Time course of myogenic and metabolic gene expression in response to acute exercise in human skeletal muscle

Yifan Yang, Andrew Creer, Bozena Jemiolo, and Scott Trappe

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Yang, Yifan, Andrew Creer, Bozena Jemiolo, and Scott Trappe. Time course of myogenic and metabolic gene expression in response to acute exercise in human skeletal muscle. J Appl Physiol 98: 1745–1752, 2005. First published December 23, 2004; doi: 10.1152/japplphysiol.01185.2004.—The aim of this study was to examine the time course activation of select myogenic (MRF4, Myf5, MyoD, myogenin) and metabolic (CD36, CPT1, HKII, and PDK4) genes after an acute bout of resistance (RE) or run (Run) exercise. Six RE subjects (25 ± 4 yr, mean ± SD, 74 ± 14 kg, 1.71 ± 0.11 m) and six Run subjects (25 ± 4 yr, 72 ± 5 kg, 1.81 ± 0.07 m, 63 ± 8 ml·kg⁻¹·min⁻¹) were studied. Eight muscle biopsies were taken from the vastus lateralis (RE) and gastrocnemius (Run) before, immediately after, and 1, 2, 4, 8, 12 and 24 h after exercise. RE increased mRNA of MRF4 (3.7– to 4.5-fold 2–4 h post), MyoD (5.8-fold 8 h post), myogenin (2.6- and 3.5-fold 8–12 h post), HKII (3.6- to 10.5-fold 2–12 h post), and PDK4 (14- to 26-fold 2–8 h post). There were no differences in Myf5, CD36, and CPT1 mRNA levels 0–24 h post-RE. Run increased mRNA of MyoD (5.0- to 8.0-fold), HKII (12- to 16-fold), and PDK4 (32- to 52-fold) at 8–12 h postexercise. There were no differences in Myf5, myogenin, CD36 and CPT1 mRNA levels 0–24 h post-Run. These data indicate a myogenic and metabolic gene induction with exercise. The timing of the gene induction is variable and generally peaks 4–8 h postexercise with all gene expression not significantly different from the preexercise levels by 24 h postexercise. These data provide basic information for the timing of human muscle biopsy samples for gene-expression studies involving exercise.

MRF4; Myf5; MyoD; myogenin; FAT/CD36; CPT1; HKII; PDK4

With the mapping of the human genome and advancement in molecular biology techniques, there is a growing interest involving gene expression and regulation with exercise. One of the goals of our laboratory is to better understand and relate various molecular adaptations at the cellular level in skeletal muscle tissue to whole body adaptations in response to acute and chronic exercise in humans. There are a host of myogenic and metabolic genes that play critical roles in cell regulation and many more that have yet to be defined. Important regulators of muscle cell growth and/or differentiation include muscle regulatory factor 4 (MRF4), myogenic factor 5 (Myf5), myogenic differentiation (MyoD), and myogenin (24). Some genes that are important regulators of carbohydrate or fat metabolism include fatty acid translocase (FAT/CD36), carnitine palmitoyltransferase 1 (CPT1), hexokinase II (HKII), and pyruvate dehydrogenase kinase 4 (PDK4) (15).

It has been suggested that training adaptations at the cellular level are the result of cumulative effects of transient changes in gene transcription after each acute bout of exercise (7, 21). In human studies, transient changes in mRNA levels of the above myogenic (10, 38) or metabolic (17, 18, 26, 27, 35) genes after an acute bout of exercise have been reported. These studies were limited to time points that did not extend beyond 6 h after exercise. Although this information has provided valuable information regarding the molecular response in the first few hours after exercise, data beyond these time points are warranted for a more complete understanding for gene induction with exercise in human skeletal muscle.

More recently, human and animal studies have examined gene expression beyond 6 h after exercise. These studies found no increase in HKII and PDK4 mRNA beyond 8 h after high-intensity intermittent one-legged knee extension (22) or 3 h of knee extension (29, 36). No change in mRNA levels of MRF4 and MyoD beyond 12 h after an acute bout of resistance exercise has been reported (2, 30, 36, 40). In contrast, Bickel et al. found an increase in myogenin mRNA 24 h after a single (2) or second (2, 3) electromyostimulation-induced resistance exercise bout. Animal studies investigating gene expression of the above myogenic (9) and metabolic (23) genes after an acute bout of exercise have reported that HKII mRNA levels return to baseline within 24 h after run exercise (23). Conversely, myogenin mRNA was elevated or remained elevated at 24 h after electromyostimulation-induced resistance exercise (9). Thus there does not appear to be universal agreement on the timing aspects related to gene induction with exercise in skeletal muscle. Certainly, mode and intensity of exercise as well as the muscle and species studied contribute to these varied results.

