Glucose ingestion during exercise blunts exercise-induced gene expression of skeletal muscle fat oxidative genes

Anthony E. Civitarese,1 Matthijs K. C. Hesselink,2 Aaron P. Russell,3 Eric Ravussin,1 and Patrick Schrauwen4

1Pennington Biomedical Research Center, Baton Rouge, Louisiana; Departments of 2Movement Sciences and 4Human Biology, Nutrition and Toxicology Research Institute, Maastricht University, Maastricht, The Netherlands; and 3Romande Rehabilitation Clinic, SUVACare, Sion, Switzerland

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Civitarese, Anthony E., Matthijs K. C. Hesselink, Aaron P. Russell, Eric Ravussin, and Patrick Schrauwen. Glucose ingestion during exercise blunts exercise-induced gene expression of skeletal muscle fat oxidative genes. Am J Physiol Endocrinol Metab 289: E1023–E1029, 2005. First published July 19, 2005; doi:10.1152/ajpendo.00193.2005.—Ingestion of carbohydrate during exercise may blunt the stimulation of fat oxidative pathways by raising plasma insulin and glucose concentrations and lowering plasma free fatty acid (FFA) levels, thereby causing a marked shift in substrate oxidation. We investigated the effects of a single 2-h bout of moderate-intensity exercise on the expression of key genes involved in fat and carbohydrate metabolism with or without glucose ingestion in seven healthy untrained men (22.7 ± 0.6 yr; body mass index: 23.8 ± 1.0 kg/m2; maximal O2 consumption: 3.85 ± 0.21 l/min). Plasma FFA concentration increased during exercise (P < 0.01) in the fasted state but remained unchanged after glucose ingestion, whereas fat oxidation (indirect calorimetry) was higher in the fasted state vs. glucose feeding (P < 0.05). Except for a significant decrease in the expression of pyruvate dehydrogenase kinase-4 (P < 0.05), glucose ingestion during exercise produced minimal effects on the expression of genes involved in carbohydrate utilization. However, glucose ingestion resulted in a decrease in the expression of genes involved in fatty acid transport and oxidation (CD36, carnitine palmitoyltransferase-1, uncoupling protein 3, and 5′-AMP-activated protein kinase-α2; P < 0.05). In conclusion, glucose ingestion during exercise decreases the expression of genes involved in lipid metabolism rather than increasing genes involved in carbohydrate metabolism.

Skeletal muscle gene expression; exercise-diet interaction

Exercise activity, in addition to enabling rapid restoration of preexercise CHO and lipid energy stores.

There are many possible sites of cellular control that contribute to improved substrate uptake, transport, and oxidation under periods of increased muscular contraction. Recent interest has focused on the impact of muscular activity on the transcription and expression of genes encoding key proteins involved in CHO metabolism and mitochondrial pathways. Typically, there is a transient increase in the cellular abundance of many genes after exercise, with expression peaking within 4 h (31, 43) and returning toward or to baseline levels within 24 h (48). This transient increase in mRNA species is coupled to the metabolic and functional adaptations that occur after repeated exercise sessions. Thus the intensity, type, and duration of the exercise session differentially influence both the species of gene transcripts and the extent to which gene expression is increased.

The specificity of the genes responding to an exercise session indicates that additional influences such as diet may interact to regulate postexercise gene expression. Several studies have addressed the impact of diet on human skeletal muscle gene expression. For example, glucose ingestion during exercise may create an unusual situation in which genes involved in fat metabolism may be stimulated by exercise but inhibited by the preferential usage of CHO. Our laboratory (5) and others (15) have shown that a preexercise glucose load reduces fat availability to the muscle (secondary to insulin-mediated decrease in adipose tissue lipolysis), thus reducing the entry of long-chain FA in the mitochondria. Consistent with these data, after an intense exercise session to lower muscle glycogen concentrations, as little as 48 h of a high-CHO or a high-fat diet to either restore or maintain suppressed muscle glycogen resulted in coordinated alterations in genes encoding key proteins for either CHO or lipid metabolism (15). Only one study has addressed whether the proceeding diet influences gene expression in the recovery period after exercise. In this study, intense exercise and a 24-h dietary manipulation resulted in either low or high muscle glycogen levels. Low muscle glycogen levels were found to increase the transcriptional activation of a range of metabolic genes in the recovery period after exercise (15).

