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Time course of proteolytic, cytokine, and myostatin gene expression after acute exercise in human skeletal muscle

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Louis E, Raue U, Yang Y, Jemiolo B, Trappe S. Time course of proteolytic, cytokine, and myostatin gene expression after acute exercise in human skeletal muscle. *J Appl Physiol* 103: 1744–1751, 2007. First published September 6, 2007; doi:10.1152/japplphysiol.00679.2007.—The aim of this study was to examine the time course induction of select proteolytic [muscle ring finger-1 (MuRF-1), atrogin-1, forkhead box 3A (FOXO3A), calpain-1, calpain-2], myostatin, and cytokine (IL -6, -8, -15, and TNF-α) mRNA after an acute bout of resistance (RE) or run (RUN) exercise. Six experienced RE (25 ± 4 yr, 74 ± 14 kg, 1.71 ± 0.11 m) and RUN (25 ± 4 yr, 72 ± 5 kg, 1.81 ± 0.07 m) subjects had muscle biopsies from the vastus lateralis (RE) or gastrocnemius (RUN) before, immediately after, and 1, 2, 4, 8, 12, and 24 h postexercise. RE increased ($P < 0.05$) mRNA expression of MuRF-1 early (3.5-fold, 1–4 h), followed by a decrease in atrogin-1 (3.3-fold) and FOXO3A (1.7-fold) 8–12 h postexercise. Myostatin mRNA decreased (6.3-fold; $P < 0.05$) from 1 to 24 h postexercise, whereas IL-6, IL-8, and TNF-α mRNA were elevated 2–12 h. RUN increased ($P < 0.05$) MuRF-1 (3.6-fold), atrogin-1 (1.6-fold), and FOXO3A (1.9-fold) 1–4 h postexercise. Myostatin was suppressed (3.6-fold; $P < 0.05$) 8–12 h post-RUN. The cytokines exhibited a biphasic response, with immediate elevation ($P < 0.05$) of IL-6, IL-8, and TNF-α, followed by a second elevation ($P < 0.05$) 2–24 h postexercise. In general, the timing of the gene induction indicated early elevation of proteolytic genes, followed by prolonged elevation of cytokines and suppression of myostatin. These data provide basic information for the timing of human muscle biopsy samples for gene expression studies involving exercise. Furthermore, this information suggests a greater induction of proteolytic genes following RUN compared with RE.

muscle ring finger-1; atrogin-1; myostatin; interleukin-6; interleukin-8

IN RESPONSE TO EXERCISE, TRANSIENT and varied changes in gene expression lead to specific training adaptations that have been well documented (59). Given this transient nature, it is important to elucidate the time course of gene expression to better understand the exercise response at the molecular level. To date, most human research has focused on gene expression at a single time point following exercise, which has varied from immediate postexercise through 24 h. However, this may potentially leave gaps in the existing knowledge regarding the timing of peak gene expression. Recently, a more detailed time course for myogenic mRNA in the 24 h following exercise (62) and electrical stimulation (3) has provided insight into the induction of key regulatory genes involved with muscle growth. Furthermore, these data have helped elucidate the optimal timing for muscle biopsy studies in humans examining the transient myogenic response to exercise.

While several investigations have focused on molecular events leading to muscle growth with exercise (3, 43, 62), there are limited human data available addressing the proteolytic events of the muscle following exercise (63). To provide a better understanding of the muscle remodeling process, the time course for genes associated with muscle proteolysis is warranted. Recently, several genes involved with catabolic events following exercise have been identified. For this study, we examined three categories of genes involved with proteolysis and inflammation with exercise. The first of these categories is proteolytic genes. Proteolytic events involve the ubiquitination and subsequent degradation of proteins marked by E3 ligases (18, 44). Exercise-responsive genes involved in the muscle cell ubiquitin/proteolysis pathway (UPP) include muscle ring finger-1 (MuRF-1), and atrogin-1 (muscle atrophy F-box), both of which share a common transcription factor, forkhead box 3A (FOXO3A). Also included in this proteolytic category are calpain-1 and calpain-2, which assist in disassembly of sarcomere proteins (24). The second category consists of the gene, myostatin, which is a negative regulator of muscle growth. Suppression of myostatin has been found to induce hypertrophy and hyperplasia in rodents (33, 53). The third category involves cytokines related to muscle inflammation. Cytokines released from muscle, specifically IL-6, -8, and -15, recently termed myokines (39), have been found to facilitate myriad cellular responses to exercise, including proteolytic suppression, angiogenesis, and regulation of muscle glycogen (39, 40). The cytokine TNF-α is associated with trauma or disease and has been found to lead to muscle catabolism (45).

