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Time course of molecular responses of human skeletal muscle to acute bouts of resistance exercise

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Bickel, C. Scott, Jill Slade, Ed Mahoney, Fadia Haddad, Gary A. Dudley, and Gregory R. Adams. Time course of molecular responses of human skeletal muscle to acute bouts of resistance exercise. *J Appl Physiol* 98: 482–488, 2005. First published October 1, 2005; doi:10.1152/jappphysiol.00895.2004.—Resistance exercise (RE) training, designed to induce hypertrophy, strives for optimal activation of anabolic and myogenic mechanisms to increase myofiber size. Clearly, activation of these mechanisms must precede skeletal muscle growth. Most mechanistic studies of RE have involved analysis of outcome variables after many training sessions. This study measured molecular level responses to RE on a scale of hours to establish a time course for the activation of myogenic mechanisms. Muscle biopsy samples were collected from nine subjects before and after acute bouts of RE. The response to a single bout was assessed at 12 and 24 h postexercise. Further samples were obtained 24 and 72 h after a second exercise bout. RE was induced by neuromuscular electrical stimulation to generate maximal isometric contractions in the muscle of interest. A single RE bout resulted in increased levels of mRNA for IGF binding protein-4 (84%), MyoD (83%), myogenin (~3-fold), cyclin D1 (50%), and p21-Waf1 (16-fold), and a transient decrease in IGF-I mRNA (46%). A temporally conserved, significant correlation between myogenin and p21 mRNA was observed ($r = 0.70$, $P \leq 0.02$). The mRNAs for mechano-growth factor, IGF binding protein-5, and the IGF-I receptor were unchanged by RE. Total skeletal muscle RNA was increased 72 h after the second serial bout of RE. These results indicate that molecular adaptations of skeletal muscle to loading respond in a very short time. This approach should provide insights on the mechanisms that modulate adaptation to RE and may be useful in evaluating RE training protocol variables with high temporal resolution.

neuromuscular electrical stimulation; myogenin; p21; MyoD

IT IS GENERALLY ACCEPTED THAT resistance exercise (RE) elicits adaptations that result in increased skeletal muscle size and changes in contractile characteristics. The majority of these adaptations take the form of increases in myofiber size (e.g., Ref. 20) and alterations in the mix of contractile and metabolic proteins present in these cells [e.g., isoform shifts, etc. (14)]. Alterations in the types and amounts of cellular proteins in myofibers can involve changes in gene transcription and translation. In vitro and in vivo studies have identified a number of specific mRNAs in skeletal muscles that change in response to increased loading (15, 18, 21, 24, 37, 40).

The concept underlying RE training, designed to induce skeletal muscle hypertrophy, is that the correct selection of program variables, e.g., frequency, intensity, and duration of

individual sessions, results in cellular and molecular responses that promote the growth process. We have shown that individual sessions of high-force actions common during an RE session elicit cellular and molecular responses in rodents that have characteristic temporal patterns of increase and decline (24). In those studies, we also found that subsequent sessions of high-force actions result in the summation of these responses. The magnitude of summation is sensitive to the time interval allowed between RE sessions (24). In a subsequent study, we found that molecular responses to high-force RE sometimes differ between skeletal muscle of spinal cord-injured and ambulatory individuals (13) but are generally similar.

In vitro and in vivo studies indicate that two primary processes are involved with the compensatory hypertrophy response in mammalian skeletal muscles. The first are anabolic processes necessary for the accretion of protein to support myofiber enlargement. The second involves proliferation of satellite cells that appear to be necessary to provide myonuclei to the enlarging myofibers (7, 41, 42, 45–47, 49). The cellular and molecular mechanisms underlying these processes have been extensively characterized in a number of cell types, particularly in cell culture settings. In particular, the steps associated with the initiation of cellular proliferation are generally well established (e.g., can be found in cell biology textbooks). Based on these well-characterized processes, we have previously used representative mRNAs as markers of potential myogenic responses to high-force actions of skeletal muscle in rodents and humans (2–4, 13, 24). For example, increased expression of myogenin is often associated with committed stages of myoblast differentiation and fusion (22, 27, 44, 48, 53). Similarly, the cyclin-dependent kinase inhibitor p21 is associated with withdrawal from the cell cycle and, often, subsequent cell differentiation (22, 33). Together, these markers are often used as an indication of myogenic cell differentiation in settings associated with muscle formation or muscle hypertrophy (2, 4, 22, 24, 33). Potential alterations in cellular proliferation can be inferred from changes in key cell cycle regulatory agents such as cyclin D1 (38). In the present study, we used this myogenic marker approach to characterize the molecular level responses of human skeletal muscles to acute bouts of RE (maximal isometric contractions).