To date, no studies have examined the effects of resistance or run exercise on both myogenic and metabolic gene expression in human skeletal muscle. Given that most key myogenic and metabolic genes appear to return to baseline within 24 h, it would be informative to know when these regulators peak after an acute bout of exercise in humans. Such knowledge could aid the timing of biopsies for future human-based exercise studies and provide a greater understanding of the molecular responses to exercise. Therefore, the purpose of this study was to examine the time course of select myogenic and metabolic gene induction after an acute bout of resistance (RE) or run (Run) exercise at different time points over a 24 h-period. The selected myogenic genes were MRF4, Myf5, MyoD, and myogenin, whereas the selected metabolic genes were CD36, CPT1, HKII, and PDK4. To our knowledge, this is the first human study to investigate gene expression in the gastrocnemius muscle after an acute bout of aerobic exercise. The gastrocnemius and vastus lateralis were selected in this study.
because they were best suited to study for RE and Run, respectively.

MATERIALS AND METHODS

Subjects. A total of 12 nonsmoking, nonobese, and physically active volunteers participated in this research. Six volunteers (2 women and 4 men) participated in the RE protocol, whereas the other six volunteers (1 woman and 5 men) participated in the Run protocol (Table 1). Subjects involved in RE group had been performing resistance exercise approximately two times per week. Subjects involved in the Run group had been running 3–5 times per week. All subjects were given oral and written information about the experimental procedures and potential risks before giving their informed consent approved by the Institutional Review Board of Ball State University.

Experimental design. This study consisted of a resistance exercise (RE) bout and a submaximal running (Run) bout performed by two different groups of subjects (recruited separately). All subjects were familiarized with the trial procedures and equipment and had their body mass, height, and skinfolds measured before the trial. In the RE group, subjects performed three sets of 10 repetitions at 70% of concentric one-repetition maximum (1-RM) of bilateral knee extensions on a Cybex Eagle knee extensor (Cybex, Medway, MA). Subjects were tested for their 1-RM Lifted after the trial. Subjects involved in the Run group had been running 3–5 times per week. All subjects were given oral and written information about the experimental procedures and potential risks before giving their informed consent approved by the Institutional Review Board of Ball State University.

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1-RM, concentric 1-repetition maximum of bilateral knee extension. MHC, myosin heavy chain; VO2max, maximal O2 uptake.

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Fig. 1. Schematic of experiment design. EX, exercise, either 3 sets of 10 repetitions at 70% of concentric 1-repetition maximum of bilateral knee extensions for the resistance exercise (RE) group or 30 min of treadmill running at 75% of maximal O2 uptake (VO2max) for the run exercise (Run) group.
tational signals (DNA contamination or RNA degradation) below the ribosomal bands and no shifts to lower fragments.

Reverse transcription. Oligo(dT)-primed first-strand cDNA were synthesized using SuperScript II RT (Invitrogen, Carlsbad, CA). This system was optimized for sensitive RT-PCR on low amounts of RNA. A first reaction mix of 10 μl for each sample, consisting of 1 μl of RNA extract, 1 μl of 10 mM dNTP, 1 μl of oligo(dT)12–18 (0.5 μg/μl), and 7 μl of DNase- and RNase-free water, was incubated at 65°C for 5 min and then placed on ice for 1 min. A second reaction mix of 9 μl consisting of 2 μl of 10× RT buffer, 4 μl of 25 mM MgCl2, 2 μl of 0.1 M DTT, and 1 μl of RNaseOUT recombinant RNase inhibitor, was then added to the first reaction mix and incubated at 42°C for 2 min. Finally, 1 μl (50 units) of SuperScript II RT was added to each tube (giving a total volume of 20 μl), incubated at 42°C for 50 min and then at 70°C for 15 min to terminate the reaction, and chilled on ice thereafter. Produced cDNA samples were diluted to a final volume of 60 μl (1 μl of RNA extract: 60 μl of total reaction volume). All incubations were done in the Peltier thermal cycler with eight cycles of 30 s hold at each degree. A single melt peak observed as it had been shown that its expression remained stable using the same Run protocol (14). The reaction mix consisted of 2.5 μl of 10× SYBRgreen real-time PCR buffer (Biosource, Camarillo, CA), 0.625 μl of 10 mM dNTPs, 0.4 μl of 5 U/μl platinum Taq DNA polymerase (Invitrogen), 1 μl each of forward and reverse primers in the case of the eight genes of interest (GOI) or 2.5 μl of 10× primer pairs for GAPDH in the case of GAPDH, 2.5 μl of cDNA, and RNase-free water to 25 μl. All primers used in this study were “mRNA specific” and designed for gene-expression analysis (MRF4, MyoD, and myogenin: Ref. 30; Myf5: GenBank NM 005593; CD36: GenBank NM 000072, Primer ID 4557419a2; CPT1: Ref. 28; HKII and PDK4: Ref. 22; Table 2).