The purpose of this investigation was to establish the time course of selected genes involved with catabolic events in skeletal muscle following run and resistance exercise. Identification of the time course of genes involved with muscle proteolysis and inflammation in conjunction with the already established time course of myogenic and metabolic genes (3, 62) will provide further insight into the muscle response and adaptation process following an acute bout of run or resistance exercise. An additional objective of this project was to compare the observed gene induction between two distinct modes of exercise that result in differing adaptations when performed chronically. Finally, this study will build on previous research (3, 62) to provide more insight for the optimal timing of muscle biopsies associated with human exercise physiology experiments investigating proteolytic, myostatin, and cytokine gene induction following a single bout of exercise.

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MATERIALS AND METHODS

Subjects

Twelve nonsmoking, nonobese, and physically active volunteers participated in this research. Six volunteers (2 women and 4 men) participated in the resistance exercise (RE) protocol, and six volunteers (1 woman and 5 men) participated in the submaximal running (RUN) protocol (Table 1). Subjects involved in the RE group had been performing resistance exercise approximately two times per week. Subjects involved in the RUN group had been running three to five 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

All subjects were familiarized with the procedures and equipment and had their anthropometric measures made before the trial. In the RE group, subjects performed 3 sets of 10 repetitions at 70% of concentric one repetition maximum of bilateral knee extensions on a Cybex Eagle Knee Extensor (Cybex, Medway, MA). Subjects were tested for their one repetition maximum on the same device approximately 1 wk before the trial. For the RUN trial, subjects performed 30 min of treadmill running at 75% of maximum O_2 uptake. Subjects completed a maximum O_2 uptake test using an incremental treadmill test to voluntary exhaustion on the same treadmill ~10 days before the trial. Oxygen uptake was measured using indirect calorimetry, as previously described (62).

For both RE and RUN, subjects refrained from physical activity for at least 48 h before the preexercise muscle biopsy and rested in a supine position for at least 30 min before each muscle biopsy, with the exception of the immediate postexercise time point. All trials began at ~6 to 7 AM. After the first and through the 8-h postexercise muscle biopsies, subjects rested quietly in the laboratory. Thereafter, subjects were allowed normal ambulation and returned to the laboratory for the 12- and 24-h postexercise muscle biopsies. Subjects also fasted with ad libitum water intake at least 8 h before lying down for the preexercise muscle biopsy and through the 8-h postexercise muscle biopsy. Thereafter, they were fed standardized meals. The 24-h postexercise muscle biopsy was performed after an overnight fast of at least 8 h.

Muscle Biopsies

Eight muscle biopsies (2) were taken from the vastus lateralis of RE subjects and from the gastrocnemius (lateral head) of RUN subjects pre-, immediately post-, and 1, 2, 4, 8, 12, and 24 h postexercise. The muscle biopsies alternated between legs, with each biopsy being more proximal than the one before on the same lower limb. Previous research has shown that the multiple biopsy technique does not alter exercise-specific gene expression and provides support for the present multiple biopsy study design (1, 15, 30). Following each muscle biopsy, the muscle sample was placed in 0.5 ml of RNA*Later* (Ambion, Austin, TX) and stored at −20°C until RNA extraction. Muscle biopsies were taken from different muscles between modes of exercise. However, both the vastus lateralis and gastrocnemius are mixed muscle, and it was our intention to measure gene expression in the muscles that are engaged in the specific mode of exercise and that have been commonly studied in exercise physiology research.

Table 1. *Subject characteristics*

	RE	RUN
Age, yr	25±4	25±4
Weight, kg	74±14	72±5
Height, m	1.71±0.11	1.81±0.07
%Body fat	13±6	12±8
%MHC I	33.0±10.1*	56.2±3.0
%MHC IIa	51.5±5.9*	40.5±3.4
%MHC IIx	15.5±5.5*	3.3±5.2
1 RM, kg	99±27	
%1 RM lifted	70±1	
$\dot{V}O_{2max}$, l/min		4.5±0.7
$\dot{V}O_{2max}$, ml·kg^{-1}·min^{-1}		63±8
Avgerage trial, %$\dot{V}O_{2max}$		75±4

Values are means± SE. RE, resistance exercise; RUN, running exercise; MHC, myosin heavy chain; 1 RM, concentric one repetition maximum of bilateral knee extension; $\dot{V}O_{2max}$, maximum O_2 uptake. *$P < 0.05$ from RUN.

