletal muscle PDH in response to two high-fat diets consisting of different fat compositions. The diets both had 75% of the total energy derived from fat and differed only with respect to the content of n-3 fatty acids, predominantly eicosapentaenoic acid and docosahexaenoic acid. The primary finding is that a high-fat diet with 15% of the fat energy replaced by fish oils attenuated but did not eliminate the high-fat diet-induced increase in PDK activity in human skeletal muscle. These data demonstrate that fat is not uniform in its effects on the PDH complex. However, in the basal state, PDHa activity was not different between the two diets. HF used in the present study has also been utilized in previous studies examining the adaptive increase in skeletal muscle PDK activity (23, 24). In these studies, it was unclear whether the observed effect of high-fat feeding was due to an excess supply of fat or due to severe CHO restriction (~5% of the total energy). Here, the two experimental diets varied only in terms of the composition of dietary fat, with no difference in the proportions of total fat, CHO, or protein. Thus the different outcomes of these two diets suggest that it is the composition of dietary fat and not solely the high-fat content or restricted levels of CHO that plays a significant role in eliciting the chronic increase in PDK activity observed in this and previous studies. However, it is possible that the differential response to n-3 fatty acids acts through changes in insulin sensitivity. Alterations in insulin concentration or insulin sensitivity cause profound changes in PDK4 expression (18).

**Dietary fat composition and PDK activity.** The fourfold increase in PDK activity observed after HF was similar to that evoked by 28 days of high-fat feeding in rat skeletal muscle (8, 14) and after 3 days of high-fat feeding in human skeletal muscle (23, 24). It has been suggested that increased PDK activity contributes to the decreased capacity for muscle CHO oxidation through downregulation of PDHa (24). The reduced increase in PDK activity observed when 15% of the fat calories are replaced by n-3 fatty acids is consistent with results from a rodent model that completely suppressed PDK activity after 28 days of high-fat feeding with the substitution of only 7% of the fat with n-3 fatty acids (8). However, it is unclear why the increased dosage of n-3 fatty acids in the present study did not fully reverse the adaptive increase in PDK activity in human skeletal muscle but suggests a species difference in the effect of dietary fat on regulation of muscle CHO oxidation or is possibly due to the decreased duration of the dietary intervention (3 vs. 28 days in the rat study).

PDK isoform protein was not measured in the present study, but both animal and human studies have demonstrated that the high-fat diet-induced increase in PDHa activity is accompanied by an increase in the PDH4 isoform, which has the lowest sensitivity to pyruvate inhibition (23, 30). Thus the increase in PDK4 content renders the PDH complex even less sensitive to pyruvate inhibition, such that oxidative disposal of glucose would be decreased compared with the normal situation. Although there are no human studies that have examined oxidative disposal of glucose in humans after n-3 fatty acid diet, Jucker and coworkers (17) examined the response of high safflower oil- or fish oil-fed rats to a hyperinsulinemic/euglycemic clamp chal-

**Table 4. Muscle acetylated metabolites**

<table>
<thead>
<tr>
<th></th>
<th>Pre-HF</th>
<th>Post-HF</th>
<th>Pre-N3</th>
<th>Post-N3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetyl-CoA</td>
<td>7±3</td>
<td>17±5</td>
<td>13±1</td>
<td>16±1</td>
</tr>
<tr>
<td>Free CoASH</td>
<td>124±20</td>
<td>97±13</td>
<td>107±16</td>
<td>117±9</td>
</tr>
<tr>
<td>Acetyl-CoA-to-CoASH ratio</td>
<td>0.14±0.02</td>
<td>0.17±0.03</td>
<td>0.14±0.03</td>
<td>0.14±0.02</td>
</tr>
<tr>
<td>Acetylcarnitine</td>
<td>2.3±0.8</td>
<td>4.6±1.7*</td>
<td>1.5±0.5</td>
<td>5.1±0.8*</td>
</tr>
<tr>
<td>Free carnitine</td>
<td>20±2</td>
<td>16±2*</td>
<td>20±2</td>
<td>17±2*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Acetyl-CoA and free CoA (CoASH) are expressed in μmol/kg dry wt; acetylcarnitine, free carnitine are expressed in mmol/kg dry wt. *Significantly different from prediet value (P < 0.05).