Despite the capacity of diet and muscle substrate stores to influence gene expression, no published study has described the actions of glucose ingestion immediately proceeding, during, and after exercise on the skeletal muscle mRNA levels of...
genes involved in CHO metabolism. This is despite the widespread recommendations for athletes to ingest CHO-rich beverages during and immediately after exercise cessation to treat dehydration and restore glycogen levels. Accordingly, the present study examined the transient regulation of key metabolic genes during endurance exercise with or without glucose ingestion within the first few hours postexercise.

METHODS

Subjects. As previously described, seven healthy (22.7 ± 0.6 yr; body mass index: 23.8 ± 1.0 kg/m²; maximal O₂ consumption (V₀₂ max): 3.85 ± 0.21 l/min) untrained male volunteers participated in this study (36). None of the subjects participated in endurance sports, but all were moderately active. All subjects gave their written informed consent, and the study was approved by the Medical-Ethical Committee of Maastricht University.

Experimental design. All participants were studied on two occasions in a blinded, randomized crossover design with either the water or the glucose trial. These two trials were separated by 1 wk. The protocol for trial 1 was as follows. Prior to the experiment, V₀₂ max was determined by an incremental exercise test on an electromagnetically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands). On test days, after an overnight fast, subjects had a percutaneous muscle biopsy taken from the vastus lateralis muscle, and muscle tissue was immediately frozen in liquid nitrogen. A Teflon cannula was inserted in an antecubital vein for blood sampling (time 0–60 min). After ingestion of 1.4 g/kg body wt glucose solution (dissolved in water to a 20% solution and flavored with 1 ml of lemon juice) and 1 h of bed rest, subjects started exercising at 50% of maximal power output (at time 0 min) for 2 h. Blood samples were taken at time 30, 60, 90, 120, 180, 240, 300, and 360 min. Subjects ingested an additional 0.35 g/kg body wt glucose solution (dissolved in water to a 10% solution and flavored with 1 ml of lemon juice) at time –10, 30, 60, 90, 180, 240, and 300 min. Immediately after cessation of exercise, a second muscle biopsy was taken (time = 120 min) followed by two more at 1 h (time = 180 min) and 4 h (time = 360 min) postexercise. To avoid the confounding and independent effects of repeated muscle sampling (42), muscle biopsy samples were taken in a randomized order at four different sites and to the opposite leg to trial 1. Indirect calorimetry was performed continuously at rest and in the last 10 min of every half-hour during exercise. From the recorded V₀₂ and VCO₂ (Oxycon Pro, Mijnhardt, The Netherlands), CHO and fat oxidation rates and energy expenditure were calculated using the formulas of Peronnet and Massicott (28). The protocol for trial 2 was as follows. This was performed exactly the same as trial 1; however, subjects remained fasted before, during, and after exercise and received lemon-flavored water without glucose.

Blood analysis. Blood was collected in EDTA tubes and immediately centrifuged at 4,000 rpm for 10 min at 4°C. Plasma was frozen in liquid nitrogen and stored at −80°C. Plasma glucose was determined using the hexokinase method (Roche, Basel, Switzerland) and plasma free fatty acid (FFA) using the Wako NEFA C test kit (Wako Chemicals, Neuss, Germany).

Real-time quantitative RT-PCR. Total RNA was extracted using the acid-phenol method of Chomczynski and Sacchi (4), with an additional DNase digestion step with concomitant acid-phenol extraction and ethanol precipitation. Primer and probe sequences were designed using Primer Express software version 1.0 (PerkinElmer, Norwalk, CT) and are given in Table 1. Gene expression was measured using quantitative RT-PCR on an ABI Prism 7700 sequence detector. Briefly, 20 ng diluted RNA was added to 2× TaqMan One-Step RT-PCR Master Mix (Applied Biosystems). PCR conditions consisted of a two-step PCR. Step 1 included a 30-min reverse transcription at 48°C and another at 95°C for 10 min to activate AmpliTaq Gold DNA Polymerase. Step 2 consisted of 40 cycles of a denaturing step at 95°C for 15 s and an extension step at 60°C for 1 min. All samples for each gene were run in duplicate simultaneously to control for amplification efficiency. To compensate for RNA input variation and the efficiency of reverse transcription, cyclophilin B mRNA was quantitated, and results were normalized to these values. To ascertain whether cyclophilin B gene expression was modified by acute exercise and glucose ingestion, absolute gene expression levels were quantitated and analyzed as previously described (43). No exercise-dietary effect was detected on the absolute expression of cyclophilin B, implying similar initial mRNA concentrations between samples.

