Regulation of metabolic genes in human skeletal muscle by short-term exercise and diet manipulation

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Arkinstall, Melissa J., Rebecca J. Tunstall, David Cameron-Smith, and John A. Hawley. Regulation of metabolic genes in human skeletal muscle by short-term exercise and diet manipulation. Am J Physiol Endocrinol Metab 287:E25–E31, 2004. First published February 3, 2004; 10.1152/ajpendo.00557.2003.—Changes in dietary macronutrient intake alter muscle and blood substrate availability and are important for regulating gene expression. However, few studies have examined the effects of diet manipulation on gene expression in human skeletal muscle. The aim of this study was to quantify the extent to which altering substrate availability impacts on subsequent mRNA abundance of a subset of carbohydrate (CHO)- and fat-related genes. Seven subjects consumed either a low- (LOW; 0.7 g/kg body mass CHO) or high- (HIGH; 10 g/kg body mass CHO) CHO diet for 48 h after performing an exhaustive exercise bout to deplete muscle glycogen stores. After intervention, resting muscle and blood samples were taken. Muscle was analyzed for the gene abundances of GLUT4, glycogenin, pyruvate dehydrogenase kinase-4 (PDK-4), fatty acid translocase (FAT/CD36), carnitine palmitoyltransferase I (CPT I), hormone-sensitive lipase (HSL), β-hydroxyacyl-CoA dehydrogenase (β-HAD), and uncoupling protein-3 (UCP3), and blood samples for glucose, insulin, and free fatty acid (FFA) concentrations. Glycogen-depleting exercise and HIGH-CHO resulted in a 300% increase in muscle glycogen content (P < 0.001) relative to the LOW-CHO condition. FFA concentrations were twofold higher after LOW- vs. HIGH-CHO (P < 0.05). The exercise-diet manipulation exerted a significant effect on transcription of all carbohydrate-related genes, with an increase in GLUT4 and glycogenin mRNA abundance and a reduction in PDK-4 transcription after HIGH-CHO (all P < 0.05). FAT/CD36 (P < 0.05) and UCP3 (P < 0.01) gene transcription were increased following LOW-CHO. We conclude that 1) there was a rapid capacity for a short-term exercise and diet intervention to exert coordinated changes in the mRNA transcription of metabolic related genes, and 2) genes involved in glucose regulation are increased following a high-carbohydrate diet.

SKELETAL MUSCLE REPRESENTS the largest component of fat-free mass (FFM) in humans and is the major site of insulin-stimulated glucose disposal (10). At rest, glucose availability exerts the dominant influence on the mix of oxidized fuels in healthy individuals (33). However, within 10–12 h of fasting, fatty acid (FA) oxidation accounts for ~80% of resting energy requirements (9). Manipulation of dietary macronutrient content is associated with marked changes in substrate stores, metabolic flux, and subsequent fuel oxidation (5, 8, 17, 32).

Changes in dietary intake alter the concentration of bloodborne nutrients and hormones and, via substrate availability, regulate the short-term macronutrient oxidative and storage profile of skeletal muscle. Perturbations in muscle and blood substrates alter the uptake and flux of these fuel-specific intermediates within related metabolic pathways. This immediate response serves to redirect enzymatic processes involved in substrate metabolism and the subsequent concentration of particular proteins critical for metabolic pathway function. Altering substrate availability impacts not only restoring energy metabolism but also regulatory processes underlying gene expression (25, 27). To bring about such modifications, a number of highly coordinated processes occur, including gene transcription, RNA transport from the nucleus, protein synthesis and, in some cases, posttranslational modification of the protein. However, the initiation of gene transcription is strongly related to changes in dietary intake and composition (20).