**Table 5. Muscle glycogen and glycolytic intermediates**

<table>
<thead>
<tr>
<th></th>
<th>Pre-HF</th>
<th>Post-HF</th>
<th>Pre-N3</th>
<th>Post-N3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-6-phosphate</td>
<td>1.6±0.4</td>
<td>1.4±0.1*</td>
<td>1.6±0.5</td>
<td>1.2±0.4*</td>
</tr>
<tr>
<td>Glycogen</td>
<td>497±72</td>
<td>414±70</td>
<td>407±55</td>
<td>448±47</td>
</tr>
<tr>
<td>Glucose-3-phosphate</td>
<td>1.0±0.3</td>
<td>0.74±0.15</td>
<td>0.9±0.3</td>
<td>0.55±0.15</td>
</tr>
<tr>
<td>Lactate</td>
<td>4.4±1.1</td>
<td>2.7±0.7*</td>
<td>5.0±1.2</td>
<td>1.8±0.7*</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.45±0.10</td>
<td>0.26±0.05*</td>
<td>0.35±0.06</td>
<td>0.25±0.06*</td>
</tr>
</tbody>
</table>

Values are means ± SE. All measures are expressed in mmol/kg dry wt. *Significantly different from prediet value (P < 0.05).
Increase in fat oxidation is an important regulator of muscle and blood parameters and regulation of PDHa activity. Increased fat oxidation is an important regulator of PDK activity and is believed to increase PDK4 gene expression through stimulation of peroxisomal proliferator-activated receptor (PPAR)-α and possibly through PPAR-δ, which has much greater abundance in skeletal muscle than either PPAR-α or PPAR-γ (21, 34). Previously, we observed increased reliance on fat oxidation for energy at rest as early as 24 h on a high-fat diet (23). Both high-fat diets were accompanied by a threefold increase in β-hydroxybutyrate levels, suggesting that was increased lipid oxidation in the liver in response to both diets (23, 24). Muscle glycogen concentration was unchanged by diet and was consistent throughout the study. Glucose-6-phosphate concentrations were decreased by HF and were decreased to a greater extent after N3, whereas plasma and muscle lactate decreased similarly after both diets. Taken together, these data are consistent with decreased reliance on CHO metabolism and increased reliance on fat metabolism. The increased reliance on fat fuel appeared to be similar in both conditions, and, therefore, the differential effect on increased PDK activity must be due to some modulation attributed to the differential effect of the n-3 fatty acids. One possibility is that n-3 fatty acids would behave differently in providing stimulating ligands for the muscle PPARs and, therefore, may not increase PDK gene expression to the same extent as other fatty acids (26).

PDHa activity. After N3, PDK activity was decreased compared with HF, but there was no differential effect on PDHa activation in the basal state. Both diets decreased PDHa activity by ~50%. This was an unexpected result, as previous work with high-fat diets without n-3 fatty acid supplementation demonstrated that alterations in PDK activity were reflected inversely in PDHa activity in resting human skeletal muscle (24). However, this finding correlates well with work in rodent muscle, which demonstrated that n-3 supplementation of a high-fat diet did not fully reverse the diet-induced suppression of PDHa activity, even though PDK activity had returned to control values (8). Despite this, they observed improved insulin-stimulated glucose uptake, probably because there was decreased PDK4 isoform, which is relatively insensitive to pyruvate inhibition (8, 14). It would seem that the dissociation between maximal PDK activity and activation of the complex occurs only in a n-3-substituted fat diet, suggesting that, in the basal or resting state, the intramitochondrial effectors are not different between the two fat diets and have similar effects on the PDH complex conversion from the inactive to the active form of the enzyme. In this study, the acetyl-CoA-to-CoASH ratio was unchanged in response to both diets, and it is, therefore, unlikely that it played a role in PDHa regulation, and intramuscular pyruvate concentrations were depressed after both high-fat diets. It is possible that, at the low concentrations of muscle pyruvate observed in the basal state after the high-fat diet, the differential response in activating PDHa between N3 and HF was too minor to be observed. However, at higher muscle pyruvate concentrations induced in response to either exercise, a meal, or a hyperinsulinemic clamp, increased oxidative metabolism of CHO after the n-3-substituted diet would be apparent. Indeed, in rat muscle, the differential effect of N3 was only observed with alterations in insulin-stimulated glucose disposal and not in the basal state (8). Future work is needed to confirm that there is less PDK4 expression after an n-3-substituted diet and that the PDH complex could activate more readily after the n-3-substituted diet with insulin stimulation or exercise in human skeletal muscle.