Following a protocol similar to that used in our previous rodent studies, we found that RE induced similar molecular level changes in human skeletal muscles in response to a single exercise bout (24).

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The *in vivo* validation of loading-sensitive cellular and molecular responses will help to elucidate the processes that have been assumed to take place as a result of RE training but, until now, have not been systematically investigated. This understanding will, in turn, allow for the further elucidation of the regulatory mechanisms that control and coordinate the suite of adaptations that comprise the compensatory hypertrophy response and, at least in a laboratory setting, provide a mechanism by which different RE training protocols and modalities (e.g., static vs. dynamic, concentric vs. eccentric, etc.) could be objectively evaluated.

METHODS

Subjects. Nine (age 25 ± 4 yr, height 178 ± 8 cm, weight 70 ± 3 kg, means \pm SE; 6 men, 3 women) able-bodied subjects participated in this study. The subjects had no history of lower extremity pathology and signed informed consent before testing. Subjects were recreationally active and not currently involved in lower extremity RE. The methods were approved by the Institutional Review Boards of The University of Georgia, Shepherd Center, and the University of California-Irvine.

RE protocol. Neuromuscular electrical stimulation (NMES) was chosen to ensure maximal activation of the vastus lateralis (VL) muscle and to avoid the complication associated with voluntary muscle activation (e.g., recurrent inhibition, motor unit cycling, etc.). It has previously been demonstrated that this method of muscle activation compares favorably with voluntary muscle contraction for the development of muscle hypertrophy and increased strength (e.g., Ref. 43). The VL muscle was stimulated, as described previously (5, 16, 29, 43). Subjects were seated in a custom-built chair with the hip and knee secured at $\sim 70^\circ$ of flexion. The leg was firmly secured to a rigid lever arm with an inelastic strap to ensure that the knee extensors could only perform isometric contractions. As with our previous animal studies, isometric mode contractions were used to minimize the potential for skeletal muscle injury (24). The moment arm was established by placing a load cell (model 2000A, Rice Lake Weighing Systems, Rice Lake, WI) parallel to the line of pull and perpendicular to the lever arm. Torque was recorded from the load cell by using a MacLab analog-to-digital converter (model ML 400, ADInstruments, Milford, MA) sampling at 100 Hz and interfaced with a portable Macintosh computer (Apple Computer, Cupertino, CA). Two 8×10 -cm surface electrodes (Uni-Patch, Wabasha, MN) were placed on the skin over the proximal and distal portions of the VL muscle. Using magnetic resonance imaging, our laboratory has previously demonstrated that this electrode placement will maximally recruit primarily the VL muscle (6, 29). A commercial stimulator (Thera-Touch model 4.7, Rich-Mar, Inola, OK) was used for NMES. The initial torque was determined in the following manner. Subjects performed a maximum voluntary contraction (MVC) for isometric knee extension before NMES. The subjects were highly motivated, and all had prior experience with performing knee extension MVC. We have previously established that the VL muscle constitutes roughly 30% of the total quadriceps group cross-sectional area (28). Accordingly, NMES current sufficient to elicit $\sim 30\%$ of the observed isometric knee extension MVC (generated by the entire quadriceps group) was determined and used for the subsequent NMES protocol. This 30% index value provides a means to avoid the unnecessary delivery of excess current that may activate synergistic muscles that were not the focus of this study. The NMES protocol consisted of 5-s contractions separated by 15 s for 30 min at these previously determined current levels. Contractions were evoked with 50-Hz trains of 450- μ s biphasic pulses.

Biopsy technique. Biopsies were taken from the VL by using the percutaneous biopsy technique, as done previously (16, 28). To reduce the number of serial biopsies, the preexercise sample was collected

from the nonexercised muscle. To minimize the potential for interference, any serial biopsy samples were collected at least 2 cm from previous biopsy sites. Samples were immediately cooled with liquid nitrogen and then stored at -70°C until analyzed.