To date, there have been few investigations to determine the responsiveness and coordination of changes in mRNA abundances following alterations in diet. A previous study from our laboratory supports a role for a short-term (5-day) low-carbohydrate, high-fat diet to upregulate lipid-related genes in skeletal muscle (6). Accordingly, one might predict that this relationship is reciprocal, with high carbohydrate eliciting greater levels of mRNA abundance of carbohydrate-related genes and a suppression of lipid-related genes. However, the coordinated control of the specific mRNA levels involved in key regulatory steps in the oxidative and storage pathways for carbohydrate and fat in response to dietary alterations in human skeletal muscle is yet to be investigated. Accordingly, the aim of the present study was to quantify the extent to which altering muscle and blood substrate availability impacts on the subsequent transcription of metabolic genes. We hypothesized that high carbohydrate availability (i.e., exhaustive glycogen-depleting exercise followed by a high-carbohydrate diet) would increase transcription of carbohydrate-related genes while simultaneously decreasing the transcription of genes underlying lipid metabolism.

METHODS

Subjects and Experimental Design

Seven male subjects who were moderately trained in cycling exercise participated as subjects in this study, which was approved by the Human Research Ethics Committee of RMIT University. The experimental design required subjects to perform two exercise-diet interventions [low-carbohydrate (LOW-CHO) vs. high-carbohydrate (HIGH-CHO)] in a random order, separated by 7 days. The subjects’ age, body mass (BM), and peak O2 uptake (VO2peak) were 33 ± 5 yr.

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the power output was lowered to 85, 75, and 65% of VO2 peak. This high-intensity, low-intensity regimen was maintained until subjects were unable to complete 2 min of exercise at 95% of VO2 peak. At this time, the power output was lowered to 85, 75, and finally 65% of VO2 peak while the same work-to-rest interval was maintained. Exercise was terminated when subjects could not complete 2 min of cycling at 65% of VO2 peak.

During the 48-h period immediately following the exercise-depletion protocol, subjects consumed either a LOW-CHO (0.7 g/kg BM of CHO, 4.4 g/kg BM of fat, 4 g/kg BM of protein) or HIGH-CHO (10 g/kg BM of CHO, 1 g/kg BM of fat, 1.9 g/kg BM of protein) isoenergetic (~235 KJ/kg BM) diet. All meals and snacks were provided to subjects to maximize dietary compliance. The morning after completion of a 48-h dietary intervention, subjects reported to the laboratory between 0700 and 0800 in a 10- to 12-h overnight-fasted state. A resting venous blood sample (10 ml) was collected via venipuncture into the antecubital space of one arm. Local anesthesia (2–3 ml of 1% xylocaine (Lignocaine)) was then administered to the skin, subcutaneous tissue, and fascia of the vastus lateralis muscle in preparation for a resting-muscle biopsy. Muscle (~100–150 mg) was removed using a UCH biopsy needle (Popper, NY), with suction applied, and immediately frozen in liquid nitrogen. Samples were stored at −80°C until subsequent analysis.

**Statistical Analysis**

Differences between dietary treatments for skeletal muscle glycogen content and resting blood substrate levels (plasma glucose, insulin, and FFA concentrations) were analyzed using paired-sample t-tests. The mRNA data for each dietary intervention were expressed in arbitrary units after normalization relative to β-actin and analyzed using paired-sample t-tests and effect size statistics. Statistical significance for these measures was established at the level of P < 0.05. Lacking any information on the smallest substantial changes in gene transcription, we converted the observed differences in mRNA abundance (arbitrary units) between dietary treatments to Cohen effect sizes by calculating the mean difference between groups divided by the average of the groups’ standard deviation. We then interpreted the magnitude of the effect size by using conventional threshold values of 0.2 as the smallest effect, 0.5 as a moderate effect, and 0.8 as a large effect size (7). All values are expressed as means ± SE.

