Effect of carbohydrate ingestion on exercise-induced alterations in metabolic gene expression

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Submitted 17 February 2005; accepted in final form 30 May 2005


SKELETAL MUSCLE POSSESES a high degree of plasticity and can adapt to both the physical and metabolic challenges it faces. The molecular adaptations underpinning the plasticity of skeletal muscle include changes in the expression of many metabolic genes. It is thought that the cumulative effects of transient changes in gene expression mediate adaptations to chronic stimuli. An acute bout of exercise is sufficient to increase the expression of a variety of metabolic genes, such as GLUT4, pyruvate dehydrogenase kinase 4 (PDK-4), uncoupling protein-3 (UCP3), and peroxisome proliferator-activated receptor-γ coactivator 1 (PGC-1). Reducing muscle glycogen levels before exercise potentiates the effect of exercise on many genes. Similarly, altered substrate availability induces transcription of many of these genes. The purpose of this study was to determine whether glucose ingestion attenuates the exercise-induced increase in a variety of exercise-responsive genes. Six male subjects (28 ± 7 yr; 83 ± 3 kg; peak pulmonary oxygen uptake = 46 ± 6 ml·kg⁻¹·min⁻¹) performed 60 min of cycling at 74 ± 2% of peak pulmonary oxygen uptake on two separate occasions. On one occasion, subjects ingested a 6% carbohydrate drink. On the other occasion, subjects ingested an equal volume of a sweet placebo. Muscle samples were obtained from vastus lateralis at rest, immediately after exercise, and 3 h after exercise. PDK-4, UCP3, PGC-1, and GLUT4 mRNA levels were measured on these samples using real-time RT-PCR. Glucose ingestion attenuated (P < 0.05) the exercise-induced increase in PDK-4 and UCP3 mRNA. A similar trend (P = 0.09) was observed for GLUT4 mRNA. In contrast, PGC-1 mRNA increased following exercise to the same extent in both conditions. These data suggest that glucose availability can modulate the effect of exercise on metabolic gene expression.

transcription; skeletal muscle

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Blood Analyses

Blood was sampled from an antecubital vein, and plasma was immediately separated by centrifugation and stored for later analysis. Plasma glucose was determined by using an EML 105 analyzer (Radiometer, Copenhagen, Denmark). Plasma insulin was analyzed by using a Phadesep kit (Pharmacia, Uppsala, Sweden). Plasma nonesterified fatty acid concentration was measured using a Wako NEFA-c kit (Wako Chemical, Neuss, Germany).

Muscle Biopsies

Muscle samples were obtained from the vastus lateralis before, immediately following, and 3 h after exercise using the percutaneous needle biopsy technique with suction (7). Muscle samples were immediately frozen in liquid nitrogen and stored for later analysis.

Glycogen Determination

Approximately 20 mg of muscle were freeze dried and dissected of connective tissue before being hydrolyzed in 1 M HCl. Glycogen concentration was determined by using an enzymatic technique with fluorometric detection, as previously described (21).

Real-time RT-PCR

Total RNA was extracted from ~10 mg of muscle using the acid guanidium thiocyanate-phenol-chloroform extraction technique with modifications (FastRNA Kit-Green; Bio101, Carlsbad, CA). Oligo (dT) single-stranded cDNA was synthesized by using avian myeloblastosis virus Reverse Transcriptase Kit (Promega A3500, Madison, WI). Forward and reverse primers complimentary to the human GLUT4, PDK-4, PGC-1, and UCP3 genes (Table 1) were designed by using Primer Express software (PE Biosystems, Foster City, CA). Real-time RT-PCR was performed by using the GeneAmp 5700 sequence detector and software (PE Applied Biosystems, Scoresby, Victoria, Australia). Changes in gene expression were normalized to the housekeeping gene cyclophilin.

