Transcriptional regulation of gene expression in human skeletal muscle during recovery from exercise

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Pilegaard, Henriette, George A. Ordway, Bengt Saltin, and P. Darrell Neuffer. Transcriptional regulation of gene expression in human skeletal muscle during recovery from exercise. Am J Physiol Endocrinol Metab 279: E806–E814, 2000.—Exercise training elicits a number of adaptive changes in skeletal muscle that result in an improved metabolic efficiency. The molecular mechanisms mediating the cellular adaptations to exercise training in human skeletal muscle are unknown. To test the hypothesis that recovery from exercise is associated with transcriptional activation of specific genes, six untrained male subjects completed 60–90 min of exhaustive one-legged knee extensor exercise for five consecutive days. On day 5, nuclei were isolated from biopsies of the vastus lateralis muscle of the untrained and the trained leg before exercise and from the trained leg immediately after exercise and after 15 min, 1 h, 2 h, and 4 h of recovery. Transcriptional activity of the uncoupling protein 3 (UCP3), pyruvate dehydrogenase kinase 4 (PDK4), and heme oxygenase-1 (HO-1) genes (relative to β-actin) increased by three- to sevenfold in response to exercise, peaking after 1–2 h of recovery. Increases in mRNA levels followed changes in transcription, peaking between 2 and 4 h after exercise. Lipoprotein lipase and carnitine palmitoyltransferase I gene transcription and mRNA levels showed similar but less dramatic induction patterns, with increases ranging from two- to threefold. In a separate study, a single 4-h bout of cycling exercise (n = 4) elicited from 5 to >20-fold increases in UCP3, PDK4, and HO-1 transcription, suggesting that activation of these genes may be related to the duration or intensity of exercise. These data demonstrate that exercise induces transient increases in transcription of metabolic genes in human skeletal muscle. Moreover, the findings suggest that the cumulative effects of transient increases in transcription during recovery from consecutive bouts of exercise may represent the underlying kinetic basis for the cellular adaptations associated with exercise training.

metabolic genes; metabolism; messenger ribonucleic acid; training adaptation

SKELETAL MUSCLE CELLS possess a remarkable capacity to respond and adapt to the physical and metabolic challenges imposed by contractile activity (39). The acute responses to exercise are dedicated to coupling energy supply processes to the metabolic demand for ATP, which can increase by >100-fold in human skeletal muscle (18). Likewise, the adaptive responses to exercise performed over several months include marked changes in key enzymatic, regulatory, and myofibrillar proteins, all of which serve to improve the efficiency of substrate utilization relative to metabolic demand during exercise (30).

The molecular mechanisms mediating the cellular adaptations to exercise training in skeletal muscle remain poorly defined. Training-induced increases in the mRNA levels for a number of metabolic genes have been reported, implying that pretranslational regulation takes place in response to exercise. Regulation at the level of transcription was first described in rats, where transcription of the GLUT-4 and citrate synthase genes was found to be transiently increased during recovery from exercise (25). These findings led to the suggestion that the cellular adaptations elicited by exercise training may arise from the cumulative effects of transient changes in gene transcription occurring specifically during recovery from each exercise session (25, 39). In agreement with this hypothesis, transient increases have since been reported for GLUT-4 mRNA in humans (21), lipoprotein lipase (LPL) mRNA and protein in humans (31) and rats (13), hexokinase II (HKII) mRNA and enzyme activity in rats (27), heme oxygenase-1 (HO-1) mRNA in rats (9), and uncoupling protein 3 (UCP3) mRNA in mice (34) during recovery from exercise.