The PCR parameters were initial denaturing at 95°C for 2 min to activate the platinum Taq DNA polymerase followed by 45 cycles of 20 s at 95°C, 20 s at 60°C (61.5°C for CD36), and 20 s at 72°C, with fluorescence gain for SYBRgreen set at 8. A melting curve analysis was generated by the Roto-Gene software after the end of the final cycle for each sample, by continuously monitoring the SYBRgreen fluorescence throughout the temperature ramp from 72 to 99°C in 1° increments and 5 s hold at each degree. A single melt peak observed for each sample was used to validate that only a single product was present.

Table 2. Primer sequences used for real-time PCR

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<td>PDK4</td>
<td>5′ TCCAGTGGCAAGAGGCTCT 3′</td>
<td>5′ TGGCAAGGCGTAAGCACAAGAA 3′</td>
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Relative quantification of real-time PCR assay. The influence of the RE and Run exercises on the human muscle gene expression was evaluated by a relative quantification method. This method is based on the fact that the difference in threshold cycles (ΔC_T) between GOI and HKG is proportional to the relative expression level of the GOI. To ensure the accuracy of the obtained data, the reaction efficiencies of the two assays should be similar or a correction factor must be introduced into the calculation (see Validation of the 2^−ΔΔC_T method).

In the present study the change in expression of target gene normalized to GAPDH was monitored over 24 h. Real-time PCR using SYBRgreen detection was performed on the corresponding cDNA (in duplicates) synthesized from each sample. The data were analyzed using the following equation:

\[
\text{Fold change} = 2^{-\Delta\Delta C_T}(\text{Ref. 19})
\]

where \( \Delta C_T = (C_T \text{ target} - C_T \text{ reference}) \text{time x } - (C_T \text{ mean target} - C_T \text{ mean reference}) \text{time 0} \), time x is any of the time point of interest, and time 0 represents the 1 × expression of the target gene normalized to GAPDH (19). The mean \( C_T \) values for both the target and GAPDH genes were determined at time zero (Pre) and were used in the equation. The fold change in target gene, normalized to GAPDH and relative to the expression at time zero, was calculated for each sample by use of the above equation.

With the use of this analysis, the value of the mean fold change at time zero should be very close to 1 (i.e., because \( 2^{0} = 1 \)). The verification of the mean fold changes at time zero is a convenient method to check for errors and variation among the samples at time zero (4, 6, 14, 19). A value that is very different from 1 suggests a high degree of experimental variation. In this study the fold change for the target genes normalized to GAPDH at time zero was 1.09 ± 0.005 for RE and Run together.

Validation of the 2^−ΔΔC_T method. Serial dilutions (1, 0.5, 0.250, 0.125, 0.062, 0.031) of cDNA for GAPDH and target genes were amplified by real-time PCR using gene-specific primers. The amplification calculated by Rotor-Gene software was specific and highly efficient (1.007 ± 0.001) for all genes. The \( R^2 \) was 0.981 ± 0.001 (RE) and \( R^2 = 0.989 ± 0.001 \) (Run) and intra-assay coefficient of variation values were 8.01 and 8.98% for the RE and Run groups, respectively.