Total RNA Extraction, RNA Quality Check, and Reverse Transcription

Each muscle sample was removed from the RNA*Later* and placed in a mixture of 0.8 ml of TRI Reagent and 4 μl of PolyAcryl Carrier (Molecular Research Center, Cincinnati, OH). The tissue was homogenized, and total RNA was extracted according to the manufacturer's protocol. The RNA pellet was dissolved in 30 μl of nuclease-free water and stored at −80°C. One microliter of total RNA from each sample was analyzed using the RNA 6000 Nano LabChip kit on an Agilent 2100 Bioanalzyer (Agilent Technologies, Palo Alto, CA). The high quality of RNA was confirmed by the presence of ribosomal peaks, with no additional signals from DNA contamination or RNA degradation. On average, the yield of total RNA from 10–15 mg of muscle tissue was 158.94 ng/μl (SE ± 9.83) for RUN and 157.38 ng/μl (SE ± 12.82) from RE subjects.

Oligo(dT) primed first-strand cDNA were synthesized using SuperScript II RT (Invitrogen, Carlsbad, CA), according to the manufacturer's protocol. This system was optimized for sensitive RT-PCR on low amounts of RNA. Produced cDNA samples were diluted to a final volume of 60 μl (1 μl of RNA in water for 60 μl of total volume). All incubations were done in the Peltier Thermal Cycler DNA engine (MJ Research, Waltham, MA) to provide temperature homogeneity and identical temperature ramping for all samples.

Real-Time PCR

Quantification of mRNA content for all genes of interest (GOIs) and GAPDH was performed in duplicate in a Rotor-Gene 3000 Centrifugal Real-Time Cycler (Corbett Research, Mortlake, NSW, Australia). Housekeeping gene GAPDH was used for internal control, as we have reported in our previous papers that its expression remained stable under the RUN and RE protocols used in our laboratory (25). The PCR reaction mix for each GOI and GAPDH consisted of SYBRgreen JumpStart Taq (Sigma, St. Louis, MO), forward and reverse primers in final concentration of 0.2 mM, DNase-free water, and cDNA template (2.5 μl) in total reaction volume of 25 μl. All primers used in this study were mRNA specific (on different exons and crossing over an intron) and designed for gene expression real-time PCR analysis using Vector NTI Advance 9 software (Invitrogen) (Table 2).

The PCR parameters were as follows: initial denaturing at 95°C for 2 min followed by 45 cycles of 20 s at 95°C, 20 s at annealing temperature specific to each primer set (Table 2), and 20 s at 72°C, with SYBRgreen fluorescence reading. A melting curve analysis was generated, and a single melt peak was observed for each sample, validating that only one product was present.

The template quality is one of the most important determinants of reproducibility and biological relevance of quantitative RT-PCR. To account for any difference in PCR efficiencies between samples, the presence of PCR inhibitors within biological samples can be assessed. In this study, the RNA extract of each muscle sample was tested for PCR inhibitors by the SPUD assay (7, 38). A SPUD amplicon set that has no sequence identity with any known sequence within the target

Table 2. *Primer set sequences and amplicon information*

Target mRNA	PCR Primer Sequence 5′→3′	Amplicon Size, bp	Amplicon Location, bp	T_a, °C	NCBI (Reference Sequence)
FOXO3A	F GAACGTGGGGAACTTCACTGGTGCTA	98	2278–2375	59	NM_201559
	R GGTCTGCTTTGCCCACTTCCCCTT				
Atrogin-1	F TATTGCACCCTGGGGGAAGCTTTCAA	92	481–572	59	NM_058229
	R TCCAACAGCCGGACCACGTAGTTAAA				
MuRF-1	F CTCAGTGTCCATGTCTGGAGGCCGTT	147	328–474	58	NM_032588
	R GGCCGACTGGAGCACTCCTGTTTGTA				
Calpain-1	F AGTCGTGCCGCAGCATGGTGAA	93	1906–1998	58	NM_005186
	R TCCGGATGCGGTTCCACAGGAT				
Calpain-2	F CTACTCCAGCAAAACCCGGGGCAT	120	337–456	60	NM_001748
	R CAGTCACCTAGGGCTCCTTGGCAGAT				
Myostatin	F GACCAGGAGAAGATGGGCTGAATCCGTT	96	861–956	60	NM_005259
	R GCTCATCACAGTCAAGACCAAAATCCCTT				
TNF-α	F CCCAGGCAGTCAGATCATCTTCTCGAA	149	390–538	58	NM_000594
	R CTGGTTATCTCTCAGCTCCACGCCATT				
IL-6	F CTATGAACTCCTTCTCCACAAGCGCCTT	127	61–187	59	NM_000600
	R GGGGCGGCTACATCTTTGGAATCTT				
IL-8	F GCTCTGTGTGAAGGTGCAGTTTTGCCAA	135	153–287	60	NM_000584
	R GGCGCAGTGTGGTCCACTCTCAAT				
IL-15	F CCGTGGCTTTGAGTAATGAGAATTTCGAA	140	831–970	60	NM_172174
	R CCTGCACTGAAACAGCCCAAAATGAA				

FOXO3A, forkhead box 3A; MuRF-1, muscle ring finger-1; F, forward; R, reverse; T_a, annealing temperature; NCBI, National Center for Biotechnology Information.