Direct measurements of transcriptional activity in skeletal muscle of humans have not been possible because of the large amount of tissue typically required for nuclei isolation (~8 g) (25). However, a simplified procedure for isolating nuclei and an RT-PCR-based technique for performing nuclear run-on analysis were recently developed, making it possible to determine transcriptional activity of several genes from relatively small amounts of muscle tissue (16). The aim of the
The present study was to test the hypothesis that exercise induces transient increases in transcription of metabolically related genes in human skeletal muscle during recovery from exercise. Two different exercise protocols were applied: an exhaustive one-legged knee extensor exercise (60–90 min) performed after a short-term training period (4 consecutive days) and a single low-intensity cycling exercise bout performed for 4 h. Skeletal muscle biopsies were obtained before and immediately after exercise, as well as during the ensuing 4 h of recovery. Transcriptional activity and mRNA content were determined for four metabolic genes [HKII, glycogen synthase (GYS), LPL, and muscle carnitine palmitoyltransferase (CPT I)], two putative regulatory genes [UCP3 and pyruvate dehydrogenase kinase 4 (PDK4)], and two stress-responsive genes [HO-1 and α-B-crystallin (αBC)].

MATERIALS AND METHODS

Subjects

Ten healthy, male subjects ranging in age from 22 to 28 yr, with an average height of 185 cm (range: 175–192 cm) and a mean weight of 81 kg (range: 65–110 kg), participated in the study. The subjects were habitually physically active without taking part in competitive sports, and they maintained their normal activity pattern during the experimental period. The subjects were given both oral and written information about the experimental procedures before they gave their informed consent. The study was approved by the Copenhagen Ethics Committee.

Experimental Design

One-legged knee extensor exercise. Six subjects participated in a 5-day exercise training protocol consisting of dynamic one-legged knee extensor exercise on a modified Krogh cycle ergometer (1). One to two weeks before exercise training was initiated, a one-legged knee extensor exercise test was performed, in which the resistance load was gradually increased every 2 min. The highest resistance that the subjects could perform for 2 min was set as the maximal workload. On five consecutive days, the subjects completed an exhaustive one-legged knee extensor exercise bout at 70% of the 2-min maximal resistance. The exercise time was improved from 61.7 ± 1.6 min (means ± SE) on day 1 to 77.3 ± 3.3 min on day 5. On day 5, the subjects arrived at the laboratory in the morning, having fasted overnight, and muscle biopsies were taken immediately at the end of the bout and after 15 min, 1 h, 2 h, and 4 h of recovery from the exercise. The subjects were allowed water ad libitum but remained fasted throughout the exercise and recovery periods.

Muscle Biopsies

Muscle samples were obtained from the middle portion of the vastus lateralis muscle by use of the percutaneous needle biopsy technique with suction. The major part of the muscle biopsy (~110 mg) was used for the isolation of nuclei, whereas the remaining portion was frozen in liquid N2 for RNA isolation.

Nuclei Isolation

The method used for isolating nuclei from small amounts of skeletal muscle was developed on the basis of established techniques, as previously described in detail (16). In short, muscle samples (~110 mg) were rapidly placed in 35 ml of ice-cold buffer A [15 mM HEPES, pH 7.5, 60 mM KCl, 3 mg/ml BSA, 300 mM sucrose, 5 mM each of EDTA and EGTA, 1 mM dithiothreitol (DTT), 0.5 mM spermidine, 0.15 mM spermine, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and 2 μg/ml leupeptin] for 1 min, minced, and homogenized for 20 s by use of a Polytron 2100 (setting 1.5). After incubation on ice for 5 min and centrifugation at 700 g at 4°C for 10 min, the resulting crude nuclear pellets were gently resuspended in 10 ml of ice-cold buffer B (like buffer A, except for 0.1 mM each of EDTA and EGTA and addition of 0.5% Triton X-100), and nuclei were spun down. Thereafter, nuclei were gently resuspended in 10 ml of ice-cold buffer C (like buffer A except for 0.1 mM each of EDTA and EGTA and the addition of 5 mM MgOAc) and pelleted again. The final nuclear pellets were gently resuspended in 200 μl of storage buffer (40% glycerol, 75 mM Hepes, pH 7.5, 60 mM KCl, 15 mM NaCl, 5 mM MgOAc, 0.1 mM each of EDTA and EGTA, 1 mM DTT, 0.5 mM spermidine, 0.15 mM spermine, and 2 μg/ml each of leupeptin and aprotinin), quick-frozen in liquid nitrogen, and stored at −70°C.

