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Journal

Journal of Applied Physiology, 94(6)

ISSN

8750-7587 1522-1601

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Publication Date

2003-06-01

DOI

10.1152/japplphysiol.00014.2003

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Acute molecular responses of skeletal muscle to resistance exercise in able-bodied and spinal cord-injured subjects

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Submitted 7 January 2003; accepted in final form 6 February 2003

Bickel, C. Scott, Jill M. Slade, Fadia Haddad, Gregory R. Adams, and Gary A. Dudley. Acute molecular responses of skeletal muscle to resistance exercise in able-bodied and spinal cord-injured subjects. *J Appl Physiol* 94: 2255–2262, 2003. First published February 28, 2003; 10.1152/jappphysiol.00014.2003.—Spinal cord injury (SCI) results in muscle atrophy, which contributes to a number of health problems, such as cardiovascular deconditioning, metabolic derangement, and osteoporosis. Electromyostimulation (EMS) holds the promise of ameliorating SCI-related muscle atrophy and, therefore, improving general health. To date, EMS training of long-term SCI subjects has resulted in some muscle hypertrophy but has fallen short of normalizing muscle mass. The aim of this study was to compare the molecular responses of vastus lateralis muscles from able-bodied (AB) and SCI subjects after acute bouts of EMS-induced resistance exercise to determine whether SCI muscles displayed some impairment in response. Analysis included mRNA markers known to be responsive to increased loading in rodent muscles. Muscles of AB and SCI subjects were subjected to EMS-stimulated exercise in two 30-min bouts, separated by a 48-h rest. Needle biopsy samples were obtained 24 h after the second exercise bout. In both the AB and SCI muscles, significant changes were seen in insulin-like growth factor binding proteins 4 and 5, cyclin-dependent kinase inhibitor p21, and myogenin mRNA levels. In AB subjects, the mRNA for mechano-growth factor was also increased. Before exercise, the total RNA concentration of the SCI muscles was less than that of the AB subjects but not different postexercise. The results of this study indicate that acute bouts of resistance exercise stimulate molecular responses in the skeletal muscles of both AB and SCI subjects. The responses seen in the SCI muscles indicate that the systems that regulate these molecular responses are intact, even after extended periods of muscle unloading.

mechano-growth factor; insulin-like growth factor I; insulin-like growth factor binding protein; myogenin

INACTIVITY CAN LEAD TO A LOSS of muscle mass and function. Complete spinal cord injury (SCI) leads to inactivation and profound muscle unloading. The muscles of SCI patients are characterized by severe atrophy, as well as extensively altered metabolic and contractile protein profiles (6, 19, 40). For example, within several

months of SCI, muscles and their constituent myofibers can be reduced to ~41% of the size of those of able-bodied (AB) individuals (16, 15). The loss in muscle mass and function can contribute to a number of health problems, such as decreased cardiorespiratory fitness, impaired glucose tolerance, and osteoporosis (37, 39).

Recognition of potential benefits to be garnered by maintaining or increasing muscle mass in SCI patients has led to a number of studies aimed at using electromyostimulation (EMS) to induce contractile activity in the muscles of these patients. In AB subjects, EMS-induced resistance exercise has been shown to result in increases in muscle size and performance (13, 14, 41). Studies have also shown that the muscles of SCI patients can respond to EMS-mediated contractile activity with some degree of appropriate adaptation, including a modest hypertrophy (11, 17, 31, 36, 38, 40). However, the absolute changes in mass seen in long-term SCI subjects tend to be relatively small as a result of the atrophied state of the muscle at the start of training (e.g., Refs. 17, 31, 35, 36). For example, Mohr et al. (36) reported that EMS cycling evoked a 12% increase in cross-sectional area of the quadriceps femoris muscle in SCI subjects. Assuming the atrophied muscle was 40% of its preinjury size, a 12% increase would result in muscle that remained less than one-half the size of that expected for an AB subject. In contrast to the relatively small effects of EMS in long-term SCI patients, we instituted EMS training ~48 wk after the injury and were able to increase muscle mass to a state more directly comparable to that of ambulatory subjects in only 8 wk (22). Others have also reported that training initiated soon after SCI (~15 wk) maintained lower extremity muscle mass (9). It is not clear whether the differences between the ability of EMS training to rebuild vs. maintain skeletal muscle are a function of alterations in the physiology of the muscles or differences in the method of training used in the various studies. One possibility is that some of the molecular and cellular response systems involved with the response of muscle to increased loading are less responsive in the muscles of SCI subjects.