**RESULTS**

Resting Muscle Glycogen, Plasma Glucose, Insulin, and FFA Concentrations

Figure 1 displays the effects of short-term exercise and diet manipulation on resting muscle glycogen content. As expected, consumption of a HIGH-CHO diet after glycogen-depleting exercise resulted in significantly higher (~300%) muscle glycogen content compared with concentrations observed following a LOW-CHO diet (570 ± 103 vs. 171 ± 40 mmol glucose/
Table 1. Gene primer sequences

<table>
<thead>
<tr>
<th>Gene</th>
<th>GenBank Accession No.</th>
<th>Reverse: 5'-</th>
<th>Forward: 5'-</th>
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<tr>
<td>β-Actin</td>
<td>X00351</td>
<td>-TGG GTG TGG</td>
<td>CGC AGA GAA CAC AGC</td>
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<tr>
<td>GLUT4</td>
<td>NM_001042</td>
<td>-3</td>
<td>GAG GAG ATT ACT-3'</td>
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<tr>
<td>Glycogenin</td>
<td>AH00714</td>
<td>-3</td>
<td>TGG CAC AGG CAG GGT</td>
</tr>
<tr>
<td>PDK-4</td>
<td>NM_002612</td>
<td>-3</td>
<td>TGG CTC CCG TTC TG</td>
</tr>
<tr>
<td>FAT/CD36</td>
<td>L06850</td>
<td>-3</td>
<td>CTC GAG GAA CCA AAG</td>
</tr>
<tr>
<td>CPT I</td>
<td>Y08683</td>
<td>-3</td>
<td>AGT TTT CCT GAA CCA</td>
</tr>
<tr>
<td>HSL</td>
<td>L11706</td>
<td>-3</td>
<td>CGA TTT TCT GAA CCA</td>
</tr>
<tr>
<td>β-HAD</td>
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<td>-3</td>
<td>CGA TTT TCT GAA CCA</td>
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<tr>
<td>UCP3</td>
<td>XM_055241</td>
<td>-3</td>
<td>CCT CTT GAT GTT CCG</td>
</tr>
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</table>

PDK-4, pyruvate dehydrogenase kinase-4; FAT/CD36, fatty acid translocase; CPT I, carnitine palmitoyltransferase I; HSL, hormone-sensitive lipase; β-HAD, β-hydroxyacyl-CoA dehydrogenase; UCP3, uncoupling binding protein-3.

The overall effect of short-term exercise-diet manipulation on mRNA abundance of metabolic genes: GLUT4, glycogenin, pyruvate dehydrogenase kinase (PDK-4), fatty acid translocase (FAT/CD36), carnitine palmitoyltransferase I (CPT I), hormone-sensitive lipase (HSL), β-hydroxyacyl-CoA dehydrogenase (β-HAD), and uncoupling binding protein-3 (UCP3) is displayed in Table 3, whereas the fold changes in mRNA abundance for these genes are displayed in Figs. 2 and 3.

**DISCUSSION**

Several experimental models have been used to alter substrate availability and examine the effects of these perturbations on genes underlying fuel utilization. Periods of fasting lasting up to 72 h have been employed to investigate the responses of genes aligned with fat-regulatory processes (25, 27, 34). Although this approach markedly influences circul-
ing metabolites (i.e., increases FFA and glucagon while decreasing glucose and insulin concentrations), muscle substrate availability remains largely unaffected. On the other hand, exercise has often been employed as a means to reduce endogenous fuel stores (i.e., muscle glycogen) to determine the level of activation of metabolic genes that encode for various regulatory proteins in the immediate (1–4 h) postexercise recovery period (21, 26, 35). Interpretations of the results from this latter model are somewhat complicated because contraction per se has an independent effect on gene transcription (for review see Ref. 14).

In the present study, we used an exercise protocol that initially reduced endogenous glycogen stores and then fed rested subjects either a low- or high-carbohydrate diet for 2 days to manipulate subsequent muscle and blood substrate availability. Our first major finding was that short-term (48-h) alteration of dietary carbohydrate intake after glycogen-depleting exercise resulted in marked alterations in the expression of metabolic genes within human skeletal muscle. This finding demonstrates the rapid capacity of whole body substrate availability to modify the abundances of genes necessary to adapt and maintain the oxidative and storage profile to accommodate the changed nutrient supply. The second novel finding was that a subset of carbohydrate-related genes demonstrated greater responsiveness to short-term exercise and diet manipulation than a subset of lipid genes: all three carbohydrate-related genes under investigation (GLUT4, glycogenin, and PDK-4) exhibited large alterations in their mRNA abundances as a consequence of altered whole body substrate availability.