Nuclear Run-on Reaction

To determine transcription of specific genes, an RT-PCR-based nuclear run-on technique was used, as previously described (16). Briefly, nuclei were thawed on ice, and equal volumes of nuclei and reaction buffer (20% glycerol, 100 mM KCl, 10 mM MgCl2, 4.5 mM DTT, 1.2 mM ATP, 0.6 mM each of CTP, GTP, and UTP, 0.5 mM spermidine, 0.15 mM spermine, and 80 units/ml RNase inhibitor) were mixed and incubated at 22°C. DNA and protein were digested by successive incubations with DNase I (RNase free) and proteinase K (in the presence of SDS), after which the nascent RNA was extracted by addition of Trizol (GIBCO-BRL) and chloroform. After centrifugation (12,000 g, 15 min, 4°C), isopropanol was added to the aqueous phase, and nascent RNA transcripts were precipitated overnight at −20°C. To ensure complete digestion of DNA, samples were subjected to a second DNase I digestion, Trizol-chloroform extraction, and isopropanol precipitation (−20°C, 30 min). The final RNA transcript pellets were rinsed twice with 75% EtOH and resuspended overnight at 4°C in 10 mM Tris (pH 8.0) and 0.1 mM EDTA.

Isolation of Genomic DNA

To provide an index of relative nuclei yield among samples, genomic DNA (gDNA) was isolated from a portion (40 μl) of
each nuclei preparation, as previously described (16). Final gDNA pellets were resuspended overnight at 4°C in 50 μl TE (10 mM Tris and 1 mM EDTA in nuclease-free water, pH 8.0). The relative gDNA content of the nuclei samples was determined by PCR amplification of the β-actin gene (described in the RT and PCR sections) and used to normalize dilution volumes of the RNA transcripts from the nuclei run-on reaction.

RNA Isolation

Total RNA was isolated from ~25 mg of muscle tissue by a modified guanidinium thiocyanate (GT)-phenol-chloroform extraction method adapted from Chomczynski and Sacchi (6). The samples were placed in 2 ml of cold GT solution and homogenized for 20 s with a Brinkman Polytron (setting 4.5). Total RNA was extracted by addition of 200 μl of 2 M NaOAc, pH 4.0, 2 ml diethyl pyrocarbonate-saturated phenol (Fisher BP226–100) and 500 μl chloroform:isoamyl-ÖH (49:1), vigorous shaking, incubation on ice for 15 min, and centrifugation at 12,000 g for 20 min at 4°C. Total RNA was precipitated from the aqueous phase by addition of an equal amount of isopropanol and incubation for 15 min at −20°C, followed by centrifugation for 10 min at 12,000 g. To facilitate localization of the RNA pellet, 100 μg of yeast tRNA were added to the aqueous phase before precipitation. To solubilize contaminating DNA, the resulting pellet was incubated for 15 min in 1 ml of 4 M lithium chloride and centrifuged for 5 min at 12,000 g at 4°C. The resulting pellets were resuspended in 500 μl of GT solution, followed by a second isopropanol precipitation. After a rinse in 75% EtOH, the final pellets were resuspended overnight at 4°C in 50 μl of nuclease-free H₂O containing 0.1 mM EDTA.

RT

RT of both nascent RNA from the nuclear run-on reactions and of total RNA samples was performed using the SuperScript II RNase H⁻ system (GIBCO-BRL), as previously described (16). RT-RNA products from the nuclear run-on reactions were diluted with nuclease-free H₂O on the basis of the relative gDNA content of each nuclei preparation (see above), with the average volume set to 150 μl. For RT-RNA, relative differences in total RNA yield among samples were determined by PCR amplification of the β-actin mRNA in each RT-RNA sample (diluted to 5 μl/μl total RT-RNA). Final dilution volumes were adjusted on the basis of relative β-actin mRNA content, with the average set to 10 μl/μl RT-RNA.