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Skeletal muscle can respond to changes in loading state via alteration in myofiber size as well as qualitative changes in contractile and metabolic characteristics. As in the case of SCI, unweighting and inactivity result in a decrease in muscle size as a result of myofiber atrophy (16). Conversely, increased muscle loading can result in myofiber hypertrophy and alterations in the expression profile of contractile and metabolic proteins (12). It is clear that changes in loading patterns result in the adaptation of only the affected muscles. This has led to the recognition that specific adaptations that occur in skeletal muscle appear to be regulated primarily by intrinsic mechanisms (e.g., local cellular mediators as opposed to central or circulating factors).

We have previously shown that, in rodent muscle, cellular and molecular events indicative of a hypertrophic response can be detected after a single bout of resistance-type exercise (25). In that study, we also demonstrated that multiple bouts of exercise result in the summation of these cellular and molecular responses. In the present study, we have attempted to evaluate the response of some of these molecular markers to acute bouts of EMS-induced resistance exercise in both healthy subjects and long-term SCI patients to uncover potential SCI-induced changes. Our hypothesis was that SCI-induced defects in mechanisms by which skeletal muscle adapts to increased loading would be detectable as differential molecular level responses to acute resistance exercise.

METHODS

Subjects. Seven AB (age 27 ± 1.6 yr, height 177 ± 3.3 cm, weight 80 ± 7.0 kg, 1 woman, means \pm SE) and eight SCI (age 36 ± 1.9 yr, height 178 ± 2.9 cm, weight 83 ± 6.8 kg) subjects participated in this study. SCI level of injury ranged from C₄ to T₁₀, and the average time postinjury was 9 ± 2.1 yr. All subjects were motor and sensory complete, with the exception of one who was motor complete only. SCI and AB subjects had no history of lower extremity pathology and signed informed consent before testing. AB subjects were recreationally active and not currently involved in lower extremity resistance exercise. Methods were approved by the Institutional Review Boards of the University of Georgia and Shepherd Center and the University of California-Irvine.

EMS protocol. The vastus lateralis (VL) muscle was stimulated essentially as described previously (4, 16, 30). 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 muscle injury (3). 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 proximal and distal portions of the VL. This electrode placement has previously been shown to

allow sufficient recruitment of the VL in AB subjects and is essentially as done previously (5, 30). A commercial stimulator (TheraTouch model 4.7, Rich-Mar, Inola, OK) was used for EMS. The initial torque was determined in the following manner. The AB controls performed a maximum voluntary contraction (MVC) for isometric knee extension prior to EMS. The subjects were highly motivated, and all had prior experience with knee-extension MVC. We have previously established that the VL muscle constitutes roughly 30% of the total quadriceps group cross-sectional area (28). Accordingly, electrical current sufficient to elicit $\sim 30\%$ of the observed isometric knee-extension MVC was determined and used for the subsequent EMS protocol in the AB subjects. For SCI patients, the torque was determined by increasing current incrementally until torque no longer increased, thereby ensuring that the entire VL was activated. The EMS protocol consisted of 5-s contractions separated by 15 s for 30 min at these previously determined current levels. An identical stimulation bout was performed 48 h later. For both groups, contractions were evoked with 50-Hz trains of 450- μ s biphasic pulses.

Biopsy technique. Muscle samples were obtained from all subjects immediately before the initial stimulation and 24 h after the second stimulation bout. Biopsies were taken from the VL by using the percutaneous biopsy technique, as done previously (16, 28). Samples were immediately cooled with liquid nitrogen and then stored at -70°C until analyzed.

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 (18). Extracted RNA was precipitated from the aqueous phase with isopropanol and after being washed 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 an optical density 260-nm unit equivalent to 40 $\mu\text{g}/\text{ml}$). The muscle total RNA concentration is calculated on the basis of 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 by using relative RT-PCR procedures.

RT. One microgram of total RNA was reverse transcribed for each muscle sample by using the SuperScript II RT from GIBCO-BRL and a mix of oligo(dT) (100 ng/reaction) and random primers (200 ng/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 insulin-like growth factor (IGF)-I, mechano-growth factor (MGF), IGF-I receptor, IGF binding proteins (IGFBP-4 and -5), myogenin, cyclin D1, and p21. The sequence for the various primers used for the specific target mRNAs is shown in Table 1. These primers were designed by 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.