Although mRNA abundance is a strong determinant of protein synthesis, this relationship is neither simple nor linear (13). The half-lives of many mRNAs are relatively short compared with their target proteins, with transcription gene activation sometimes occurring before sustained and measurable increases in protein (15). Although we acknowledge that the extent to which a protein might be modified in response to an adaptive stimulus cannot be predicted from an increase in mRNA, we (6) and others (25) have previously reported that increased abundance of genes encoding for substrate metabolism is accompanied by a concomitant increase in the cellular content of the transcribed protein. More to the point, the dietary intervention employed in the present investigation lasted for 2 days, with subsequent changes in mRNA likely to represent new “steady-state” levels rather than a transient activation of gene transcription (23).

To the best of our knowledge, this is the first study to measure GLUT4 mRNA in humans in response to short-term exercise and dietary manipulation, although GLUT4 gene expression (21) and protein content (28) have previously been reported to increase immediately after a single bout of moderate-intensity exercise. After glycogen-depleting exercise and 2 days of a diet high in carbohydrate, resting muscle glycogen content was elevated ~300% above concentrations following a low-carbohydrate diet, with

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Table 3. Effect of short-term exercise-diet manipulation on mRNA abundance of metabolically related genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Dietary Carbohydrate</th>
<th>Effect Size</th>
<th>Effect Magnitude</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>GLUT4</td>
<td>0.009±0.003</td>
<td>0.023±0.006</td>
<td>1.2</td>
</tr>
<tr>
<td>Glycogenin</td>
<td>0.819±0.164</td>
<td>1.349±0.167</td>
<td>1.5</td>
</tr>
<tr>
<td>PDK-4</td>
<td>0.068±0.025</td>
<td>0.021±0.007</td>
<td>1.1</td>
</tr>
<tr>
<td>FAT/CD36</td>
<td>0.367±0.051</td>
<td>0.196±0.044</td>
<td>1.4</td>
</tr>
<tr>
<td>CPT I</td>
<td>0.151±0.023</td>
<td>0.137±0.028</td>
<td>0.2</td>
</tr>
<tr>
<td>HSL</td>
<td>0.023±0.014</td>
<td>0.009±0.004</td>
<td>0.6</td>
</tr>
<tr>
<td>β-HAD</td>
<td>1.67±0.248</td>
<td>1.268±0.242</td>
<td>0.6</td>
</tr>
<tr>
<td>UCP3</td>
<td>0.214±0.045</td>
<td>0.137±0.042</td>
<td>0.7</td>
</tr>
</tbody>
</table>

All values expressed as means ± SE are arbitrary units; n = 7.

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Fig. 2. Effect of short-term exercise-diet manipulation on skeletal muscle mRNA abundance of GLUT4 (A), glycogenin (B), and pyruvate dehydrogenase kinase-4 (PDK-4; C) expressed as fold change relative to HIGH-CHO trial. Values expressed as means ± SE; n = 7. *P < 0.05.
GLUT4 mRNA abundance also substantially higher than values measured after the low-carbohydrate diet. A similar trend was observed for glycogenin, the primer for glycogen synthesis in muscle and liver and a potential determinant of maximum glycogen storage capacity (24). The coordinated increase in GLUT4 and glycogenin mRNA abundance might be expected to enhance protein expression and thus facilitate muscle glycogen storage.