Experimental timeline. See Fig. 1. At *time 0*, a biopsy sample was collected from the VL of the nontreatment leg. This approach was used to ensure that any changes seen in the 12-h biopsy samples could not be attributable to a previous biopsy procedure. The use of the contralateral limb as a control has been previously validated (e.g., Ref. 35). The subjects then completed the initial 30-min bout of NMES-induced RE. At 12 h postexercise, a biopsy sample was collected from the VL of the exercised leg. At 24 h, a second biopsy was collected from the previously exercised VL, and the NMES-induced RE exercise was then repeated. Biopsy samples were then collected from the exercised VL 48 and 96 h post-NMES-induced RE, relative to *time 0* (24 and 72 h after the second bout of RE).

Total RNA isolation. Measurements of total RNA content provide insights on the translational capacity of tissue. Total RNA was extracted from preweighed frozen muscle samples by using the TRI Reagent (Molecular Research Center, Cincinnati, OH), according to the company's protocol, which is based on the method described by Chomczynski and Sacchi (17). Extracted RNA was precipitated from the aqueous phase with isopropanol and, after washing with ethanol, dried and suspended in a known volume of nuclease-free water. The RNA concentration was determined by optical density at 260 nm (using a 260-nm optical density unit equivalent to 40 $\mu\text{g}/\text{ml}$). The muscle total RNA concentration is calculated based on total RNA yield and the weight of the analyzed sample. The RNA samples were stored frozen at -80°C to be used subsequently in determining specific mRNA expression with the use of relative RT-PCR procedures.

RT. One microgram of total RNA was reverse transcribed for each muscle sample using the SuperScript II RT from GIBCO BRL and a mix of oligo(dT) (100 ng per reaction) and random primers (200 ng per reaction) in a 20- μ l total reaction volume at 45°C for 50 min, according to the provided protocol. At the end of the RT reaction, the tubes were heated at 90°C for 5 min to stop the reaction and then were stored at -80°C until they were used in the PCR reactions for specific mRNA analyses (see below).

PCR. A relative RT-PCR method using 18S as an internal standard (Ambion, Austin, TX) was applied to study the expression of mRNAs for IGF-I, mechano-growth factor (MGF), IGF-I receptor, IGF binding proteins (BP)-4 and -5, myogenin, cyclin D1, and p21. The sequence for the various primers used for the specific target mRNAs has been published previously (13). Primers were designed using Primer Select computer program (DNA Star), purchased from Life Technology, GIBCO, and were tested for their compatibility with the alternate 18S primers.

In each PCR reaction, 18S ribosomal RNA was coamplified with the target cDNA (mRNA) to serve as an internal standard and to allow correction for differences in starting amounts of total RNA.

For the 18S amplification, we used the Alternate 18S Internal Standards (Ambion), which yields a 324-bp product. The 18S primers were mixed with competitors at an optimized ratio that could range from 1:4 to 1:10, depending on the abundance of the target mRNA. Inclusion of 18S competitors was necessary to bring down the 18S

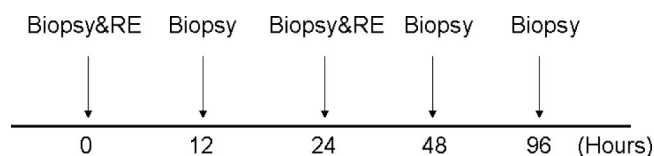


Fig. 1. Experimental protocol timeline. Resistance exercise (RE) = 30 min of neuromuscular electrical stimulation-induced RE. Note that the 48- and 96-h samples are relative to *time 0* and as such were 24 and 72 h after the second bout of RE.

signal, which allows its linear amplification to the same range as the coamplified target mRNA (Ambion, Relative RT-PCR kit protocol).

For each specific target mRNA, the RT and PCR reactions were carried out under identical conditions by using the same reagent premix for all of the samples to be compared in the study. To validate the consistency of the analysis procedures, at least one representative from each group was included in each RT-PCR run.

One microliter of each RT reaction (0- to 10-fold dilution, depending on target mRNA abundance) was used for the PCR amplification. The PCR reactions were carried out in the presence of 2 mM MgCl₂ by using standard PCR buffer (GIBCO), 0.2 mM dNTP, 1 μM specific primer set, 0.5 μM 18S primer-competimer mix, and 0.75 unit of DNA Taq polymerase (GIBCO) in 25-μl total volume. Amplifications were carried out in a Stratagene Robocycler with an initial denaturing step of 3 min at 96°C, followed by 25 cycles of 1 min at 96°C, 1 min at 55°C (55–60°C depending on primers), 1 min at 72°C, and a final step of 3 min at 72°C. PCR products were separated on a 2–2.5% agarose gel by electrophoresis and stained with ethidium bromide, and signal quantification was conducted by laser scanning densitometry, as reported previously (52).