Table 1. Real-time RT-PCR primer sequences and Genbank Accession numbers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genbank Accession Number</th>
<th>Forward Primer (5’-3’)</th>
<th>Reverse Primer (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUT4</td>
<td>NM_001042</td>
<td>CTTCATCATGGGAGATGGTTT</td>
<td>AGGACGCCAAATAGAAGAAG</td>
</tr>
<tr>
<td>PDK-4</td>
<td>NM_002612</td>
<td>CCGAGAAGGAGCAGCATT</td>
<td>GCATTGGCTGAGCAAACTGAGTA</td>
</tr>
<tr>
<td>PGC-1</td>
<td>AF_09330</td>
<td>CAGGCGGAAACCAAGACTTATCTCT</td>
<td>CAGACCTTAAGGTGGAGCTATAGTC</td>
</tr>
<tr>
<td>UCP3</td>
<td>XM_055241</td>
<td>CGTGGTAGATTCTGAACTCATTG</td>
<td>CGGGAGGTCCCGPAACATCTG</td>
</tr>
<tr>
<td>Cyclophilin</td>
<td>XM_004890</td>
<td>CCCGACTTTTCTTGTGCAGAT</td>
<td>CCAAGTGCCTCAGGACGCAAA</td>
</tr>
</tbody>
</table>

PDK-4, pyruvate dehydrogenase kinase 4; PGC-1, peroxisome proliferator-activated receptor-γ coactivator 1; UCP3, uncoupling protein-3.

Statistics

All values reported are the means ± SE. Gene expression data are represented with resting samples assigned the arbitrary value of 1.0 and postexercise samples expressed relative to rest. Mean values for the two experimental conditions were compared by using two-way repeated-measures ANOVA (Sigma Stat 3.0). Specific differences were analyzed by using Newman Keuls post hoc test, with a significance level of 0.05.

RESULTS

Blood Analyses

Glucose. No differences were observed in plasma glucose between Con and Glu (Table 2).

Insulin. Plasma insulin was higher in Glu compared with Con (treatment effect, P < 0.05; Table 2).

Free fatty acids. Plasma free fatty acids were significantly elevated (P < 0.05) after 120 min in Con (Table 2), but they were significantly lower in Glu throughout recovery.

Muscle Glycogen

From resting levels (682 ± 82 mmol/kg dry wt Con, 517 ± 73 mmol/kg dry wt Glu), muscle glycogen decreased (P < 0.05) in both groups immediately following exercise (224 ± 58 mmol/kg dry wt Con, 218 ± 58 mmol/kg dry wt Glu) and remained lower (P < 0.05) 3 h after exercise (213 ± 37 mmol/kg dry wt Con, 294 ± 30 mmol/kg dry wt Glu). No differences were observed between Con and Glu.

Gene Expression

GLUT4 mRNA. Exercise resulted in an approximate twofold elevation in GLUT4 mRNA that persisted 3 h after exercise,
whereas glucose supplementation tended \((P = 0.09)\) to attenuate this response (Fig. 1A).

**PDK-4 mRNA.** PDK-4 mRNA was unchanged immediately after exercise in both Con and Glu. Three hours after exercise, PDK-4 mRNA had increased 23-fold \((P < 0.05)\) in Con, while there was no change in Glu (Fig. 1B).

**PGC-1 mRNA.** PGC-1 mRNA was unchanged in both conditions immediately following exercise, but increased threefold in Con \((P < 0.05)\) and 3.8-fold \((P < 0.05)\) in Glu 3 h after exercise (Fig. 2A). No differences were observed between Con and Glu.

**UCP3 mRNA.** Immediately after exercise, UCP3 mRNA was unchanged in both Glu and Con. Three hours after exercise, UCP3 mRNA was increased sevenfold \((P < 0.05)\) in Con, whereas there was no change in Glu (Fig. 2B).

**Cyclophilin.** The expression of the housekeeping gene cyclophilin did not change \((P = 0.96)\) in either condition (data not shown), as assessed by two-way ANOVA on the \(2^{-\Delta\Delta C_t}\) values, which convert the log scale cycle threshold \((C_T)\) values to the linear form.