Fig. 3. Effect of short-term exercise-diet manipulation on skeletal muscle mRNA abundance of fatty acid translocase (FAT/CD36; A), carnitine palmitoyltransferase I (CPT I; B), hormone-sensitive lipase (HSL; C), β-hydroxyacyl-CoA dehydrogenase (β-HAD; D), and uncoupling binding protein-3 (UCP3; E) expressed as fold change relative to HIGH-CHO trial. Values expressed as means ± SE; n = 7. *P < 0.05, **P < 0.01.
Induction of PDK-4 is a primary means by which glucose oxidation is suppressed in skeletal muscle during periods of low carbohydrate availability, largely as a means to conserve limited glucose stores. The transcriptional activation of PDK-4 has previously been determined after 3 days of a low-carbohydrate, high-fat diet (25) and also after prolonged fasting (27). In agreement with Peters (25), we show that substrate availability had a large effect on PDK-4 mRNA. In the present study, intake of a diet low in carbohydrate induced a threefold increase in PDK-4 compared with the high-carbohydrate diet. Peters reported a significant increase in both PDK-4 mRNA and protein after 1 day of a low-carbohydrate diet. In that study, the induction of mRNA correlated with a decrease in the resting respiratory exchange ratio, indicating a shift to greater fat metabolism (25). Interestingly, neither PDK-4 mRNA nor protein was increased further after an additional 2 days of a low-carbohydrate diet despite progressive increases in plasma FFA levels (25). Although muscle glycogen was not determined in that investigation (25), little variation in content would be expected, as muscle glycogen levels can be maintained for at least 3 days in subjects who do not participate in vigorous exercise (12).

In the present study, the intake of a low-carbohydrate diet following glycogen-depleting exercise was also associated with a twofold elevation in FFA concentrations. Accordingly, we are unable to ascertain whether muscle energy stores per se and/or the prevailing concentrations of circulating metabolites exerted the predominant effect on PDK-4 transcription. However, Pilegaard et al. (26) have previously reported that the transcriptional activation of PDK-4 in response to a bout of exercise is enhanced in skeletal muscle when starting muscle glycogen content is low. Taken collectively, these observations suggest that the transient transcriptional activation of PDK-4 may be coordinately linked to signaling mechanisms that are sensitive to muscle glycogen content and/or FFA availability.

Although the subset of glucoregulatory genes determined in the present study responded in a coordinated manner to changes in whole body carbohydrate availability, the fat genes under investigation were selectively altered in response to exercise-dietary intervention. Several genes specific to lipid metabolism were chosen for investigation as indexes of putative fatty acid transport/uptake (FAT/CD36), mitochondrial fatty acid transporters (CPT I), and substrate-level oxidative enzymes (β-HAD and HSL). Whereas 2 days of a low-carbohydrate diet following glycogen-depleting exercise induced only small to moderate effects on CPT I, HSL, and β-HAD mRNA, the transcription of FAT/CD36 and UCP3 was significantly increased after this intervention. We have previously reported that 5 days of a low-carbohydrate, high-fat diet failed to increase the abundance of CPT I and plasma membrane fatty acid-binding protein mRNA expression, while markedly increasing FAT/CD36 mRNA and protein (6). However, in that study, well-trained athletes maintained a vigorous exercise program while consuming the diet; the subjects’ high fitness levels and intense training are likely to have influenced the extent and degree of gene expression observed (6). FAT/CD36 is present in tissues with a high FA demand and turnover (3), and although its contribution to skeletal muscle FA uptake is not well characterized, it does appear to have a role as a regulator of intracellular FA uptake during periods of increased supply and/or demand (2).

UCP3 is a member of a family of mitochondrial carrier proteins with a number of hypothetical roles, including contributing to the uncoupling of respiration (4), FA export from the mitochondrial matrix (19), or reactive oxygen species regulation (31). Previous studies have reported an induction of UCP3 mRNA in response to fasting (27, 34) and high-fat feeding (18, 30). The results from the present study confirm and extend previously observed diet-induced alterations in UCP3 gene expression (30) to show that UCP3 mRNA levels are moderately elevated (twofold) within 48 h of switching to a low-carbohydrate diet.

In conclusion, this is the first study to quantify the extent to which short-term exercise and diet manipulation impact the subsequent transcription of a subset of both carbohydrate- and fat-related metabolic genes. We found a rapid capacity for alterations in dietary macronutrient content to modulate changes in the expression of mRNA following a bout of glycogen-depleting exercise. However, substrate availability only exerted large and uniform effects on a subset of glucoregulatory genes (GLUT4, glycogenin, and PDK-4). The transcription of genes encoding for lipid transport and oxidation in skeletal muscle varied, with only FAT/CD36 and UCP3 exhibiting significant increases in mRNA abundance in the face of low-carbohydrate availability.

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