The RT and PCR methods used in this work have been extensively validated in previous publications (13, 52). Each specific mRNA signal is normalized to its corresponding 18S. For each primer set, PCR conditions (cDNA dilutions, 18S competitor-primer mix, MgCl₂ concentration, and annealing temperature) were set to optimal conditions, normalized so that the target mRNA product yields were in the linear range of the semilog plot when the yield is expressed as a function of the number of PCR cycles, and, for a given condition, 18S and target mRNA PCR yields were tightly correlated to input cDNA.

Statistical analysis. All values are reported as means ± SE. Treatment effects were determined by ANOVA with repeated measures by using the Prism software package (Graphpad). Pearson's correlation analysis was used to assess the relationship between p21 and myogenin by using the Prism software package. For all statistical tests, the 0.05 level of confidence was accepted for statistical significance.

RESULTS

Analysis of the torque output records indicated that, over the two exercise sessions, the subjects produced $29 \pm 3\%$ of their previously measured isometric MVC during the NMES-induced high-force actions. The NMES current used to induce these isometric actions was 151 ± 15 mA.

Components of the IGF-I axis. Expression and/or accumulation of mRNA for IGF-I was decreased by ~46% 12 h after a single session of NMES-induced high-force isometric actions (Fig. 2A). The level of MGF mRNA, the loading-sensitive isoform of IGF-I (25), was unchanged at all time points (not shown). The mRNA for IGFBP-4 was increased by 84% by a single session of NMES-induced high-force isometric actions, but this response decayed such that it was no longer elevated 24 h postexercise (Fig. 2B). The imposition of a second session of NMES-induced high-force isometric actions 24 h after the first resulted in an additional increase in IGFBP-4 mRNA to approximately twofold above the preexercise value that was sustained through 96-h postexercise (Fig. 2B). There were no changes in the levels of IGFBP-5 or the IGF-I receptor mRNAs at any time point (not shown).

Myogenic regulatory factors. The expression and/or accumulation of MyoD mRNA was increased by 83% 12 h after a single session of NMES-induced high-force isometric actions (Fig. 3A). The level of mRNA for myogenin was increased 2.8- to 3.1-fold at 12, 24, and 48 h postexercise (Fig. 3B).

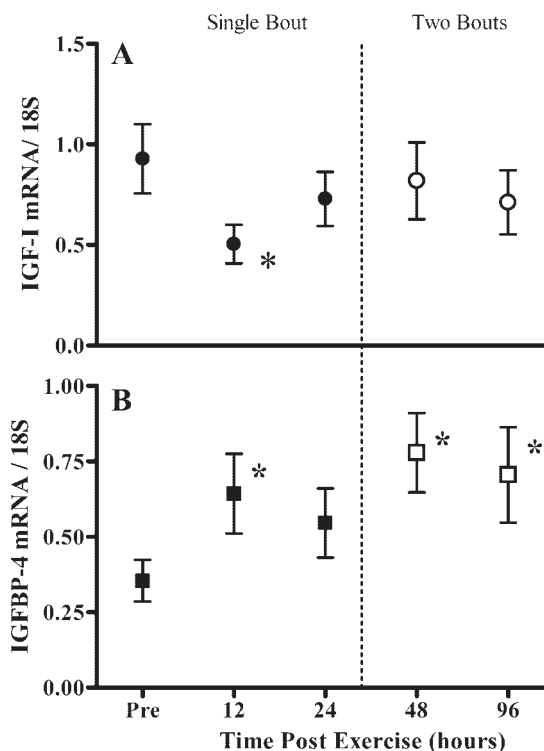


Fig. 2. Components of the IGF-I axis. RE induced changes in the expression and/or accumulation of the mRNA for IGF-I (A) and IGF binding protein-4 (IGFBP-4; B). Values are means ± SE. Pre, preexercise. Solid symbols, samples collected at the indicated time points following a single bout of RE; open symbols, samples collected after the imposition of a second bout of RE, ~24 h after the initial bout (time 0, Fig. 1). * $P < 0.05$ vs. Pre. Representative gel images are presented as supplemental data (<http://jap.physiology.org/cgi/content/full/00895.2004/DC1>).