RNA was amplified in the presence of human muscle tissue cDNA. The assay generated average cycle threshold (C_T) values of 20.39 ± 0.02 characteristic to an uninhibited SPUD assay (20.33 ± 0.04), showing that no inhibitors were present in the cDNA generated from the muscle tissue samples in the present study.

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. The data were analyzed using the Livak and Schmittgen equation, where the fold change $= 2^{-\Delta\Delta C_T}$ (29). Principles of this method, validation of internal control (GAPDH), and amplification efficiencies equality based on amplification of serial dilution of cDNA for each PCR run were determined and performed, as previously described (43, 62).

The amplification calculated by Rotor-Gene software for each PCR run, based on the dilution curve, was specific and highly efficient. The mean efficiency (±SE) of the PCR in the RE group was 1.06 ± 0.01 (R^2 = 0.99 ± 0.00) for GAPDH, and 1.02 ± 0.01 (R^2 = 0.99 ± 0.00) for GOI, with percent coefficient of variation of 0.13 ± 0.06 for duplicates. The mean efficiency of PCR in the RUN group was 1.07 ± 0.01 (R^2 = 0.99 ± 0.00) for GAPDH, and 1.03 ± 0.01 (R^2 = 0.99 ± 0.00) for GOI, with percent coefficient of variation 0.13 ± 0.06 for duplicates.

Myosin Heavy Chain Composition

Muscle samples were homogenized in 40 volumes of cold homogenizing buffer containing 250 mM sucrose, 100 mM KCl, 20 mM imidazole, and 5 mM EDTA (pH 6.8) and then spun for 30 min at 20,000 *g* and 4°C and subjected to SDS-PAGE to determine myosin heavy chain (MHC) composition, as described previously (55, 60, 62). 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. Following electrophoresis, the gels were silver stained, as described by Giulian et al. (17). MHC isoforms were identified according to migration rate and compared with molecular weight standards. The MHC was categorized as MHC I, IIa, or IIx, and percentages were determined by densitometry (ChemImager 4000, Alpha Innotech, San Leandro, CA).

Statistical Analysis

For each gene, data were checked for normality and equality of variances. When normality was present, the fold changes in mRNA expression ($2^{-\Delta\Delta C_T}$ data) were compared using one-way repeated-measures ANOVA. When assumptions for parametric analyses were violated, the changes in mRNA levels with respect to the preexercise levels at the various time points postexercise for each studied gene were analyzed using the nonparametric Friedman test. Significant differences between postexercise level and preexercise level were determined using the pairwise comparison post hoc analysis. Significance was set at $P < 0.05$ (two-tailed). Statistical analyses were performed using SPSS 10.0 for Windows software package. Data are presented as means ± SE.

RESULTS

mRNA Induction With RE

Proteolytic. Proteolytic gene expression is shown in Fig. 1. Following RE, MuRF-1 mRNA increased ($P < 0.05$) 3.5-fold at 1 h, 3.4-fold at 2 h, and 2.0-fold at 4 h postexercise. Atrogin-1 mRNA decreased ($P < 0.05$) 3.3-fold at 8 h and 1.9-fold at 12 h postexercise. FOXO3A gene expression exhibited a trend ($P = 0.06$) to decrease 1.7-fold at both 8 h ($P = 0.06$) and 12 h ($P = 0.07$) postexercise. All proteolytic mRNA expression returned to preexercise values by 24 h postexercise. There were no changes in calpain-1 or calpain-2 mRNA (data not shown).

Myostatin. Myostatin gene expression is shown in Fig. 2. Myostatin mRNA expression decreased ($P < 0.05$) 2.5-fold to 6.3-fold 1–24 h post-RE, with the greatest suppression from 2 to 8 h post-RE.

Cytokines. Cytokine mRNA expression of the interleukins IL-6, IL-8, and IL-15 is shown in Fig. 3. Cytokine expression peaked between 4 and 8 h postexercise. An increase ($P < 0.05$) in IL-6 mRNA expression 4.4-fold to 791-fold was seen 4–24 h post-RE, with peak expression 4 h postexercise. IL-8 mRNA expression increased ($P < 0.05$) 2.7-fold to 759-fold 2–24 h