**DISCUSSION**

Exercise has previously been shown to increase the transcription and expression of a variety of metabolic genes (3, 24), including PDK-4, PGC-1, and UCP3. Furthermore, studies have found that altered substrate availability (17, 22, 32) and reduced muscle glycogen levels before exercise (23) can also influence the expression of some of these genes, suggesting that the availability of substrate could be modulating the effect
of exercise. As such, the purpose of this study was to examine the effect of glucose ingestion on GLUT4, PDK-4, PGC-1, and UCP3 gene expression in humans following exercise. It was found that glucose ingestion attenuated the exercise-induced increase in PDK-4 and UCP3 mRNA expression. Similar trends were observed with GLUT4 mRNA; however, these were not statistically significant. In contrast, PGC-1 expression was increased in both conditions. These results do not appear to be influenced by muscle glycogen, as no differences in muscle glycogen were found between groups. It has previously been shown that glucose ingestion attenuates the exercise-induced increase in UCP3 gene expression in humans (27) and GLUT4 gene expression in rodents (13). However, data from the present study suggest that alterations in substrate availability can also modulate the exercise-induced increase in PDK-4 mRNA, whereas PGC-1 mRNA expression is increased by exercise, independent of changes in substrate.

Oxidative glucose metabolism is regulated at the rate-limiting and irreversible step where pyruvate is converted to acetyl-CoA. The enzyme catalyzing this reaction, PDH, is regulated by a number of factors, including the PDKs. PDK-4 is able to phosphorylate the αE1 subunit of PDH, resulting in reduced PDH activity and oxidative glucose metabolism (4). Increases in PDK-4 transcription have previously been observed in the period following exercise in humans, with this response further enhanced when muscle glycogen levels were low (23). It has been hypothesized that the induction of PDK-4 gene expression is a means of shifting metabolism toward greater lipid oxidation and conserving glucose when glucose availability is compromised (24). Supporting this hypothesis is an observation that increases in PDK-4 mRNA correlate strongly with inactivation of PDH (30). In the present study, exercise alone resulted in a 23-fold increase in PDK-4 mRNA 3 h following exercise. However, when subjects were supplemented with glucose throughout the exercise and recovery period, PDK-4 mRNA abundance was unchanged from resting levels, suggesting that alterations in substrate availability can influence PDK-4 expression. This is supported by evidence that PDK-4 expression is suppressed by insulin (15) and induced by fasting, possibly through enhanced rates of lipid oxidation (22). Consistent with these data, glucose supplementation in the present study resulted in reduced plasma free fatty acid and increased plasma insulin concentrations compared with the control trial. These factors could describe a potential mechanism by which PDK-4 expression was suppressed following glucose supplementation. Indeed, it is proposed that PDK-4 expression is regulated by PPAR-α (11) and the forkhead homologue in rhabdomyosarcoma (FKHR) transcriptional regulator (8). PPAR-α requires fatty acid ligands for transcriptional activation, while FKHR, a member of the FOXO family of transcription factors, is phosphorylated and inactivated by Akt following insulin treatment (26). While not assessed in the present study, it could be possible that glucose supplementation during exercise differentially regulates PPAR-α and FKHR, which, in turn, influences PDK-4 gene expression.

Exercise resulted in a sevenfold elevation in UCP3 mRNA 3 h after exercise. However, when subjects were supplemented with glucose, UCP3 mRNA was unchanged from resting levels. This is consistent with previous research showing that glucose ingestion suppressed UCP3 mRNA immediately after 4 h of exercise (27). Despite much research, the exact role of UCP3 in muscle is unclear. The paradoxical observation that UCP3 expression is enhanced with exercise and starvation, both conditions that demand tightly controlled metabolic coupling (10), suggests that UCP3 might not be involved in uncoupling activity. Following observations that circulating free fatty acids are able to induce the expression of UCP3 (33), it has been suggested that UCP3 could be involved in regulating lipid metabolism. Recent studies propose that UCP3 is an exporter of fatty acid anions from the mitochondria (10) and that UCP3 expression is regulated by the fatty acid-sensitive PPAR-δ (29). By supplementing subjects with glucose in the present study, the exercise-induced rise in circulating free fatty acids was reduced. This could, in turn, remove the stimulus for increased UCP3 gene expression. While this was not directly assessed in this study, it potentially describes the mechanism by which glucose supplementation was able to attenuate the increase in UCP3 mRNA following exercise.