Markers of cellular proliferation and differentiation. The mRNA for cyclin D1, a non-muscle-specific marker of cellular proliferation, was increased by 50% at 12 h after the first session of NMES-induced isometric actions (Fig. 4A). The mRNA for p21, a general marker of cellular differentiation, was increased 16- to 20-fold in the postexercise samples at 12, 24, and 48 h postexercise (Fig. 4B). There was a positive relation between myogenin and p21 mRNA when analyzed by individual data point (Fig. 5A) or by time point (Fig. 5B).

Total RNA concentration. Total muscle RNA increased 96 h postexercise (Fig. 6).

DISCUSSION

On a cellular level, the implicit aim of RE training programs, designed to induce skeletal muscle hypertrophy, is the optimal activation of the anabolic and myogenic mechanisms that result in the accretion of the proteins necessary to support an increase in myofiber size. Because these cellular-level mechanisms must be activated to achieve meaningful adaptations, their activation must precede these adaptations. As a matter of course, studies that involve the development of measurable adaptations, such as increased skeletal muscle or myofiber size, will not provide information on the dynamic state that exists as the adaptation processes are initiated.

In this study, we have used previously established molecular markers, representative of several cellular-level myogenic processes, to elucidate the time course of the response of human

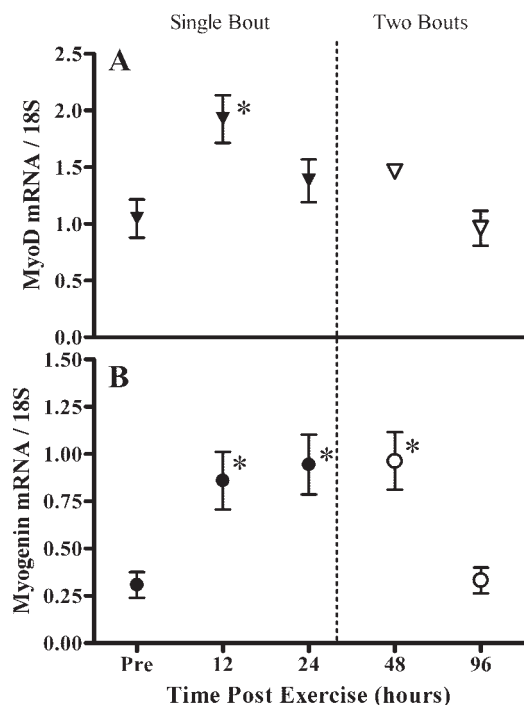


Fig. 3. Myogenic regulatory factors. RE induced changes in the amount of mRNA for MyoD (A) and myogenin (B). Values are means \pm SE. See Fig. 2 legend for explanation of symbols. * $P < 0.05$ vs. Pre. See supplemental data for representative gel images.

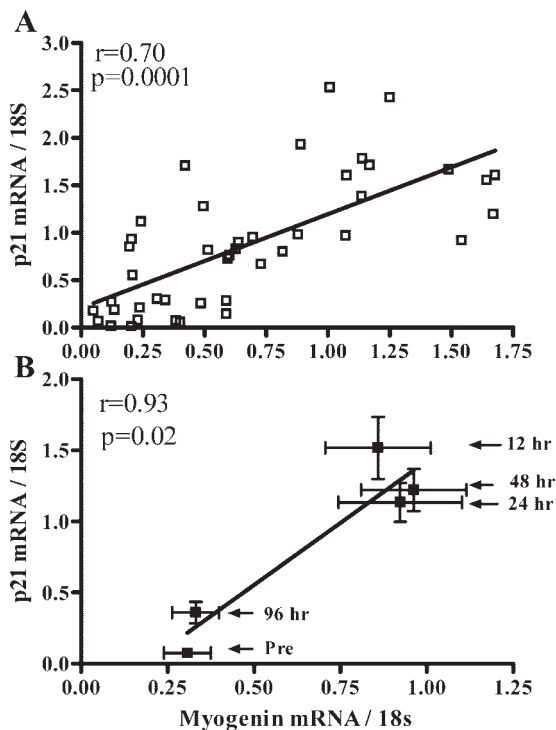


Fig. 5. Relationship between myogenin and p21. There were significant positive correlations between the mRNA for p21 and myogenin. A: individual data points. B: data grouped by time point as indicated ($n = 9$, per data point). Values are means \pm SE.