Table 1. *The sequence of the specific sets of primers used in mRNA RT-PCR analyses*

Target mRNA	PCR Primer Sequence 5'→3'	Product Size, bp	GenBank Accession No.
IGF-I	5' sense: GTGGAGACAGGGGCTTTTATTTTC 3' antisense: CTTGTTTCCTGCACTCCCTCTACT	251	M37483
MGF	5' sense: ACCAACAAGAACACGAAGTC 3' antisense: CAAGGTGCAAATCACTCCTA	282	U40870
IGF-I receptor	5' sense: ACAAAGGGCCATCGTTTCATCC 3' antisense: GCACAGCAGGGGCACTCG	195	NM_000875
IGFBP-5	5' sense: AGATCTTCCGGCCCAACACA 3' antisense: GTACACAGCAGGGGCAACAT	242	NM_000599
IGFBP-4	5' sense: CTCATCCCCATCCCCAACTG 3' antisense: CTGCTGGCAGGCCCTCACTCT	200	M38177
Myogenin	5' sense: AGAAGGGGAGAGGAACAGC 3' antisense: AAGAAATCACCCCAAGAGG	205	NM_002479
Cyclin D	5' sense: GACCCCGCACGATTTTCAT 3' antisense: GGAGGCAGTCTGGGTCACAC	269	NM_001758
p21	5' sense: GCAGCGGAACAAGGAGTCAG 3' antisense: ACACATGGGGAGCCGAGAG	233	L25610

IGF-I, insulin-like growth factor I; MGF, mechano-growth factor; IGFBP-4 and IGFBP-5, IGF binding protein-4 and -5, respectively.

For the 18S amplification, we used the Alternate 18S Internal Standards (Ambion), which yields a 324-bp product. The 18S primers were mixed with competimers at an optimized ratio that could range from 1:4 to 1:10, depending on the abundance of the target mRNA. Inclusion of 18S competimers was necessary to bring down the 18S 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 under identical conditions by using the same reagents premixed 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 2-deoxynucleotide 5'-triphosphate, 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 (45). In this approach, 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 and 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 (Fig. 1).

Statistical analysis. All values are reported as means ± SE. For each time point, treatment effects were determined by ANOVA with post hoc testing (Student Newman-Keuls) by using the Prism software package (Graphpad). Pearson's correlation analysis was used to assess the relationship between p21 and myogenin 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 bouts, the AB subjects were stimulated to produce $36 \pm 1\%$ of their previously measured isometric MVC. Over the course of the stimulation protocol, the torque of the AB subjects declined by $42 \pm 6\%$, whereas that of the SCI subjects decreased $64 \pm 9\%$.

The mRNAs for several components of the IGF-I system were altered in response to the acute bout of EMS-induced resistance exercise. Compared with the preexercise sample, the expression and/or accumulation of MGF, the loading-sensitive isoform of IGF-I (26), was significantly increased in the muscles of SCI subjects after EMS (Fig. 2A). The expression of the mRNA for IGFBP-4 was significantly increased in both the able-bodied control and SCI muscles, whereas that of IGFBP-5 was significantly depressed (Fig. 2, B and D). There were no significant alterations in the expression of the mRNAs for either IGF-I (data not shown) or type 1 IGF-I receptor (Fig. 2C).

The expression of two markers of cellular differentiation was increased as a result of EMS. The mRNA for p21, a general marker of cellular differentiation, was significantly increased in the postexercise samples from both control and SCI subjects (Fig. 3A). In addition, p21 mRNA expression was significantly higher in the muscles of SCI patients before the EMS exercise (Fig. 3A). The expression of myogenin mRNA, a putative muscle-specific marker of cellular differentiation, was increased to a similar extent in both control and SCI subjects (Fig. 3B). The observed increases in cyclin D1 expression, a marker of cellular proliferation, did not reach statistical significance in either the AB or SCI subjects (data not shown).

Compared with preexercise, the concentration of total muscle RNA was not significantly altered by EMS (Fig. 4). However, total muscle RNA concentration was