Statistical analysis. Repeated-measures ANOVA was performed to examine differences in measured parameters between the glucose and fasted treatments at any time point. Two-way ANOVA was performed to examine the interaction between diet and exercise. Because all the variables were normally distributed, Pearson correlation coefficients were calculated to determine the relationship between selected variables within each treatment trial. All data are presented as means ± SE. A P value <0.05 was considered statistically significant.

RESULTS

Energy and substrate metabolism. As expected, blood glucose concentration decreased during the fasted state, relative to

Table 1. Gene primer and probe sequences

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense Primer (5’-3’)</th>
<th>Antisense Primer (5’-3’)</th>
<th>Probe (5’-FAM-BHQ1′3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophilin B</td>
<td>GCG ATG GAG GCG TTT TTG GGT</td>
<td>CGA CAG TCA GCA ATG TTG ATC</td>
<td>TCG ACG AAT GCC ACC ACC AAG A</td>
</tr>
<tr>
<td>CPT</td>
<td>GAG GCC TCA ATG ACC AGA ATG</td>
<td>TGG GAC TCG TGT GTA GAT GAA</td>
<td>CAG CTT GCA TCC GTC CTC GGA</td>
</tr>
<tr>
<td>β-HAD</td>
<td>TGG CTT CCC GGC TGG TTG CTG</td>
<td>TGG AGG CCG TCG ACT ATC TTC</td>
<td>CGG CAT ACA GAT GCA AAA GGA A</td>
</tr>
<tr>
<td>CD36</td>
<td>AGT CAC TGG GAC ATG ATT AAT GGT</td>
<td>CTC GAA TAC TGT GGT TTC TTT A</td>
<td>CAT AGT CAT CCT TGG CAT TTT TTG</td>
</tr>
<tr>
<td>AMPKα2</td>
<td>AAC TGG AGA GCA CCA TTA CCT ATT GTT</td>
<td>GGT GAA ACT GAA GAT GAT GTC TGT</td>
<td>TCC GTC TGT CAG GTC TGT GTC</td>
</tr>
<tr>
<td>AMPKγ3</td>
<td>TGT GGA CCG GCG TGT GT AGG CCC ACG ACC TGA</td>
<td>ACC AGG CCG ACC TGG GC</td>
<td>TGC ACT GTG CTT GAT CAA CAG A</td>
</tr>
<tr>
<td>ACC2</td>
<td>ACCAT TGG CAC TGG CTC TGG CAC</td>
<td>TCT CTC TGG CAC AAT CTA</td>
<td>GGA TTA CAG GAA ACC GCC TTG</td>
</tr>
<tr>
<td>UCP3</td>
<td>CTA GCC TGC AGA GAG ACA AA</td>
<td>CTA GTC TGG CAG CTA</td>
<td>TTA ACA GCA GCA CAT TGC</td>
</tr>
<tr>
<td>IR</td>
<td>CAA TGG GAA GAT CCC CTA CTA C</td>
<td>CAA TGG GAA GAT CCC CTA C</td>
<td>CAA GCA GCA GCA</td>
</tr>
<tr>
<td>IRS-1</td>
<td>GCG ATG GCG GTT TCA TCT</td>
<td>TAT AGT TGC TTA GCT CCT CCT CAC</td>
<td>CTT CCG CAG TGT CAC GGA TTT</td>
</tr>
<tr>
<td>IRS-2</td>
<td>GCC ACC ATC GTC GCC TGT</td>
<td>CAC TGC GAT GAA GAA GAC CTA</td>
<td>GGT GTG GAC CAG CAG CAG CAG</td>
</tr>
<tr>
<td>PI 3-kinase</td>
<td>AGC AAT CGG CGG TAT G</td>
<td>TTG GGA TTA GGG TGC</td>
<td>AAC TGG TCT ATT TTT ATT</td>
</tr>
<tr>
<td>GLUT4</td>
<td>CCT CCT ATG AAA TGG TCA TCC TGT</td>
<td>GCA COT ACA TGG GCA CCA</td>
<td>GCA TTA CTG ATT GCC GAC TAC</td>
</tr>
<tr>
<td>PDK-4</td>
<td>GAG AAT TAT GGG CCG CCT TAG</td>
<td>AAA CCA GCC AAA GGA GAA TTT</td>
<td>ACT CCA CTC GAC CAA CCC CCA</td>
</tr>
</tbody>
</table>