PCR

Transcription and mRNA content of a given gene were determined by PCR (in duplicate) with 5 μl of diluted RT product in a total volume of 50 μl of reaction mixture containing 10 mM Tris (pH 9.0), 50 mM KCl, 0.1% Triton X-100, MgCl₂ 0.3 mM each of PCR-grade dATP, dCTP, dGTP, and dTTP, 0.5 μg each of forward and reverse primers, and 1.5 units of recombinant Taq DNA polymerase (GIBCO-BRL). PCR was performed in a Peltier thermal cycle dual-block DNA engine (MJ Research) by use of the general cycle profile: 94°C for 2 min [94°C for 30 s, annealing temperature for 50 s, and 72°C for 50 s] × 10 + [94°C for 30 s, annealing temperature for 50 s, 72°C for 50 s + 20 s extension/cycle] × the remaining number of cycles. PCR primer pairs (Table 1) were constructed from human specific sequence data (Entrez-NIH) using DNA analysis computer software (Lazergene, DNASTAR). PCR products were confirmed by direct sequencing. Prior testing of annealing temperature, MgCl₂ concentration, and number of PCR cycles had been performed to establish optimal conditions within the linear range for PCR amplification. For each subject, control and recovery samples were run simultaneously to permit relative comparisons. PCR products were separated by gel (2.5% agarose) electrophoresis, stained with ethidium bromide, visualized by ultraviolet exposure with a charge-coupled device integrating camera (Gel Doc, Bio-Rad), and quantified under nonsaturated conditions by use of analysis software (Molecular Analyst, Bio-Rad).

Statistics

Transcriptional activity and mRNA contents were normalized to β-actin transcription and mRNA levels, respectively, and samples were expressed relative to the corresponding control sample, which was set to 1. Values are means ± SE. One-way ANOVA for repeated measures was used to evaluate the effect of exercise and Dunnett’s post hoc test to locate significant differences among specific time points. Student’s paired t-test was used for comparisons between control and control exercised samples. Differences were considered significant at P < 0.05.

RESULTS

One-Legged Knee Extensor Exercise

Five consecutive days of one-legged knee extensor exercise (60–90 min) induced marked increases in transcription of several genes involved in both fat and glucose metabolism. As shown in Figs. 1 and 2, activation of transcription was transient and occurred primarily during recovery from exercise. Transcription of the UCP3, LPL, and CPT I genes was only slightly elevated (~2-fold, P > 0.05) immediately after exercise.

