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 binding 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 blood-borne 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 resting 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, respectively.

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80.3 ± 9.5 kg, and 4.64 ± 0.62 l/min, respectively (values are means ± SE). As muscle and blood samples were taken, all procedures and risks were carefully explained to each subject before their written consent was obtained.

Preliminary Testing

All subjects performed an incremental test to volitional fatigue (i.e., maximal test) on a Lode cycle ergometer ( Groningen, The Netherlands) under standard laboratory conditions (21–22°C, 40–50% relative humidity) for the determination of VO₂ peak. The maximal test has been described in detail previously (16). The results of the maximal test were used to determine the work rates to be employed in the exercise-depletion protocol.

Exercise Depletion Protocol and Dietary Intervention

Immediately before each dietary intervention, muscle glycogen content was depleted by means of exhaustive cycle exercise. The exercise-depletion protocol has been described in detail previously (36). Briefly, subjects reported to the laboratory and after a 5-min warm-up, commenced cycling for 2 min at ~95% VO₂ peak, followed immediately by 2 min of cycling at ~55% 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 50% 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.

Analytical Procedures

Blood substrates and hormone concentrations. Five milliliters of whole blood were placed into a tube containing fluoride EDTA, mixed, and spun in a centrifuge at 4,000 rpm for 8 min at 0°C. The plasma was later analyzed in duplicate for glucose concentration by means of an automated glucose/lactate analyzer (YSI 2300 STAT PLUS; Yellow Springs Instruments, Yellow Springs, OH). Four milliliters of whole blood were placed into a tube containing lithium heparin, mixed, and spun in a centrifuge (as above). The plasma was stored at −80°C for later analysis (in duplicate) of plasma insulin concentration by radioimmunoassay (Phadeseph, Insulin RIA; Pharmacia & Upjohn Diagnostics, Uppsala, Sweden). Blood (3 ml) for the determination of plasma free fatty acid (FFA) concentration was placed in tubes containing ethylene glycol-bis[β-aminoethyl ether]-N,N,N',N'-tetraacetic acid and reduced glutathione and spun in a centrifuge at 0°C for 15 min at 4,000 rpm. The supernatant was then stored at −80°C until analysis. Plasma FFA concentration was measured by an enzymatic colorimetric method (NEFA C code 279–75409; Wako, Tokyo, Japan).

Muscle analyses. A small piece of frozen muscle (10–20 mg) was removed under liquid nitrogen for the quantification of mRNA. At the same time, an additional portion of frozen muscle (10–20 mg) was chipped, freeze-dried, and dissected of all visible blood, connective tissue, and fat, and powdered for subsequent determination of muscle glycogen concentration in duplicate as glucose residues after hydrolysis in 2 M HCl at 100°C for 2 h (22).

Total RNA isolation and reverse transcription. Total RNA from ~10 mg of wet muscle was isolated using FastRNA Kit-Green (BIO 101, Vista, CA) protocol and reagents. Total RNA concentration was determined spectrophotometrically at 260 nm. First-strand cDNA was generated from 0.5 μg of RNA by use of AMV RT (Promega, Madison, WI) as described previously (37). The cDNA was stored at −20°C for subsequent analysis.

mRNA quantification. A real-time polymerase chain reaction (PCR) mix of 0.5X SYBR green PCR master mix (Applied Biosystems, Foster City, CA), forward and reverse primers (3 μM), and 12 ng of cDNA was run for 40 cycles of PCR in a volume of 20 μl. Because SYBR green indiscriminately binds to double-stranded DNA and other products such as primer dimers, the samples were subjected to a heat dissociation protocol after the final cycle of PCR to ensure that only one product was detected. Heat dissociation of oligonucleotides detects differences in melting temperature and produces a single dissociation peak for each nucleotide within a 2°C difference in melting temperature (29).

Real-time RT-PCR analysis. Primers were designed using Primer Express software package version 1.0 (Applied Biosystems) from gene sequences obtained from GenBank. A BLAST (1) search for each primer confirmed homologous binding to the desired mRNA of human skeletal muscle. Primer sequences are shown in Table 1.