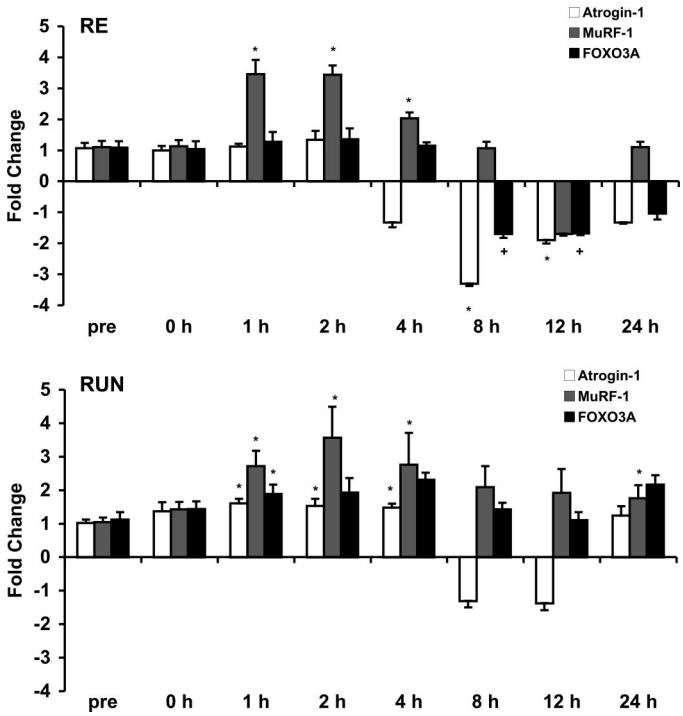

Fig. 1. Fold changes in proteolytic mRNA expression following an acute bout of resistance exercise (RE; *top*) or running exercise (RUN; *bottom*) normalized to GAPDH mRNA and relative to preexercise levels. Total RNA was extracted from the muscle biopsies of the vastus lateralis (RE) or gastrocnemius (RUN). MuRF-1, muscle ring finger-1; FOXO3A, forkhead box 3A. Values are means ± SE. *$P < 0.05$ from preexercise mRNA expression. $^{+}$Trend ($P \leq 0.07$) from preexercise mRNA expression.

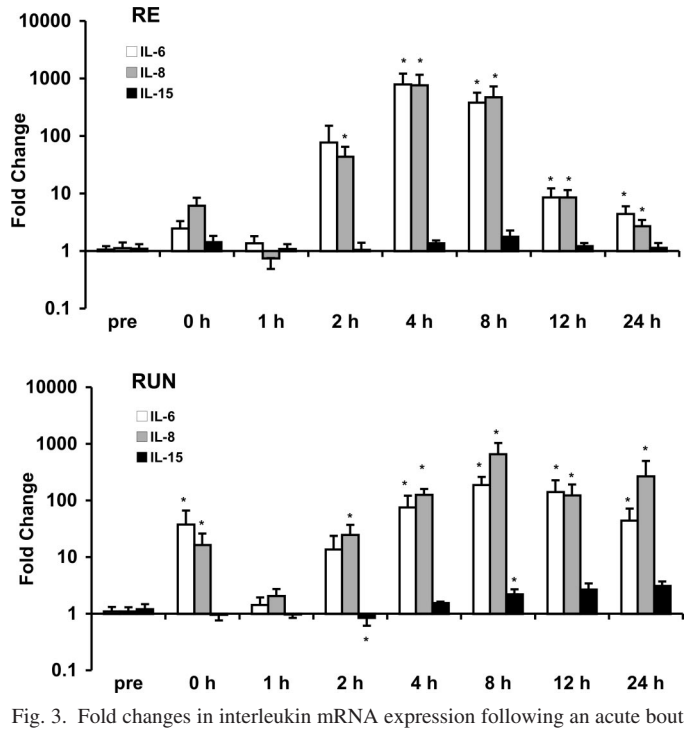

Fig. 3. Fold changes in interleukin mRNA expression following an acute bout of RE (*top*) or RUN (*bottom*) normalized to GAPDH mRNA and relative to preexercise levels. Total RNA was extracted from the muscle biopsies of the vastus lateralis (RE) or gastrocnemius (RUN). Values are means ± SE. *$P < 0.05$ from preexercise mRNA expression.

postexercise, with the peak at 4 h post-RE. There was no change in IL-15 mRNA in the 24 h following the acute bout of RE. TNF-α expression is shown in Fig. 4. TNF-α mRNA increased ($P < 0.05$) 2.1-, 3.9-, 5.1-, 6.3-, and 2.0-fold immediately and at 2, 4, 8, and 24 h postexercise, respectively.

mRNA Induction With RUN

Proteolytic. Proteolytic gene expression of MuRF-1, atrogin-1, and FOXO3A following RUN is shown in Fig. 1. MuRF-1 mRNA increased ($P < 0.05$) 2.7-, 3.6-, and 1.8-fold at 1, 2, and 4 h postexercise, respectively. Atrogin-1 mRNA increased ($P < 0.05$) 1.6-fold at 1 h, 1.5-fold at 2 h, and 1.5-fold 4 h after exercise. FOXO3A gene expression increased ($P < 0.05$) 1.9-fold at 1 h postexercise. Calpain-1 decreased ($P < 0.05$) 1.3-fold 1 h postexercise, while calpain-2 mRNA increased ($P < 0.05$) 2.6-fold at 24 h postexercise (data not shown).