CPT 1a, carnitine palmitoyltransferase-1 (molecule isoform); β-HAD, β-hydroxyacyl-CoA dehydrogenase; CD36, fatty acid transport protein; AMPKα2, 5-AMP-activated protein kinase α2-subunit; AMPKγ3, 5′-AMP-activated protein kinase γ3-subunit; ACC2, acetyl-CoA carboxylase 2; UCP3, uncoupling protein-3; IR, insulin receptor; IRS-1, insulin receptor substrate-1; IRS-2, insulin receptor substrate-2; PI 3-kinase (p38), p38 subunit of phosphatidylinositol 3-kinase; GLUT4, glucose transporter 4; PDK-4, pyruvate dehydrogenase kinase-4.
the fed state ($P < 0.01$; Fig. 1A), and plasma FFA concentration increased during exercise in the fasted state and remained unchanged after glucose ingestion ($P < 0.01$; Fig. 1B). There was no treatment effect on energy expenditure (Fig. 1C), whereas respiratory quotient (RQ) was lower in the fasted state vs. glucose feeding ($P < 0.01$; Fig. 1D). Accordingly, fat oxidation was 37 ± 9% higher in the fasted state relative to the glucose trial (data not shown; see Ref. 36).

**mRNA quantification.** The effect of short-term moderate-intensity exercise with or without glucose consumption on the mRNA level of genes involved in CHO metabolism is shown in Fig. 2, whereas the effect on genes involved in fat metabolism is shown in Fig. 3.

**CHO metabolism gene expression.** Exercise with glucose ingestion did not alter the mRNA expression of the insulin receptor (IR; data not shown), insulin receptor substrate-2, (IRS-2; data not shown), IRS-1, and PI 3-kinase over time compared with exercise in the fasted state (Fig. 2). However, exercise did increase PI 3-kinase mRNA expression 4 h after exercise in either treatment group (Fig. 2B). With regard to
GLUT4, glucose feeding did blunt the significant increase in the expression level of GLUT4 that was observed after exercise in the fasted state ($P < 0.05$), indicating a treatment effect. Furthermore, glucose ingestion was associated with a time-by-treatment suppression of pyruvate dehydrogenase-4 (PDK-4) mRNA compared with fasting exercise ($P < 0.01$).

**Fat metabolism gene expression.** Exercise with glucose ingestion caused a time-by-treatment suppression of CD36 ($P < 0.05$), CPT I ($P < 0.05$), UCP3 ($P < 0.01$), and 5'-AMP-activated protein kinase $\alpha_2$-subunit (AMPK$\alpha_2$) mRNA ($P < 0.05$) levels compared with the fasted state (Fig. 3). No exercise-diet interaction was observed for acetyl-CoA carboxylase 2 (ACC2), -HAD, and AMPK$\gamma_3$ (data not shown).