In addition, the validation of the reference gene was performed to ensure that its expression was unaffected by experimental variation. In this study the fold change for GAPDH across the time points for RE was 1.08 ± 0.0118 and for Run was 1.06 ± 0.01 with a slope of −0.0131.

Fiber-composition analysis. Muscle samples were homogenized in 40 volumes of cold homogenizing buffer containing (in mM) 250 sucrose, 100 KCl, 20 imidazole, and 5 EDTA (pH 6.8) and spun for 30 min at 20,000 g and 4°C. The supernatant was then removed and the pellet resuspended in 40 volumes of cold homogenization buffer (34). An equal volume of sample buffer [10% SDS, 6 mg/ml EDTA, 0.06 M Tris (pH 6.8), 2 mg/ml bromphenol blue, 15% glycerol, and 5% β-mercaptoethanol] was added, and the samples were stored at −20°C until assayed. To determine myosin heavy chain (MHC) composition, homogenized samples were subjected to SDS-PAGE as described previously (37). Briefly, samples were run overnight at 4°C on a Hoefer SE 600 gel electrophoresis unit utilizing a 3.5% (wt/vol) acrylamide stacking gel with a 5% separating gel. After electrophoresis, the gels were silver stained as described by Giulian et al. (8). MHC isoforms were identified according to migration rate and comigration with muscle weight standards. The MHC was categorized as MHC I, Ila, or IIX, and percentages were determined by densitometry (Chemilnager 4000, Alpha Innotech, San Leandro, CA).

Statistical analysis. Data were checked for normality and equality of variances. Assumptions for parametric analyses were violated and data transformation was not feasible. As such, the changes in mRNA levels with respect to the preexercise levels at the various time points postexercise for each studied gene were analyzed by using the non-parametric Friedman test. Significant differences between postexercise levels and preexercise level for each gene were determined by Dunn post hoc analysis. Significance was set at \( P < 0.05 \) (two-tailed). Statistical analyses were performed by using SPSS 10.0 for Windows and Prism 4 for Windows (Graphpad, San Diego, CA) software packages. Data are presented as means ± SE.

RESULTS

mRNA changes with RE. The fold changes in mRNA levels of the select myogenic and metabolic genes normalized to GAPDH and relative to preexercise levels for RE are shown in Fig. 3. Increased gene expression of five genes was observed within 4–8 h post-RE. MRF4 mRNA increased (\( P < 0.05 \)) 3.7- and 4.5-fold at 2 and 4 h postexercise, respectively. Myogenin mRNA increased (\( P < 0.05 \)) 3.5- and 2.6-fold at 8 and 12 h postexercise, respectively, whereas MyoD mRNA increased (\( P < 0.05 \)) 5.8-fold at 8 h postexercise. HKII mRNA increased (\( P < 0.05 \)) 3.6- and 5.2-fold at 2 and 4 h postexercise, respectively. HKII peaked (\( P < 0.05 \)) 10.5-fold at 8 h postexercise and remained elevated (\( P < 0.05 \)) at 12 h postexercise (3.8-fold). PDK4 mRNA increased (\( P < 0.05 \)) 14-, 26-, and 20-fold at 2, 4, and 8 h postexercise, respectively. There were no differences in Myf5, CD36, and CPT1 mRNA levels 0–24 h after RE from preexercise levels. The mRNA levels of all GOI were not significantly different from the preexercise levels by 24 h post-RE.

mRNA changes with Run. The fold changes in mRNA levels of the select myogenic and metabolic genes normalized to GAPDH and relative to preexercise levels for Run are shown in Fig. 4. MyoD, HKII, and PDK4 mRNA increased (\( P < 0.05 \)) at 8 and 12 h postexercise. MyoD mRNA peaked (\( P < 0.05 \)) 8.0-fold at 8 h postexercise and remained elevated (\( P < 0.05 \)) 12 h postexercise (5.0-fold). HKII mRNA increased (\( P < 0.05 \)) 16- and 12-fold, whereas PDK4 mRNA increased (\( P < 0.05 \)) 32- and 52-fold, at 8 and 12 h postexercise, respectively. There were no differences in Myf5, myogenin, MRF4, CD36, and CPT1 mRNA levels 0–24 h after Run from preexercise levels. The mRNA levels of all GOI were not significantly different from the preexercise levels by 24 h post-Run.