Myostatin. Myostatin mRNA expression is shown in Fig. 2. Myostatin mRNA decreased ($P < 0.05$) 2.9-fold at 8 h, and 3.6-fold at 12 h postexercise.

Cytokines. Cytokine mRNA expression exhibited a biphasic response following RUN, with significant elevation immediately post-RUN, a drop to preexercise expression 1 h postexercise, and an increase to peak expression between 4 and 12 h postexercise (Fig. 3). An increase ($P < 0.05$) in IL-6 mRNA of

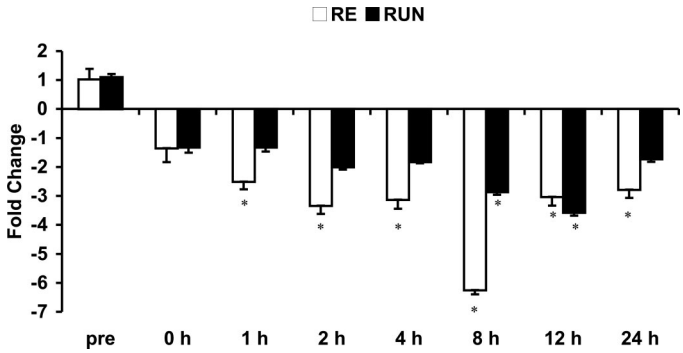

Fig. 2. Fold changes in myostatin mRNA expression following an acute bout of RE or RUN normalized to GAPDH mRNA and relative to preexercise levels. Total RNA was extracted from the muscle biopsies of the vastus lateralis (RE) or gastrocnemius (RUN). Values are means ± SE. *$P < 0.05$ from preexercise mRNA expression.

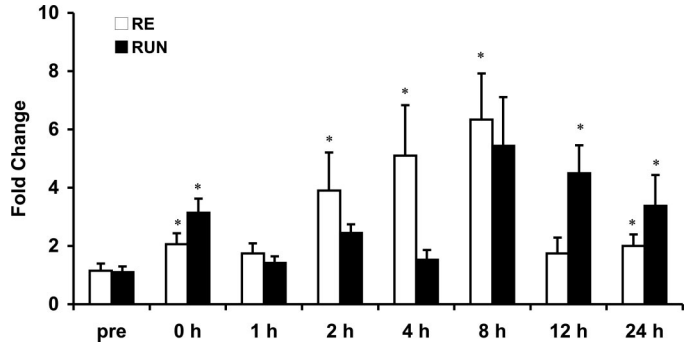

Fig. 4. Fold changes in TNF-α mRNA expression following an acute bout of RE or RUN normalized to GAPDH mRNA and relative to preexercise levels. Total RNA was extracted from the muscle biopsies of the vastus lateralis (RE) or gastrocnemius (RUN). Values are means ± SE. *$P < 0.05$ from preexercise mRNA expression.

37-, 75-, 187-, 140-, and 44-fold was seen immediately and 4, 8, 12, and 24 h following exercise, respectively. IL-8 mRNA increased ($P < 0.05$) 16-fold immediately following RUN, and also increased 25- to 653-fold between 2 and 24 h post-RUN, with peak expression at 8 h postexercise. IL-15 mRNA levels decreased ($P < 0.05$) 1.2-fold 2 h postexercise and increased slightly ($P < 0.05$) 2.2-fold at 8 h postexercise. Expression of TNF-α mRNA is shown in Fig. 4. TNF-α mRNA increased ($P < 0.05$) 3.1-, 2.5-, 4.5-, and 3.4-fold immediately and 2, 12, and 24 h postexercise, respectively.

DISCUSSION

The main findings from this time course investigation of select proteolytic, myostatin, and cytokine mRNA in response to an acute bout of RUN or RE were as follows: *1*) the timing of gene induction was variable and moderately different between modes, with changes in mRNA expression generally peaking 2–8 h post-RE and 2–24 h post-RUN; *2*) at 24 h postexercise, TNF-α, IL-6, and IL-8 were still elevated with RE and RUN, MuRF-1 and calpain-2 were elevated with RUN, and myostatin was still suppressed with RE; and *3*) generally, running induced a greater number of genes associated with muscle catabolism compared with the RE bout.