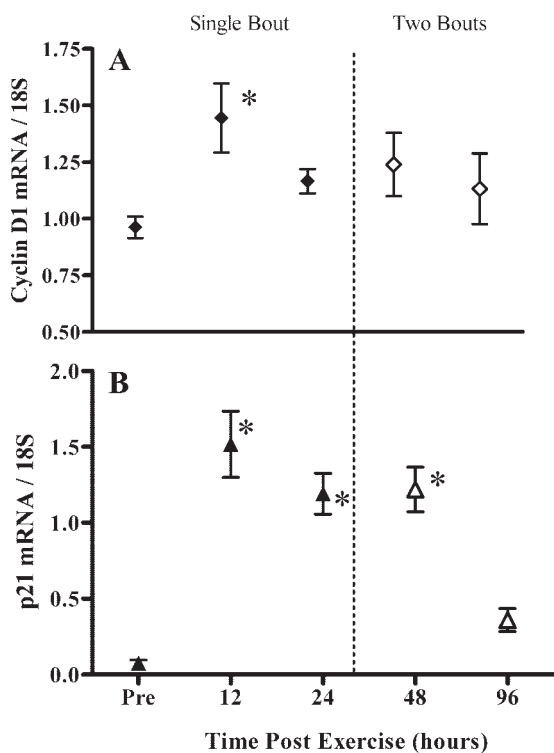


Fig. 4. Markers of cellular proliferation and differentiation. RE induced changes in the amount of mRNA for cyclin D1 (A) and cyclin-dependent kinase inhibitor p21 (B). Values are means \pm SE. See Fig. 2 legend for explanation of symbols. * $P < 0.05$ vs. Pre. See supplemental data for representative gel images.

skeletal muscle to acute sessions of high-force isometric actions with a temporal resolution of hours rather than weeks. In contrast to voluntary muscle actions, NMES results in consistent, maximal activation of all effected myofibers with each stimulation. The design of the present study was closely patterned on our previous work conducted in rodents in an effort to translate those findings from the rat model to humans (3, 24).

Potential weaknesses. Some compromises were made during the translation of the experimental design from rodents to humans. Specifically, a second session of high-force isometric actions was performed in the same subjects after just two single-session time point samples were obtained. This was

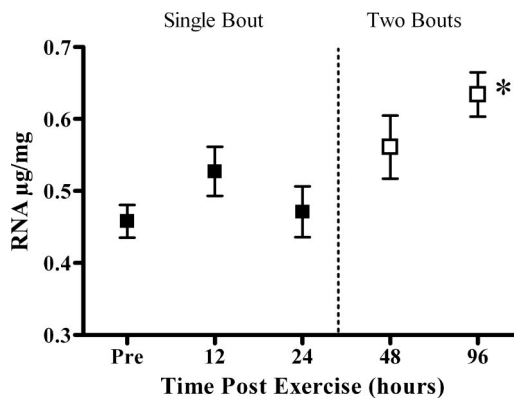


Fig. 6. RNA concentration. RE resulted in an increase in the concentration of total RNA at 96 h postexercise. Values are means \pm SE. See Fig. 2 legend for explanation of symbols. * $P < 0.05$ vs. Pre.

done to avoid having to use a second cohort of subjects to complete this study. However, this approach sacrificed the potential to observe the decline in responses over time. In addition, fewer time points were used to reduce the number of biopsy samples that each subject was required to provide. Due to the size of the biopsy samples, the number and type of analysis targets were also reduced. Due to this limitation, we focused on mRNA-level analyses, which provided the greatest number of data points for a given tissue sample.

RE. The high-force muscle contractions are common in RE and, as used in this study, were very similar to that used in our laboratory's previous work with rat and humans (3, 13, 24). As with our rodent and human studies, NMES was used to avoid the complications associated with voluntary contractile activity, including the problem of motor unit cycling, which would increase variability when using biopsy sampling (3, 13, 24). These foundational studies used isometric as opposed to dynamic muscle actions to reduce the possibility of skeletal muscle injury, which might tend to complicate the interpretation of results. While isometric-mode resistance training is currently less common, it has been shown to be effective in producing increases in muscle size and strength (e.g., Refs. 30, 36, 43).