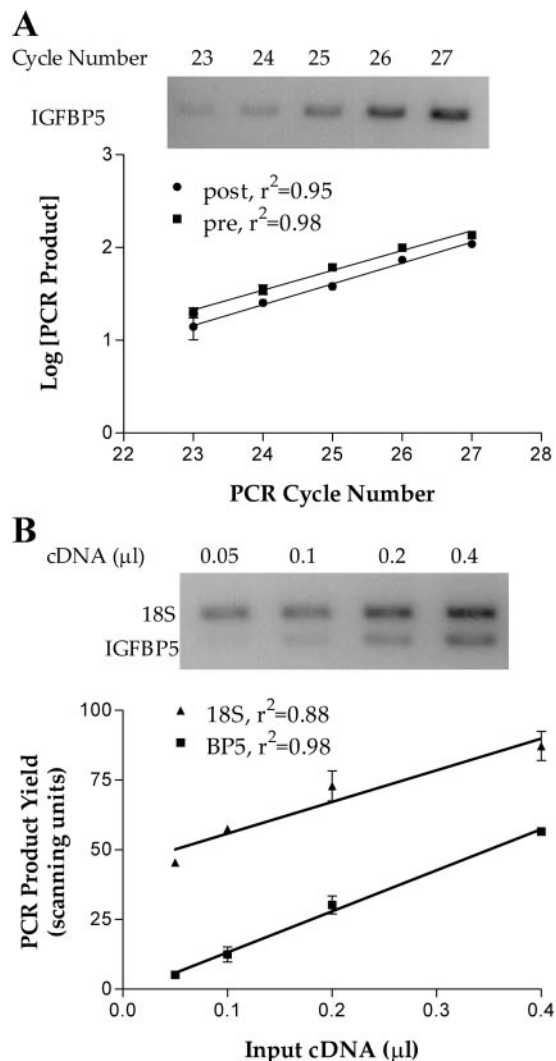


Fig. 1. Validation of the PCR. *A*: for the insulin-like growth factor binding protein-5 (BP5) mRNA product, we show the semilog plot of the PCR product for 2 control samples, preexercise (Pre) and postexercise (Post), as a function of PCR cycle number. Regression analysis shows that the relationship is significantly linear with high coefficient of determination. *B*: for a given PCR condition aimed to amplify a target cDNA (BP5 shown), final product for both 18S and target mRNA was tightly correlated with the amount of input cDNA, and the relationship between input cDNA vs. PCR product was significantly linear for the range of cDNA tested. Note that the slopes of the 18S line vs. the BP5 lines were not significantly different. This approach was used to validate the use of 18S for the correction for potential variability in the starting amount of cDNA in each case. Values are means \pm SE. IGFBP, insulin-like growth factor binding protein.

significantly lower in SCI vs. control muscles before exercise (Fig. 4). This difference was no longer significant after the EMS exercise bouts.

DISCUSSION

Individual skeletal muscles adapt to alterations in loading, allowing for the development of task-specific functional characteristics. These adaptations can include an increase or decrease in mass, as well as alterations in contractile characteristics. Enforced un-

loading and/or inactivity can lead to adaptations that have negative impacts. In the case of SCI patients, extensive loss of muscle mass can contribute to cardiovascular deconditioning, metabolic derangement, and osteoporosis (37, 39). A number of studies have attempted to evaluate the utility of EMS to increase the muscle mass of long-term SCI patients (6, 9, 11, 13–17, 19, 22, 30, 31, 36–40). Whereas the general consensus appears to be that EMS training can result in improvement in various measures of muscle function, it is not clear that the benefits are proportional to the time and effort the patients must invest in EMS training. Some portion of this uncertainty most likely results from the inconsistency of the published results. In turn, much of the variability in the published research in this field can be attributed to differences in experimental design and methodology. For example, the protocols imposed during various EMS training studies have included no loading, endurance types of activity (cycle ergometry), and a few attempts at true resistance types of exercise (e.g., Refs. 9, 31, 35–40). As a result, the ability of the muscles of long-term SCI patients to hypertrophy has not been systematically examined. Of particular interest, there have been some reports that suggest that EMS may be more effective at normalizing muscle mass and function when applied a relatively short time after the injury. This raises the possibility that the muscles of long-term SCI patients may exhibit physiological responses to EMS training that differ from those of ambulatory subjects.

Few studies involving direct comparisons between the exercise response of the muscles of SCI and AB subjects have been reported (15, 22, 30, 39). Where they exist, such studies have followed traditional approaches to the evaluation of resistance exercise and relied on end-state measures, such as strength, muscle size, or protein level biochemistry (22, 30). The temporal resolution of such measurements is generally on the scale of weeks or months, requiring many exercise sessions. In the present study, we used changes in the expression of various mRNAs as indicators of the cellular-level responses to the exercise stimulus with a temporal resolution on a scale of hours to compare the responses of AB vs. SCI subjects. These experiments were patterned on our previous work in rodents in which changes in the expression of markers of both myogenic and anabolic processes were detected after a single bout of resistance exercise (25). In that study, we found that two bouts of exercise, separated by 48 h, provided a summation of cellular and molecular signaling responses, such that the magnitude of a given response was greater than that seen after a single exercise bout. In the present study, the two-exercise-bout model was used to ensure that a sufficient stimulus was delivered.