Table 1. Primers and reaction conditions used for PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>5’ Primer</th>
<th>3’ Primer</th>
<th>AT, °C</th>
<th>MgCl₂, mM</th>
<th>Size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCP3</td>
<td>AGAACCATGCCGCCAGGGGAGAGG</td>
<td>CCCGGGGAGGCACCACCTGT</td>
<td>59.0</td>
<td>2.0</td>
<td>216</td>
</tr>
<tr>
<td>PDK4</td>
<td>GCCCGGCCGCCTGCCTGTC</td>
<td>GCCGGGACCCACTCAGCT</td>
<td>53.0</td>
<td>1.5</td>
<td>240</td>
</tr>
<tr>
<td>HKII</td>
<td>TCAACCCGCCGCAAGCAGAG</td>
<td>CGCGCGCCGCACCACT</td>
<td>55.0</td>
<td>1.5</td>
<td>287</td>
</tr>
<tr>
<td>GYS</td>
<td>CGCGCGGAGGCTATCATTAC</td>
<td>GGCGGGCTCTCTCTCCTAC</td>
<td>61.0</td>
<td>2.5</td>
<td>313</td>
</tr>
<tr>
<td>LPL</td>
<td>GTCGCCGCGTCACATTACCAAAT</td>
<td>GTCGGGCTCTCTCCTACATTCA</td>
<td>57.0</td>
<td>1.5</td>
<td>246</td>
</tr>
<tr>
<td>CPT I</td>
<td>CTGTGCCGCTGCGCCAT</td>
<td>GGGCTGCTGCGCCATG</td>
<td>57.0</td>
<td>1.5</td>
<td>269</td>
</tr>
<tr>
<td>HO-1</td>
<td>CCCCGCTGCCGCCAGGAGGCA</td>
<td>GAGTTTCAAGGGGAGGCTAGAC</td>
<td>61.0</td>
<td>1.5</td>
<td>306</td>
</tr>
<tr>
<td>αBC</td>
<td>GATCGCCGCGGCCCTTTC</td>
<td>GTTCAGCTCAGCCAGT</td>
<td>57.0</td>
<td>1.5</td>
<td>219</td>
</tr>
<tr>
<td>β-Actin</td>
<td>CCAAGGACAGGCGAGGAGGAG</td>
<td>TGCAGCCGCGGAGGAGGAG</td>
<td>61.0</td>
<td>1.5</td>
<td>219</td>
</tr>
</tbody>
</table>

AT, annealing temperature; UCP3, uncoupling protein 3; PDK4, pyruvate dehydrogenase kinase 4; HKII, hexokinase II; GYS, glycogen synthase; LPL, lipoprotein lipase; CPT I, carnitine palmitoyltransferase; HO-1, heme oxygenase-1; αBC, αB-crystallin.
changes in response to exercise for most genes (Figs. 1–3). LPL mRNA content tended to increase during the 4-h recovery period (1.9-fold), but this was not a statistically significant change. CPT I mRNA levels, however, were elevated \((P < 0.05)\) by about twofold throughout recovery, whereas no change in UCP3 mRNA occurred. PDK4 mRNA remained at control levels through the first 2 h of recovery and then increased to about eightfold above controls after 4 h of recovery. HKII mRNA increased throughout recovery, reaching about fourfold above control levels after 4 h of recovery, whereas GYS mRNA content steadily increased during the first 2 h of recovery to about threefold above control levels. HO-1 mRNA levels showed the most dramatic change, increasing to 11-fold above control levels 2 h after exercise. αBC mRNA levels did not change during recovery.

It should be noted that, 22 h after the fourth bout of one-legged knee extensor exercise (control exercised time point), transcription was not different between the exercised and the nonexercised leg for any of the genes examined, illustrating the transient nature of the transcriptional response to each exercise bout (i.e., transcription returned to baseline). However, by day 5, mRNA levels for CPT I, GYS, and HO-1 were all still elevated by about twofold in control exercised relative to control (nonexercised) samples, providing evidence of a “training response.” These data also suggest that these mRNA species have slightly longer half-lives than the other genes examined.

Prolonged Cycling Exercise

To determine the effects of an acute prolonged exercise bout, transcription and mRNA content were determined in response to a single 4-h low-intensity cycling bout (Figs. 4 and 5). Similar to the one-legged knee extensor exercise, transcription of the HKII, LPL, and UCP3 genes was not elevated immediately postexercise. However, during recovery, transcription of the HKII, LPL, and UCP3 genes steadily increased to three- to sixfold above control levels by the 4-h time point. In addition, mRNA levels for HKII, LPL, and UCP3 increased by 2- to 2.5-fold after 4 h of recovery. Recovery from prolonged exercise did not influence transcription or mRNA content of the CPT I and GYS genes. Similar to the one-legged exercise, cycling for 4 h elicited marked changes in transcription of the PDK4 gene. By the end of exercise, transcription of PDK4 was elevated by >10-fold and continued to increase during recovery to >20-fold above preexercise control levels after 2–4 h of recovery. PDK4 mRNA was on average 7- to 12-fold higher throughout the recovery period, although the timing of the adaptive response among the four subjects varied. Transcription of the HO-1 gene progressively increased to nearly 4.5-fold above controls after 4 h of recovery. HO-1 mRNA was elevated by about fourfold immediately after exercise and remained three- to fivefold above control levels throughout the 4 h of recovery period. The single 4-h cycling bout did not influence αBC transcription or mRNA levels.
DISCUSSION