DISCUSSION

The aim of the present investigation was to examine select myogenic and metabolic genes to determine peak induction over a 24-h period in response to an acute bout of resistance exercise or run exercise. The main findings from this investigation showed that 1) an increase in mRNA transcripts was observed for both RE (MRF4, myogenin, MyoD, HKII, and PDK4) and Run (MyoD, HKII, and PDK4) exercises; 2) the timing of the gene induction was variable, with peak gene expression occurring 4–8 h after the exercise bout; 3) all mRNA levels were not significantly different from the preexercise levels within 24 h after the exercise bout; and 4) no induction was observed for certain genes over a 24-h period after RE (Myf5, CD36, and CPT1) and Run (Myf5, myogenin, MRF4, CD36, and CPT1) exercises.

Resistance exercise responses. Activation of the myogenic genes in response to resistance exercise occurred as early as 2 h
MRF4 and remained elevated as late as 12 h (myogenin). Peak induction appears to occur 4–8 h after exercise with little response in the early phase immediately after resistance exercise. These data are in agreement with other studies (10, 30, 36, 38, 40), which have reported changes in select myogenic genes 2–6 h after resistive type exercise.

Psilander et al. (30) and Vissing et al. (36) found that MyoD increased immediately postexercise, which may be partly due to the feeding before the exercise (36) or differences in exercise protocol. In the study by Psilander et al. (30), the authors reported a sustained return to baseline levels of myogenic genes within 24 h after an exhaustive resistance exercise session over a 48-h recovery period, which is in agreement with the present investigation. Thus it appears that feedings before exercise can influence the gene induction response in the early phases after exercise, but this is not sustained and returns to baseline within 24 h independent of preexercise feedings. Myogenin has been reported to be elevated two- to threefold at 24 h after a single (2) or second bout (2, 3) of electromyostimulation-induced resistance exercise, which differs from previous studies (10, 30, 36, 38, 40) and the present investigation. Both of these studies (2, 3) utilized an electrical stimulation protocol to induce RE responses, which differs from the dynamic RE protocol used in the present investigation. It is reasonable to assume that the differences in myogenic gene expression in the extended recovery phase after muscle activation may be related to protocol (multiple sessions vs. single session) and the type of muscle activation (stimulation vs. voluntary lifting) used in the investigations.

Acute resistance exercise had a modest impact on the studied metabolic genes investigated in this study. To our knowledge, this is the first study to examine selected metabolic gene induction in response to resistance exercise. Markers of carbohydrate metabolism, HKII and PDK4, were elevated within 2 h of the exercise bout and peaked at 4 and 8 h, respectively. These data are in agreement with aerobic studies showing that HKII and PDK4 have a robust response to exercise (17, 18, 22, 26, 27, 29, 36). In contrast, CD36 and CPT1, which are important regulators of fat metabolism (15), were not altered in the 24 h after resistance exercise, which is in agreement with other aerobic exercise studies (27 Study B, 35). Therefore, it seems that, other than the more anaerobic nature of the RE bout, these genes (CD36 and CPT1) of fat metabolism do not appear to be influenced by acute exercise or that their mRNA levels do not change within 24 h postexercise.

Running responses. This is the first study in human skeletal muscle to examine myogenic genes in the gastrocnemius muscle with run exercise. Interestingly, 30 minutes of running at
75% of Vo2 max was sufficient to increase MyoD, a transcription factor that is involved in myogenesis. The other myogenic genes measured in this investigation, Myf5, myogenin, and MRF4, were relatively unchanged in the 24 h after the run exercise. Kadi et al. (16) found that a single bout of endurance exercise (one-legged cycle ergometry) was sufficient to increase myogenin expression in the vastus lateralis. The differences in mode of exercise or muscle phenotype make direct comparisons difficult, but the study by Kadi et al. and the present investigation indicate that endurance exercise can lead to an induction of select myogenic markers. In this regard, the mode of exercise should be considered because the mechanical stress to the muscle most likely differs between running and cycle-type exercise. In particular, running exercise is not purely metabolic because it has an eccentric component, which could trigger the myogenic response due to muscle damage. Indeed, eccentric muscle actions (25) and running (31) have been shown to alter key myogenic genes (MyoD and myogenin) in rats and early response genes in humans, respectively.