While the exercise-induced increases in PDK-4 and UCP3 mRNA were attenuated with glucose supplementation, PGC-1 mRNA was increased 3 h after exercise in both experimental conditions. This suggests that the exercise-induced increase in PGC-1 mRNA is unaltered by changes in glucose availability. This is consistent with the function of PGC-1, which is a transcriptional coactivator of a variety of transcription factors regulating genes involved in mitochondrial biogenesis and fiber-type switching (16). As such, unlike the other genes analyzed in this study, PGC-1 appears to be more critical for the longer term adaptations to exercise rather than the acute regulation of metabolism during and immediately after exercise. Consequently, it seems logical that substrate availability would not acutely influence the expression of PGC-1. This is reinforced when examining the PGC-1 promoter, which contains binding domains for many calcium-sensitive transcription factors (9). Furthermore, raising calcium concentrations in L6 myotubes increases PGC-1 expression (20). Calcium transients associated with excitation-contraction coupling are thought to be a potent regulator of both acute metabolism and gene expression during exercise and would be insensitive to alterations in substrate availability during exercise, possibly explaining the results from the present study.

Previous studies have identified that exercise induces GLUT4 gene transcription (12, 18) and that this response can be attenuated with glucose ingestion (13). In the present study, exercise resulted in a twofold elevation of GLUT4 mRNA immediately after exercise that persisted for 3 h after exercise. When subjects were supplemented with glucose, GLUT4 mRNA following exercise was similar to basal levels. While these trends (P = 0.09) were not statistically significant and are likely a type II error, they are consistent with studies examining the effects of acute exercise (12, 18) and increased glucose availability (13) on skeletal muscle GLUT4 gene expression. Various studies have presented conflicting data on the influence of insulin on the in vivo regulation of the GLUT4 gene in skeletal muscle. Andersen et al. (1) found that insulin infusion in humans increased skeletal muscle GLUT4 mRNA, whereas others have found that insulin does not change (25) or decreases (2) skeletal muscle GLUT4 mRNA. However, a recent study has found that insulin is essential for the increase in GLUT4 mRNA immediately following exercise in rats (14). Although, in 3T3-L1 adipocytes, insulin represses GLUT4 expression through nuclear factor 1, which binds to an insulin...
response element on the GLUT4 promoter (5). While the role of insulin on human skeletal muscle GLUT4 gene expression is unclear, plasma insulin levels were higher throughout the glucose trial and could possibly have had an effect on GLUT4 mRNA levels.

Changes in cellular energy balance through AMP-activated protein kinase (AMPK) signaling and calcium release from the sarcoplasmic reticulum have both been implicated in the gene expression response to exercise (19). Interestingly, these stimuli increase PGC-1, GLUT4 (19), and UCP3 (31) gene expression. While it would seem that intracellular calcium perturbations during exercise would not be affected by glucose supplementation, the effect that glucose supplementation has on AMPK activity during exercise is unknown. It could be possible that glucose supplementation attenuates the exercise-induced increase in AMPK activity, which, along with the effect of suppressed circulating free fatty acids and increased insulin levels, could result in differential regulation of exercise-induced genes. This is an area for future research.

In conclusion, the results from the present study demonstrate that glucose supplementation attenuated the increases in PDK-4 and UCP3 mRNA following exercise, but has no effect on PGC-1 mRNA. These data suggest that altered glucose availability can modulate the effects of exercise on some metabolic genes.

ACKNOWLEDGMENTS

We thank Dr. Andrew Garnham, Dr. Rod Snow, and Nick Kimber for assistance with the study.

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3. Bazzoni GF, Bellasio M, Shaw AC, and Howaldt T. In conclusion, the results from the present study demonstrate that glucose supplementation attenuated the increases in PDK-4 and UCP3 mRNA following exercise, but has no effect on PGC-1 mRNA. These data suggest that altered glucose availability can modulate the effects of exercise on some metabolic genes.