The RT-PCR-based nuclear run-on analysis employed in the present study enabled determination of the transcriptional activity of several genes from a single muscle biopsy and thus made it possible to examine the primary adaptive responses of human skeletal muscle to exercise. The data demonstrate that exercise stimulates transcription of several metabolically related genes in human skeletal muscle. For most genes examined, activation of transcription occurred specifically over the course of several hours after exercise, although some genes were also clearly induced during exercise. Importantly, transcriptional activation in response to exercise was transient, as evidenced by the fact that transcription of all genes examined returned to control levels within 22 h after exercise. When exercise was repeated over several days, however, the mRNA content of some genes was still elevated 22 h after the last exercise bout. Although further testing is required, these data support the hypothesis that transient increases in transcription during recovery from consecutive bouts of exercise result in a gradual accumulation of mRNA and, thus, may represent the underlying kinetic basis for the cellular adaptations associated with exercise training (39).

The clinical benefits of regularly performed exercise are irrefutable, both as a form of preventive medicine and as a primary intervention for the treatment of various chronic diseases (4); however, the molecular mechanisms underlying the adaptive responses to exercise are not well understood. Although exercise influences metabolism within a number of tissues and organ systems, skeletal muscle is the most relevant tissue to study because of its rate-limiting role in the clearance of dietary glucose and triglycerides (29, 35) and its capacity to adapt to habitual changes in physiological activity (3). Moreover, exercise is relatively unique as a stimulus because, although performed only for short periods of time on a periodic basis, fairly dramatic two- to threefold changes in the expression of proteins with roles in glucose and fatty acid metabolism can occur over the course of several weeks. The idea that the molecular basis for these training adaptations may actually stem from the cumulative effects of transient changes in gene expression originally arose from work in rats, in which transcription of the GLUT-4 gene was found to be transiently increased during the initial few hours of recovery from exercise (25). Similar responses have since been found for GLUT-4, HKII, and LPL mRNA and/or protein in skeletal muscle of humans during recovery from exercise (20, 21, 31). The data from the present study extend these findings to demonstrate that recovery from exercise is characterized by transient transcriptional activation of several metabolically related genes, the products of which may contribute to the enhanced rate of insulin-stimulated glucose uptake and triglyceride clearance seen after exercise (31, 42).

PDK4 was the most dramatically regulated gene analyzed in the present study. Transcription was increased immediately after exercise and continued to increase throughout the 4-h recovery period, particularly after the 4-h cycling bout, when transcription and mRNA content were elevated by >20- and >10-fold, respectively. PDK4 is one of four isozymes identified in mammalian tissues that catalyze the phosphorylation of the E1 component of pyruvate dehydrogenase (PDH) (5). Phosphorylation of E1 inactivates the PDH complex, inhibiting the conversion of pyruvate to acetyl-CoA and thus preventing the entry of glycolytic products into the mitochondria for oxidation. The PDK isozymes vary in their distribution and regulatory properties, thereby establishing an important con-
Fig. 4. Transcription and mRNA responses in skeletal muscle during recovery from a single 4-h bout of cycling exercise. Negative images of PCR products (ethidium bromide stained) generated from RT-PCR-based nuclear run-on (transcription) and mRNA analysis are shown for the indicated genes (defined in Figs. 1–3) for a single subject.