Proteolytic mRNA Response

The UPP is responsible for the majority of protein degradation in skeletal muscle (19). The UPP is highly associated with muscle atrophy (44) and has been found to be upregulated with exercise. As part of the UPP, two muscle-specific E3 ligases, MuRF-1 and atrogin-1, have been identified, and their substrates, although still under investigation, include muscle proteins (44). Both of these E3 ligases share FOXO3A as a transcription factor (47, 52).

Following both RE and RUN, the proteolytic genes showed significant elevation from 1 to 4 h. However, the RE profile of proteolytic gene expression following exercise was different from RUN in that only MuRF-1 showed an early elevation, and there was a suppression of atrogin-1 and FOXO3A several hours after exercise (Fig. 1). Little research regarding proteolytic gene expression following acute endurance exercise has been published to date. Previous human studies have reported MuRF-1 and atrogin-1 mRNA expression that corresponds to time points in the time course presented here (9, 26, 63). Recent work from our laboratory (42, 63) utilizing untrained individuals after a similar acute bout of RE further corroborate a significant increase in MuRF-1 mRNA 4 h postexercise, with mRNA expression returning to preexercise levels 24 h postexercise, with no change in atrogin-1 at either time point. However, available human research has shown increased atrogin-1 mRNA 3 h following aerobic exercise (9). A noteworthy finding from the present investigation is that the time points for changes in FOXO3A mRNA expression closely resembled the time points for changes in atrogin-1, with increases observed after RUN, and a decrease observed after RE. This finding supports the recently established relationship between FOXO transcription factors and atrogin-1 (47, 52). In general, the mRNA expression of the UPP is suggestive of a greater induction of proteolysis following RUN exercise. RUN exhibited an increase in all three UPP GOIs, whereas RE only increased MuRF-1. Furthermore, the suppression of UPP genes (atrogin-1 and FOXO3A) following RE was not present following RUN.

Calpain-1 and -2 play important roles in the degradation of muscle cytoskeletal proteins (24). Calpain mRNA displayed little change in expression in response to either running or RE, with only slight, but significant changes occurring post-RUN. Feasson et al. (10) reported similar results with no change in calpain-2 mRNA immediately following downhill running, but did observe an increase 24 h postexercise. With RE, no calpain activity was observed, which is consistent with previously published data (63).

Myostatin mRNA Response

Myostatin is a member of the transforming growth factor-β gene family and has been shown to be a negative regulator of muscle growth (33, 53). Our data indicate that, after an acute exercise bout, myostatin mRNA is decreased with both RUN and RE, but RUN did not show as great of a response as RE (Fig. 2). Existing human research has shown a suppression of myostatin following resistance exercise (26, 27, 43), but information regarding the effects of aerobic exercise, such as running, is absent. Rodent data have shown that endurance exercise leads to a suppression of myostatin mRNA (31, 32). Acute bouts of RE have been shown to suppress myostatin mRNA expression in young individuals 4 h (43) and 24 h after exercise (27). Furthermore, myostatin mRNA reduction appears to be negatively correlated to muscle mass, which may be influenced by a greater distribution of type II (fast-twitch) muscle fibers, as previous research has indicated (31). Although we did not measure the muscle mass of our subjects, the resistance-trained individuals did show a significantly higher ($P < 0.05$) percentage of MHC IIa fibers compared with the runners (Table 1) and showed a decrease in myostatin mRNA from 1 h to 24 h postexercise, compared with the runners, who only decreased myostatin mRNA 8–12 h postexercise.

Cytokine mRNA Response

Chronic pro-inflammatory cytokine elevation in response to trauma or disease is associated with muscle wasting (20, 21). However, with exercise, the role of these inflammatory markers may include potential involvement in glycogen regulation, angiogenesis, and suppression of catabolic processes (5). Due to these alternative roles in the contracting muscle, the cytokines IL-6, IL-8, and IL-15 are referred to as "myokines" (39), and increases in myokines have been found independent of other indications of muscle trauma, such as the pro-inflammatory cytokine TNF-α (5).

It has been established that IL-6 is released from skeletal muscle in response to both endurance and resistance exercise (23). Recent research has indicated that, in muscle cells, IL-6 may have a metabolic role, with particular influences involving glycogen levels of the muscle (11, 36, 37, 51). Specifically, studies have shown that, following long-duration exercise, IL-6 mRNA expression is higher in muscles that were in a low glycogen state before the start of exercise (50). Our subjects remained fasted until 8 h postexercise. Interestingly, 4–8 h postexercise is when IL-6 peaked in both RUN and RE, suggesting that lowered glycogen stores following the exercise bout may have influenced IL-6 expression. Although speculative, the second IL-6 mRNA increase of the biphasic response

in RUN may represent a lipolytic response in an attempt to spare available glucose. IL-6 has been shown to increase lipolysis in healthy humans (58), and observations of arterial fatty acid kinetics following aerobic exercise have exhibited a similar biphasic pattern through 3 h of recovery in the unfed state (57). It is also intriguing to note the much higher peak fold change with RE vs. RUN. There are several possible explanations for this observation. There are previous data suggesting that fast-twitch muscle fibers may contribute a larger amount of IL-6 than type I (23), which is consistent with the fiber-type distribution of our subjects (Table 1). In addition, other research has shown that endurance training leads to a lower induction of IL-6 mRNA postexercise, despite higher workloads (13). Although not measured in the present study, it is likely the endurance-trained muscle of the experienced runners had a higher glycogen content before exercise, potentially further attenuating the mRNA induction of IL-6.