Muscle and blood parameters and regulation of PDHa activity. Increased fat oxidation is an important regulator of PDK activity and is believed to increase PDK4 gene expression through stimulation of peroxisomal proliferator-activated receptor (PPAR)-α and possibly through PPAR-δ, which has much greater abundance in skeletal muscle than either PPAR-α or PPAR-γ (21, 34). Previously, we observed increased reliance on fat oxidation for energy at rest as early as 24 h on a high-fat diet (23). Both high-fat diets were accompanied by a threefold increase in β-hydroxybutyrate levels, suggesting that there was increased lipid oxidation in the liver in response to both diets (23, 24). Muscle glycogen concentration was unchanged by diet and was consistent throughout the study. Glucose-6-phosphate concentrations were decreased by HF and were decreased to a greater extent after N3, whereas plasma and muscle lactate decreased similarly after both diets. Taken together, these data are consistent with decreased reliance on CHO metabolism and increased reliance on fat metabolism. The increased reliance on fat fuel appeared to be similar in both conditions, and, therefore, the differential effect on increased PDK activity must be due to some modulation attributed to the differential effect of the n-3 fatty acids. One possibility is that n-3 fatty acids would behave differently in providing stimulating ligands for the muscle PPARs and, therefore, may not increase PDK gene expression to the same extent as other fatty acids (26).

Previous work has demonstrated that elevated insulin levels suppress PDK4 and PDK2 gene expression in human skeletal muscle (19). In this study, plasma insulin concentration decreased to the same extent with both high-fat diets. Recently, it has been demonstrated that n-3 polyunsaturated fatty acids prevent the defect in insulin receptor signaling caused by a regular high-fat diet (31). Therefore, the enhanced action of insulin in response to the n-3-substituted high-fat diet could be another potential mechanism for the attenuation in PDK activity in this condition.

Lipid profile. Plasma glycerol, HDL, LDL, and total cholesterol were not different between the diets. Although animal studies have documented lower total cholesterol and HDL with n-3 fatty acid supplementation, species differences have been observed and human studies have failed to demonstrate changes in these parameters (12).

After HF, plasma triglyceride concentration decreased and may have been due to an increased capacity for fat disposal after the 3-day HF. Plasma triglycerides were reduced further by ~30–40% after N3. The triglyceride-lowering effect of n-3 fatty acids has been well documented in supplementation studies of ≥2 wk in duration, although the mechanism is still under investigation (13). Decreased hepatic triglyceride secretion rates and increased chylomicron triglyceride clearance rates and lipoprotein lipase activities have been documented (11, 22). These data support data from prolonged supplementation studies and suggest that the mechanisms responsible for the hypolipidemic effect of the n-3 fatty acids are early events (3 days) and are independent of the percentage of total dietary energy coming from fat sources.

Plasma FFA concentrations increased in both high-fat diets but were not increased to the same extent after N3 compared with HF. This could result from decreased adipose tissue triglyceride breakdown, possibly through increased sensitivity to circulating insulin and suppression of adipose lipolysis. Alternatively, circulating FFAs may be cleared more rapidly by skeletal muscle, and this is supported by earlier research that provided evidence that n-3 fatty acids are oxidized to a greater extent than saturated fatty acids after a standardized meal (16).

In summary, in human skeletal muscle, the high-fat diet-induced increase in PDK activity is dependent on the composition of the dietary fat. A high-fat diet rich in n-3 fatty acids did not exhibit an increase in PDK activity to the same degree
as the regular high-fat diet, although the PDK activity was elevated compared with prediet levels. PDHα activity was decreased to a similar extent in the two high-fat diet conditions, suggesting that, in the basal state, the activity of the complex is regulated by intramitochondrial effectors, which primarily decreased concentration of muscle pyruvate caused by decreased CHO availability in both diets. Plasma triglycerides decreased with both 3-day high-fat diets but decreased to a greater extent with n-3 fatty acid substitution. These data suggest that the hypolipidemic effects of n-3 fatty acids are observed earlier than previously reported and are evident even when the diet has 75% of its total energy from fat.

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