Fig. 3. Transcription and mRNA responses in skeletal muscle during recovery from 60–90 min of exhaustive one-legged knee extensor exercise. See Fig. 1 for details of exercise protocol. Top: negative image of PCR products (ethidium bromide stained) generated from RT-PCR-based nuclear run-on (transcription) and mRNA analysis for the heme-oxygenase 1 (HO-1), αB-crystallin (αBC), and β-A genes. Analysis of each gene over time is shown for a single subject. Middle and bottom: summary of image and statistical analysis (n = 6) of changes in transcription (solid bars) and mRNA (light gray bars) of indicated genes. All data were normalized to β-actin and expressed relative to control samples (set to 1.0) as means ± SE. *Significantly (P < 0.05) different from C; † significantly (P < 0.05) different from C and CE.

trol point for coordinating overall intermediary metabolism. PDK4 is primarily expressed in heart and skeletal muscle, with recent work showing a marked induction of PDK4 expression by starvation and streptozotocin-induced diabetes in rats (40, 41), presumably as a means of conserving glucose and ensuring that 3-carbon units are diverted to the liver for gluconeogenesis. The pronounced increase in muscle PDK4 transcription and mRNA content found in the present study indicates that a similar regulatory mechanism may be operating in response to exercise in humans.

Two points are worth considering. First, the increase in PDK4 transcription and mRNA content was much higher immediately after the 4-h cycling than after the 60- to 90-min one-legged knee extensor exercise, suggesting that PDK4 expression may progressively increase with exercise duration. This in turn would be expected to cause a progressive inactivation of PDH (8) and thus may provide a mechanistic explanation for the steady decline in carbohydrate oxidation that occurs during prolonged low-intensity exercise (38). Second, recovery from exercise was associated with further increases in transcription of the PDK4 gene, providing indirect evidence that PDH activity may be inhibited during recovery from exercise. This interpretation is consistent with recent data indicating that previously exercising muscle relies on a preferential utilization of lipids for fuel during the postexercise recovery period (19), likely reflecting the high metabolic priority given to glycogen resynthesis.

Recovery from exercise resulted in transient increases in transcription and mRNA content of the UCP3 gene. These findings are in agreement with recent data from both rats and mice in which UCP3 transcription (16) and mRNA levels (7, 34) have been shown to be transiently increased in both red and white skeletal muscle during recovery from exercise. The fact that UCP3 is expressed exclusively in skeletal muscle and that UCP3 induces uncoupling activity when overexpressed in yeast or C2C12 muscle cells (10, 11, 17) has led to speculation that control of UCP3 expression and/or activity may play an important role in the regulation of adaptive thermogenesis and energy expenditure. On the other hand, UCP3 knockout mice do not display any overt phenotype; body weight gain and temperature regulation in response to various metabolic challenges are normal (12, 37). However, mitochondria from UCP3 knockouts are characterized by a higher state 3-to-state 4 respiration ratio, due entirely to a reduced proton leak, which is associated with an increased production of reactive oxygen species (ROS) (37). Although these findings collectively suggest that UCP3 is not required for long-term metabolic balance, the functional consequences of the dramatic changes in UCP3 expression that arise from different
physiological challenges (e.g., exercise, fasting, diet) on overall metabolic control and ROS formation have yet to be determined.