IGF-I axis. IGF-I has been shown to stimulate anabolic and myogenic processes associated with the development of skeletal muscle hypertrophy (1). In skeletal muscle, the IGF-I system has also been shown to be sensitive to increased loading (1, 2, 4, 24). In this tissue, the IGF-I axis consists of locally expressed IGF-I and MGF, the type I IGF-I receptor, and a number of IGF-I binding proteins. Modulation of the various components of this system appears to be important for a compensatory hypertrophic response (1, 8, 9, 12, 19, 23, 25, 32, 51). In humans, RE has been variously reported to increase (Ref. 11; lengthening muscle actions), decrease (39), or not change (13, 26) IGF-I mRNA levels. We had previously reported that our acute RE protocol resulted in increased IGF-I mRNA in rodents (24). In contrast to that result, in the present study, IGF-I mRNA was found to decrease by almost 50% at the earliest time point (Fig. 2A). Using a different exercise program (e.g., 250–350 dynamic contractions), Psilander et al. (39) also found that IGF-I mRNA was modestly decreased 1–6 h postexercise. We had previously found that IGF-I mRNA was unchanged 24 h after two bouts of RE in humans, which would agree with our current findings (13). In animal studies, increased skeletal muscle loading has been shown to result in an upregulation of the expression of IGFBP-4 and downregulation of IGFBP-5 (8, 24). In the present study, we found that the mRNA for IGFBP-4 was increased 12 h after a single bout of RE and then started to return toward control by 24 h. The second bout of RE conducted after the 24-h time point resulted in a further increase in the mRNA for IGFBP-4. In a possible contrast, Bamman et al. (11) found that RE involving lengthening skeletal muscle actions resulted in a decrease in IGFBP-4 mRNA. Unlike the results from rodent studies, we did not find that the mRNA for IGFBP-5 was depressed following RE (8, 24). Also, in contrast to our previous rodent studies, we did not see an increase in MGF mRNA following RE (24). In agreement with this result, we had previously found that two bouts of RE resulted in an increase in MGF mRNA in spinal cord injury but not control subjects (13).

Myogenic regulatory factors. The myogenic regulatory factor (MRF) family is made up of a set of transcription factors that determine the myogenic fate of cells. Although various members of this family may have overlapping functions, specific roles for the various MRFs have been proposed. MyoD is most often associated with cell determination and is often found to be highly expressed in actively proliferating myoblasts and may be critical for proliferation in response to stimuli such as skeletal muscle injury (34, 44, 48, 50). Increased expression of myogenin is often associated with committed stages of myoblast differentiation and fusion (22, 27, 44, 48, 53). Alternatively, myogenin expression may occur within myofibers and may be associated with alterations in metabolic properties (31).

In the present study, the expression and/or accumulation of MyoD mRNA was modestly increased 12 h after the initial bout of RE (Fig. 3A). However, the subsequent bout of RE did not stimulate an increase in MyoD. Psilander et al. (39) also reported a transient increase in MyoD mRNA immediately following their RE bouts.

The mRNA for myogenin experienced a fairly robust increase in response to RE that was maintained through the 24-h time point (Fig. 3B). The second bout of RE resulted in a renewed or continued elevation of myogenin mRNA that decayed by the final 96-h time point (i.e., 72 h later).

Cell proliferation and differentiation. In contrast to the MRFs, markers such as cyclin D1 and cyclin-dependent kinase inhibitor p21 are generalized or nonskeletal muscle cell-specific markers of potential proliferation (cyclin D1) or differentiation (p21; p21 is a cell cycle inhibitor). In rats, we found that cyclin D1 mRNA was relatively insensitive to a single bout of RE but responded with an approximately twofold increase following a second RE bout (24). In the present study, we found that cyclin D1 was increased only at the earliest post-RE time point and only by ~50% (Fig. 4A). An elevation of cyclin D1 expression would suggest an increase in the number of cells within the previously exercised muscles that are activated to enter the cell cycle in response to this stimulus. Visual comparisons between the cyclin D1 and MyoD data appear to suggest that these two message pools were responding in a similar manner (Figs. 3A and 4A). However, there was no significant correlative relationship between these two data sets (data not shown).

The mRNA for p21 demonstrated the most dramatic magnitude of change of any of the markers analyzed for this study (Fig. 4B). The cyclin-dependent kinase inhibitor p21 is associated with withdrawal from the cell cycle and, often, subsequent cell differentiation (22). Because there did not appear to be a high signal indicating proliferative activity, the increased expression of p21 mRNA suggests that a number of cells within the exercised skeletal muscles were most likely going through the process differentiation. As our laboratory has reported in a number of previous studies involving increased skeletal muscle loading (4, 13, 24), there was a strong correlation between the mRNA for myogenin and that of p21 (Fig. 5A). Further analysis indicated that this relationship retained temporal coherence (Fig. 5B). These results strongly suggest that at least some of the cells going through the process of differentiation were myogenic precursor cells.