Although MyoD was the only myogenic gene induced in response to run exercise, the increases were fairly robust with 5- to 8-fold changes occurring 8–12 h after the run. What these data imply for muscle adaptation to run exercise is unknown. However, chronic run training leads to an increase in MHC I fibers and a decrease in hybrid muscle fibers (12) and atrophy of the slow-twitch muscle fibers (11). With this in mind, it is unlikely that an increase in myogenic precursors with run exercise would be an indicator of muscle hypertrophy. MyoD has been shown to be more prevalent in fast-twitch muscle fibers (13) and may be involved in the regulation of muscle phenotype (5, 20). Because submaximal run exercise is more reliant on oxidative type of muscle fibers, the recruitment of glycolytic muscle fibers during this type of exercise may be enough of a metabolic challenge to induce transient changes in gene expression that would contribute to alterations in muscle phenotype that would eventually result in a more oxidative muscle. Our previous data (11, 12) coupled with the present observation that MyoD mRNA increases with acute run exercise may be an indication of transcriptional regulation of fiber-specific alterations in MHC expression.

As with the resistance exercise bout, the running bout resulted in an increase in HKII and PDK4 mRNA and a return to baseline levels within 24 h, which is in agreement with previous studies (18, 22, 26, 27 Study A, 29, 36). However, these previous studies reported earlier increases in HKII (3–6 h postexercise) and PDK4 (1–4 h postexercise) compared with...
the present study (8 h postexercise). This could be due to the longer duration (1–4 h) of endurance exercise used (17, 18, 27 Study B, 39) as opposed to our 30 min of treadmill running protocol. High-intensity intervals (22), prior exercise (26), short-term training of 5 consecutive days (27 Study A), and prior feeding of high glycemic meal (36) might also have caused an earlier increase in HKII and PDK4 mRNA with exercise.

The observation that CD36 and CPT1 did not change with the run exercise bout is in agreement with previous studies using aerobic exercise bouts lasting 1 and 4 h in duration (27 Study B, 35). When untrained subjects undergo an aerobic exercise training program (5 days to 6 wk), CD36 and CPT1 mRNA have been shown to increase (27 Study A, 32, 35). It is possible that the 30-min run at 75% of \( \dot{V}O_{2\text{max}} \) might not be a great enough stimulus to induce changes in CD36 and CPT1 mRNA levels given that our recreationally active Run subjects were aerobically fit (mean \( \dot{V}O_{2\text{max}} \) of 63 ml·kg\(^{-1}\)·min\(^{-1}\)). However, CD36 and CPT1 mRNA were not increased after an acute bout of aerobic exercise in both the trained (5–9 days of aerobic training) and untrained states (27 Study A, 35). Thus it appears that these markers of fat metabolism are not altered with acute exercise. Or perhaps they are involved in other modes of transcriptional control that fall outside the range of the 24-h period used in the present investigation.

Additional considerations. Given that different subjects, different muscles, and a different mode of exercise were used for each protocol, direct comparisons between the RE and Run protocols are difficult. However, it is interesting to note that both modes of exercise induced a response in select myogenic and metabolic genes to the acute exercise bout. Fiber-type differences between the muscles studied must also be taken into account because it has recently been shown that slow- and fast-twitch fibers differ in their gene-expression profile with exercise (14). With this in mind, the fiber-type profiles of the RE and Run subjects observed in the present investigation (Table 1) most likely contributed to the gene-expression responses of the acute exercise bout.

When these data are put in context with previous studies, it is clear that there are a host of variables that can influence the gene induction response to exercise in humans. We chose to study our subjects in the postprandial state and wait until after the 8-h biopsy before feeding. This is an important consideration because fasting and feeding have both been shown to alter metabolic gene expression (28, 33). Additionally, muscle biopsy procedures (36) and training status among subjects (39) need to be taken into consideration when comparing human studies.

In summary, this is the first study to investigate both myogenic and metabolic gene expression with acute bouts of resistance or endurance exercise. In general, there is an induction of selected myogenic and metabolic genes with both forms of exercise. Induction of these genes occurs as early as 2 h and last up to 12 h postexercise. Peak induction of the responsive myogenic and metabolic genes generally occurs between 4 and 8 h after the exercise bout. These data provide basic timeline information for studies examining aspects of gene regulation with acute exercise in humans.

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