Quantification of mRNA expression was performed in duplicate by real-time RT-PCR using the ABI PRISM 7500 sequence detection system (Applied Biosystems) as described previously (11). Fluorescent emission data were captured and mRNA levels quantitated using the critical threshold value. To compensate for variations in input RNA amounts and efficiency of reverse transcription, β-actin mRNA was quantitated, and results were normalized to these values as described previously (35). Each gene was analyzed with the incorporation of a negative control template.

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 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 glucosyl
units/kg dry mass, \( P < 0.001 \)). Table 2 displays resting concentrations of plasma glucose, insulin, and FFA following the exercise-diet interventions. Plasma glucose (4.9 ± 0.3 vs. 4.6 ± 0.4 mmol/l) and insulin concentrations (5.9 ± 1.6 vs. 4.9 ± 1.5 μU/ml) were similar after both LOW- and HIGH-CHO conditions, respectively. However, plasma FFA levels were approximately twofold higher after the LOW-compared with the HIGH-CHO trial (0.43 ± 0.17 vs. 0.24 ± 0.10 mmol/l, \( P < 0.01 \)).

**Gene Transcription**

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 Figs. 2 and 3, whereas the fold changes in mRNA abundance for these genes are displayed in Figs. 2 and 3.

The exercise-diet manipulation exerted a large effect (>0.8 units; Table 3) on the transcription of all carbohydrate-related genes, with an increase in GLUT4 (\( P < 0.05 \)) and glycogenin (\( P < 0.05 \)) mRNA abundance and a reduction in PDK-4 (\( P < 0.05 \)) transcription after HIGH-CHO. This corresponded to a ~2.5- and ~1.6-fold increase in mRNA abundance for GLUT4 and glycogenin, respectively, and a ~0.3-fold reduction in PDK-4 mRNA, following the HIGH-CHO compared with the LOW-CHO condition (Fig. 2).

The results of altering dietary carbohydrate availability after glycogen-depleting exercise on the transcriptional regulation of specific lipid-based genes (FAT/CD36, CPT I, HSL, β-HAD, and UCP3) ranged from small (0.2–0.5) to large (>0.8) effect sizes (Table 3). The largest effects of the short-term exercise-diet manipulation on mRNA abundance were observed for FAT/CD36 (1.4). A significant increase in the gene transcription of both FAT/CD36 (\( P < 0.05 \)) and UCP3 (\( P < 0.01 \)) was observed following the LOW-CHO condition, which corresponded to a fold change of ~3.0 and ~1.8, respectively (Fig. 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 circulat-
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).

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 Effect Size</th>
<th>Effect Magnitude</th>
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<tbody>
<tr>
<td>GLUT4</td>
<td>Low 0.009±0.003</td>
<td>0.023±0.006</td>
</tr>
<tr>
<td>Glycogenin</td>
<td>High 0.819±0.164</td>
<td>1.349±0.167</td>
</tr>
<tr>
<td>PDK-4</td>
<td>Low 0.068±0.025</td>
<td>0.021±0.007</td>
</tr>
<tr>
<td>FAT/CD36</td>
<td>High 0.367±0.051</td>
<td>0.196±0.044</td>
</tr>
<tr>
<td>CPT 1</td>
<td>Low 0.151±0.023</td>
<td>0.137±0.028</td>
</tr>
<tr>
<td>HSL</td>
<td>High 0.023±0.014</td>
<td>0.009±0.004</td>
</tr>
<tr>
<td>β-HAD</td>
<td>Low 1.67±0.248</td>
<td>1.268±0.242</td>
</tr>
<tr>
<td>UCP3</td>
<td>High 0.214±0.045</td>
<td>0.137±0.042</td>
</tr>
</tbody>
</table>

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

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...
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 after 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 mitochondrion 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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REFERENCES

10. DeFronzo RA, Jacob E, Jequier E, Maeder E, Wahren J, and Felber JP. The effect of insulin on the disposal of intravenous glucose: results