Total RNA. The majority of the RNA present in skeletal muscle cells consists of ribosomal RNA. Therefore, the con-

centration of RNA in skeletal muscle provides an indication of the synthetic potential of the cells that make up that tissue. However, it should be noted that potential differences in translational efficiency would not necessarily be reflected in this measurement. In a previous study, we found that the concentration of RNA in skeletal muscle of spinal cord injury subjects before the RE bouts was lower than that in skeletal muscle of able-bodied control subjects but that, after exercise, the RNA concentration was not different between these two groups (13). In skeletal muscle from the able-bodied subjects, two sessions of RE did not result in any changes in RNA when measured 24 h postexercise, a result that is similar to that seen in the present study (13). However, herein we report that two sessions of exercise did induce an increase in RNA at the 96-h time point (72 h after the second RE bout). This suggests that the anabolic potential of these skeletal muscles was increasing after just two sessions of RE but with a substantial delay relative to the molecular markers.

Exercise mode. The question arises whether the exercise mode used in this study may account for differences with published results using dynamic muscle actions. For example, in one such study, Bamman et al. (11) had subjects complete eight sets of eight squat exercises on two different occasions. In one session, shortening muscle actions were performed; in the other session, lengthening actions were performed. Muscle biopsies were collected 48 h postexercise in each case. These authors reported that the mRNA for IGF-1 was decreased and that of IGF-1 increased in response to lengthening contractions conducted at 110% of one repetition maximum (11). Compared with the present results using isometric RE (Fig. 2), this indicates that lengthening muscle contractions may lead to a difference in adaptation, either in magnitude or time course. However, it should be noted that, at the 48-h time point, following the lengthening but not shortening contractions, these subjects reported high levels of soreness, had reduced MVC, and had serum creatine kinase levels that were approximately threefold higher than baseline measurements. Therefore, it is not clear whether the changes seen were related to nascent adaptive responses or to muscle injury.

In a more recent study, Bamman et al. (10) measured the expression of MRF and cell cycle-related proteins in the muscles of young and old subjects of both genders, before and after dynamic-mode RE. In that study, the RE consisted of three sets of three different dynamic REs designed to recruit the quadriceps muscle group for shortening or shortening plus lengthening muscle contractions (10). In biopsy samples collected 24 h postexercise, no changes were detected in protein levels of the MRFs or the cyclins measured. These authors did observe a decrease in the levels of cyclin-dependent kinase inhibitor p27 in female subjects postexercise. In the context of the present study, it is not clear whether these results represent RE mode-specific differences or a latency in the translation of MRF and cyclin mRNAs.

Our laboratory recently reported that, following resistance training programs that resulted in muscle hypertrophy in rats, significantly elevated levels of MGF and IGF-1 mRNA could be detected in the muscles of animals that had been trained using either isometric or shortening but not lengthening muscle contractions (3). However, due to the experimental design, it cannot be determined whether these results represent mode-

specific responses to the last bout of RE or a different steady state induced by 10 serial bouts of RE training.

Perhaps the most compelling argument for a mode-specific difference in molecular level responses to RE comes from the work published by Hameed et al. (26). In that study, subjects completed 10 sets of knee extensor exercise at 80% of one repetition maximum with muscle biopsies of the VL collected at 2.5 h postexercise. These authors report that RE induced an increase (range, 2–864%) in the mRNA for MGF in the muscles of young but not old subjects, whereas no RE-induced change in IGF-1 mRNA was detected. Whereas the results presented herein do not include a postexercise time point before 12 h, the findings published by Hameed et al. may suggest that exercise mode plays a role in determining the pattern of molecular level responses to RE.

In summary, the results of this study indicate that a single session of RE is sufficient to stimulate molecular-level responses, which is indicative of the initiation of myogenic processes in skeletal muscle. It is important to note that the RE session used in this study consisted of a total of 90 electrically stimulated contractions with a total duty cycle of 7.5 min of contractile activity. Just two bouts of this activity, a total of 14 min of contraction spread over 2 days, was sufficient to induce an increase in the concentration of total RNA, most likely representing an increase in ribosomal RNA, and, therefore, an increase in the anabolic potential of this tissue. These results, along with a number of other published observations, indicate that the mechanisms that regulate the adaptation of skeletal muscle to increased loading respond on a relatively short time scale and that these responses may be useful in evaluating RE training variables with high temporal resolution.

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GRANTS

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