The role of IL-8 in exercised muscle has not yet been fully clarified, but recent research has suggested that IL-8 is angiogenic in nature (1, 22). Both RE and RUN showed an increase in IL-8 mRNA postexercise, and the pattern of expression was similar to that observed with IL-6. RE demonstrated a drop from peak IL-8 mRNA expression after 12 h, whereas RUN showed less of a decline in IL-8 mRNA expression postexercise and was elevated to a greater extent than RE 24 h postexercise. Several studies have shown an increase in IL-8 immediately following both resistance and endurance exercise (1, 8, 36, 37). Given the suggested angiogenic role of IL-8, it is possible that this may contribute to the increased capillarization associated with endurance training (4).

IL-15 has been shown to blunt proteolysis (6) and apoptosis (12) in skeletal muscle, as well as increase MHC and α-actin accretion within myofibers (16). Only slight changes in IL-15 were observed in the 24 h post-RUN. Limited data exist on the activity of IL-15 in response to exercise. Despite human research showing increases in plasma IL-15 following exercise (46), several studies have failed to observe any change in IL-15 mRNA expression from active muscle tissue samples following both endurance and resistance exercise (8, 36, 37). However, it should be pointed out that previous human exercise studies have only observed IL-15 immediately postexercise. Therefore, the IL-15 mRNA changes following RUN observed in this study may indicate a late response of IL-15 mRNA expression to exercise.

TNF-α has been found to increase muscle protein breakdown (21, 28) via stimulation of atrogin-1 (28) and decreases muscle protein synthesis through inhibition of the ERK1/2 signaling pathway (61). Current literature has shown modest elevation of TNF-α mRNA expression following both endurance and resistance exercise in humans (36, 37, 51), which is consistent with the current time course investigation. It should be noted, however, that previous research from our laboratory has observed no change in TNF-α in single muscle fibers following a similar bout of resistance exercise (63). Given that we used muscle homogenate in the present study, the actual source of TNF-α and the aforementioned interleukins may not have been the muscle tissue alone, but perhaps also inflammatory cells (54).

Summary and Practical Applications

To our knowledge, this is the first study to show the time course of the expression of genes associated with muscle protein breakdown and inflammation in the 24 h following an acute bout of resistance or endurance exercise. These study findings show that there was an induction of proteolytic genes early (1–4 h), followed by increases in the cytokines (2–24 h), in concert with a decrease in myostatin (2–24 h). It is also noteworthy that, despite the subjects being accustomed to their mode of exercise, the muscle responded with robust changes in mRNA expression of the selected genes. Previous research has shown that training leads an attenuated gene response to exercise (13, 48). Furthermore, the robust gene responses occurred following modest bouts of exercise, indicating that exercise of only moderate duration and intensity is sufficient to induce gene responses.

It should not be overlooked that a limitation of this study is the fact that we did not measure protein levels. We recognize that, due to posttranscriptional events (i.e., degradation, modification), there is not a 1:1 ratio between mRNA and functional proteins (34). However, it is interesting to note that the early induction of proteolytic genes corroborates well with muscle protein breakdown following resistance exercise, which has been shown to be greatly increased 3 h postexercise (41). Given the similar time course of muscle protein breakdown relative to our proteolytic gene time course, it is seemingly fair to suggest that some of the mRNA is being translated into functional proteins.

The previously established myogenic time course papers have indicated peak gene induction of myogenic genes ~4–24 h postexercise (3, 62). Interestingly, the now established proteolytic time course shows peak proteolytic gene induction 1–4 h postexercise. Taken together, it appears that the gene program for muscle turnover begins with induction of genes associated with muscle protein breakdown, which is followed by genes associated with muscle protein synthesis. Furthermore, following RUN, the induction of myogenic genes was lower than that following RE, whereas the proteolytic gene expression following RUN was greater than following RE. These combined data add insight to the muscle adaptive response at the molecular level that lead to a decreased muscle fiber size with endurance training (56), as well as the commonly associated adaptation of muscle hypertrophy that occurs with chronic resistance training (14, 35, 49).

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