**DISCUSSION**

During exercise and the subsequent recovery period, the type and quantity of fuel used depends on the interplay between the fuels available from the diet and storage depots, the intensity and duration of the activity, and the training status of the subject. Accordingly, it has long been known that a preexercise glucose load reduces fat oxidation during low- to moderate-intensity exercise (up to $\sim 60\% \text{ VO}_2\text{max}$; see Ref. 40). CHO feeding markedly influences circulating metabolites (increasing glucose concentrations and reducing FFA availability) and presumably changes the pattern of gene transcription. Several studies have shown that sustained training-induced cellular adaptation is the result of the cumulative effects of repeated exercise bouts. However, the initial signaling responses that lead to long-term adaptations can occur in response to each bout of exercise (12, 31). In this context, we examined the regulation of genes involved in CHO and fat metabolism in response to a single 2-h bout of moderate-intensity exercise and in the presence or absence of repeated glucose ingestion. Our first major finding was that glucose consumption lowered the mRNA expression of genes involved in FA transport and oxidation (CD36, UCP3, CPT I, and AMPK$\alpha_2$) and of PDK-4, a gene involved in the regulation of glycolysis. This treatment lowering of gene expression over “time” was associated with reduced FFA concentrations and an increase in CHO oxidation (higher RQ), albeit, at an exercise intensity normally favoring oxidation of lipids (40). These data suggest several important findings. Firstly, acute changes in whole body substrate flux favoring CHO metabolism can modify the expression level of genes involved in transport and oxidation of lipids immediately after exercise. Second, no time-by-treatment effect was observed in our set of CHO metabolism genes (IR, IRS-1/2, PI 3-kinase, and GLUT4), implying that an increased glucose flux during exercise does not upregulate genes involved in insulin signaling and is an effect inherent to the contracting muscle.

The mechanism by which consumption of CHO leads to a decrease in gene expression of fat metabolism during exercise is unclear; however, insulin may play a role. We have previously shown, in a similar experiment, that insulin levels remain elevated during exercise with glucose ingestion compared with exercise in the fasted state (5). Therefore, the elevated circulating insulin produced by the glucose load could have a negative feedback on gene expression. In support of this concept, Rome et al. (33) recently demonstrated that insulin acts on global gene expression by regulating the mRNA levels of genes involved in transcription, energy metabolism, and intracellular signaling. More specifically, insulin has also been
shown to suppress PDK-4 mRNA (21), which is consistent with the current findings.

Conversely, the reduced expression of lipid metabolism genes may have been due in part to suppressed lipolysis and a lowering of circulating FFA. In support of this mechanism, we have recently demonstrated that the administration of acipimox reduced both fat oxidation and UCP3 mRNA levels (13) by blocking lipolysis and reducing FFA plasma concentration. It is well established that FFAs, via their interaction with the peroxisome proliferator activator receptor (PPAR) family of nuclear hormone receptors, can regulate systemic FA metabolism via ligand-dependent transcriptional activation of target genes (20). For example, fasting increases both the lipid supply to oxidative tissue and the expression of both PDK-4 and UCP3 in the skeletal muscle of rats (11, 41) via an FFA-mediated activation of PPARα (46). Interestingly, in humans, the expression of muscle CPT I, the rate-limiting step in fat oxidation (23), and CD36, an important transporter of long-chain FA, is also regulated by PPARα (35). Importantly, in our study, increasing blood glucose concentrations during CHO ingestion was accompanied with a lowering of CD36, CPT I, and UCP3 mRNA over time and reduced lipid flux and oxidation, suggesting that a reduction in ligands for PPAR can reduce the expression of downstream targets.

The main function of PDK-4 is the suppression of glycolysis by phosphorylating and inactivating the pyruvate dehydrogenase complex (11). The activity of PDK-4 is regulated by glucose flux in the cell but may also be enhanced with increased lipid supply and utilization (11, 25). Consistent with these facts, in the present study, we observed an induction in PDK-4 transcription during the entire fasting trial that was associated with high rates of fat oxidation. Conversely, glucose feeding caused a highly significant time-by-treatment suppression of PDK-4 mRNA that was associated with increased CHO oxidation, as evidenced by an elevated RQ. These data are in agreement with previous studies that demonstrate that substrate availability has a profound effect on PDK-4 gene expression (29, 32). Recently, Arkinstall et al. (1) demonstrated that PDK-4 transcription is suppressed by 2 days of CHO feeding to replenish glycogen stores. Conversely, other studies have shown that glucose consumption is not sufficient to inhibit increased PDK-4 transcription during exercise (1, 30). This discrepancy is most likely the result of the timing of the meal ingestion. Several studies have demonstrated that a single preexercise glucose load causing hyperinsulinemia results in a rapid and sustained drop in blood glucose concentrations during submaximal exercise (6, 7). However, in this study, we provided a constant flux of ingested glucose that resulted in elevated plasma glucose concentration and oxidation during the entire glucose trial (Fig. 1A). Glycogen is also a known signaling molecule that can regulate gene transcription, although it is unlikely that it would have contributed to changes in PDK-4 mRNA expression in this current experimental paradigm. It was previously shown that the type of exercise trial used in this study does not alter glycogen oxidation (18). These data suggest that changes in PDK-4 transcription (or the subset of lipid metabolism genes examined in this study) can be repressed during exercise conditions favoring fat oxidation as a result of increased glucose flux and oxidation and are independent of muscle glycogen concentration.