The heme oxygenase family consists of one inducible (HO-1) and two constitutively (HO-2, HO-3) expressed genes, the protein products of which catalyze the conversion of heme to biliverdin and CO (23). The HO-1 isoform is induced by a variety of agents that cause oxidative stress, including heat shock, ischemia, and hypoxia (22, 24, 32). This induction is believed to serve a protective role in the cell by initiating the degradation of the prooxidant iron-bound heme while generating the reaction products biliverdin and bilirubin, both of which are potent antioxidants (33). Skeletal muscle cells appear to be sensitive to oxidative stress, because HO-1 has recently been shown to be induced by hemin and sodium nitroprusside (an NO donor) in L6.G8 muscle cells (36). In rat skeletal muscle, HO-1 is normally expressed at very low levels but increases dramatically after exhaustive exercise (9, 15). The results from the present study confirm that HO-1 is also markedly induced in skeletal muscle of humans during and after exercise. Interestingly, however, exercise elicited minimal change in the expression of αBC, a small molecular weight stress protein that has previously been shown to increase dramatically in skeletal muscle of rabbits during recovery from motor nerve stimulation (26). The rapid activation of HO-1 transcription and the more pronounced increases in HO-1 mRNA elicited by the one-legged knee extensor vs. the prolonged bout of cycling exercise also raise the possibility that HO-1 induction may be sensitive to exercise intensity. Although the physiological role of HO-1 in muscle has not been established, partially reduced ROS are produced in human skeletal muscle during intense exercise (14). Thus it is conceivable that both HO-1 and UCP3 (discussed above) may be induced in response to exercise to help minimize intracellular damage by free radicals, similar to the protective role of HO-1 in other tissues (22, 28). Moreover, it may be speculated that the elevated HO-1 mRNA content found immediately before the 5th day of exercise reflects an adaptive response that improves the capacity of the trained muscle for scavenging free radicals.

In an effort to maximize the chances of detecting significant responses at the transcriptional level, two experimental protocols were used in the present study. On the basis of work by Seip et al. (31), we speculated
that the one-legged knee extensor exercise protocol performed for 5 days would isolate a specific muscle group in the midst of a maximal "training" response, whereas the single 4-h cycling bout might elicit a maximal adaptive response to an acute endurance exercise stimulus. Although different subjects were used for each protocol, limiting direct comparison, some clear differences in the adaptive responses emerged. Transcriptional activation of UCP3, HKII, and LPL peaked much earlier in the recovery period (1–2 h postexercise) after 5 days of one-legged knee extensor exercise compared with the single 4-h cycling bout (>4 h postexercise). Obvious differences between the two protocols, such as the type, intensity, and duration of exercise, may have accounted for these findings. Alternatively, the five consecutive days of one-legged knee extensor exercise may have resulted in the accumulation of a transcription factor(s) or other regulatory protein(s) that enhanced the sensitivity of the myofibers to subsequent exercise bouts. Such a training-induced improvement in the sensitivity and/or responsiveness is consistent with the fact that CPT I and GYS transcription and mRNA showed little to no change during recovery from the 4-h cycling bout but significantly increased during recovery from the fifth bout of one-legged knee extensor exercise.

The results of the present study provide insight into the kinetics governing the induction and maintenance of the cellular adaptations associated with exercise training. It is clear that exercise represents an acute stimulus that triggers, as a primary response, a transient increase in the transcription of target genes. However, this does not translate into a one-for-one increase in gene product, because the turnover rates of both mRNA and protein are first-order processes; thus the cumulative effects of several days or weeks of transient increases in transcription are likely required for eliciting detectable changes in mRNA or protein for particular genes (39). Moreover, many of the traditional clinical benchmarks (reductions in circulating cholesterol, triglycerides, and the like) associated with exercise programs will require several weeks to months to become evident. On the basis of epidemiological data, however, recent recommendations concerning the type, intensity, duration, and frequency of exercise necessary to achieve improvements in cardiovascular risk factors have been controversial (2). The ability to assay transcriptional changes as a primary response of the cell to exercise will provide a valuable means for defining the importance of specific parameters in the regulation of target genes in humans. Moreover, defining the window during which molecular events are taking place provides the necessary framework for studies designed to identify the underlying signaling and gene regulatory events controlling the response of the muscle cell to exercise.

In summary, the results from the present study demonstrate that recovery from exercise is associated with the transient increases in transcription of several metabolically related genes in human skeletal muscle. Therefore, these findings suggest that transcriptional activation of target genes is a primary adaptive response to exercise within muscle cells and that the cumulative effects of transient increases in transcription during recovery from consecutive bouts of exercise may represent the underlying kinetic basis for the cellular adaptations associated with exercise training.

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REFERENCES