Many of the adaptations taking place during exercise are proposed to involve AMPK, a member of a metabolite-sensing protein kinase family that functions as a metabolic “fuel gauge” in skeletal muscle (9). However, the independent effect of glucose supplementation on AMPK mRNA expression during exercise is unknown. During exercise, AMPK becomes activated in skeletal muscle in response to changes in cellular energy status (e.g., increased AMP-to-ATP and creatine-to-phosphocreatine ratios; see Ref. 44). In turn, AMPK can phosphorylate ACC2, which leads to the inhibition of ACC2 activity and a consequent reduction in the malonyl-CoA content, thereby derepressing CPT I activity and increasing skeletal muscle FA oxidation (44). Although we and others (24, 26) did not observe an upregulation of AMPKα2 mRNA with exercise in the fasted state, the reduced AMPKα2 mRNA expression observed in this study with glucose feeding and the suppression of fat metabolism genes in this condition is consistent with the fact that AMPK activate pathways involved in FA metabolism when cellular substrate levels decline (2). However, we acknowledge, the extent to which a protein and posttranslational modifications might be modified in response to an exercise-diet interaction cannot be predicted from an increase in mRNA level; however, it has previously been reported that the concentration of mRNA for genes encoding for substrate metabolism is accompanied by a concomitant increase in the cellular content of the transcribed protein (3, 29) and, presumably, function. In addition, without the inclusion of control subjects with rest ± fasting or glucose, it is not possible to attribute the alterations observed after glucose feeding to a modulation of CHO treatment only. Future studies should be aimed at examining this discrepancy.

Results from this study and others (12, 31) demonstrate an increase in GLUT4 mRNA and protein (8) expression after a single bout of moderate-intensity exercise. However, the increase in GLUT4 gene expression in the postexercise period was reduced during the glucose trial. To the best of our knowledge, this is the first study to demonstrate the acute reduction in GLUT4 gene expression in response to dietary manipulation. Recent data suggest that insulin is an important regulator of GLUT4 mRNA after exercise in rats (19). However, muscle contraction during exercise has an insulin-independent effect on muscle glucose uptake (34). The two main mediators of this process are thought to involve muscle glyco- gen content and AMPK activity, as well as several downstream kinases from AMPK, including the p38 mitogen-activated protein kinase (47) and the Ca²⁺-sensitive class of atypical protein kinase C (27). It has repeatedly been shown, both in vivo and in vitro, that glucose uptake increases when muscle glycogen is low (10, 45). In this context, the coordinated decrease in AMPKα2 and GLUT4 mRNA abundance might be expected to decrease protein expression and thereby provide a negative feedback that limits further storage of muscle glycogen when glycogen concentrations and the glucose flux in the myocyte are high. This hypothesis fits well with data from the present study that demonstrate that glucose oxidation was elevated and PDK-4 and GLUT4 gene expression lowered during the postexercise period in the glucose trial.

In summary, we provide evidence that glucose ingestion during moderate-intensity exercise inhibits the expression of genes involved in the transport and oxidation of lipids (CD36, CPT I, UCP3, and AMPKα2) and downregulates PDK-4,
which is involved in glycolysis suppression. The change in mRNA expression was rapid (1–4 h) and occurred at an exercise intensity relying mostly on fat metabolism. Therefore, acute exercise with glucose ingestion does not result in adaptive changes in muscle that favor the capacity for carbohydrate oxidation/metabolism but rather suppresses the exercise-induced adaptive changes that would favor fat oxidative capacity.

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REFERENCES