A limitation of the present study is that, unlike our previous rodent studies, which provided relatively large amounts of muscle (e.g., >500 mg), analysis was limited to mRNA changes due to the smaller amount of tissue available from human biopsy samples (3). In addition, the number of biopsy samples and, therefore,

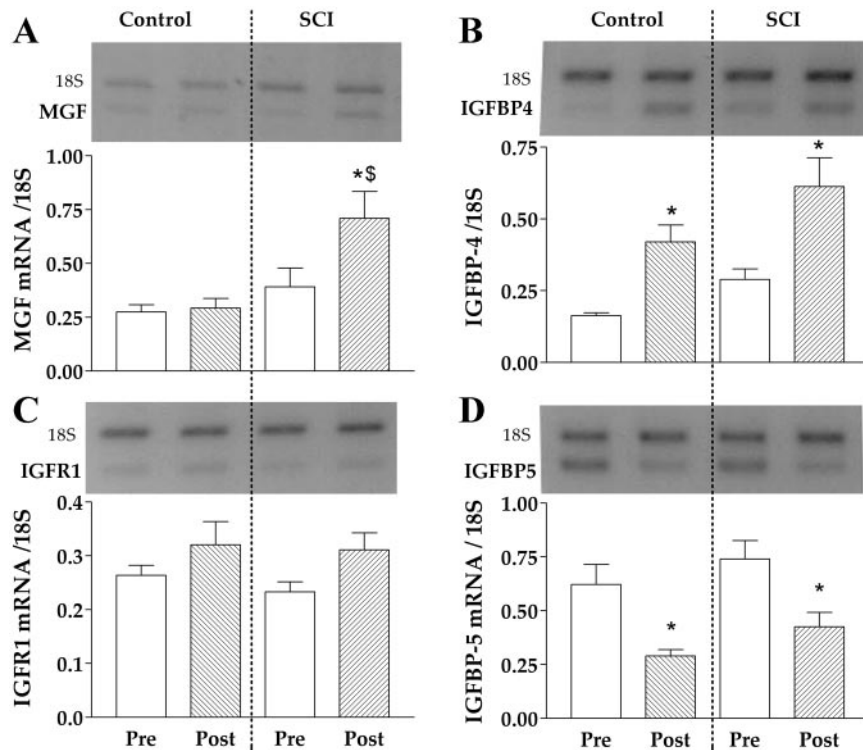


Fig. 2. Effects of electromyostimulation (EMS)-induced exercise on the expression and/or accumulation of mRNA for components of the muscle insulin-like growth factor (IGF)-I system. The mRNA levels were determined by quantitative RT-PCR, including coamplification of 18S. A: mechano-growth factor (MGF) mRNA/18S increased in Post in the muscles of spinal cord-injured (SCI) subjects. B: EMS resulted in similar increases in IGFBP-4 mRNA in the muscles of both able-bodied (control) and SCI subjects. C: mRNA for the type I IGF-I receptor (IGFR1) was unchanged after EMS. D: EMS-induced exercise resulted in an ~2-fold decrease in the mRNA for IGFBP-5. Above each plot is a representative gel image showing both 18S and target mRNA PCR products for each group. Values are means \pm SE. * $P < 0.05$ vs. Pre. \$ $P < 0.05$ vs. Control-Post.

time points when it was feasible to collect from human subjects were limited compared with those from our animal studies. Therefore, we could not be certain that we had chosen the optimal parameters (e.g., exercise stimulus, sample collection time) to ensure that the response was fully realized. Nonetheless, we were able to detect changes in a number of molecular signals indicative of a hypertrophy response in the EMS-exercised muscles of both AB and SCI subjects.

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 (1–3, 25). In muscle, the IGF-I axis consists of locally expressed IGF-I and MGF, the type I IGF-I receptor, and a number of IGFBPs. Modulation of the various components of this system appears to be important for the development of a compensatory hypertrophic response (1, 7, 8, 10, 20, 24, 26, 33, 44). In animal studies, increased muscle loading has been shown to result in an upregulation of the expression of IGFBP-4 and downregulation of IGFBP-5 (7, 25). The same pattern of changes was seen in these mRNAs in muscle samples from both the AB and SCI subjects in the present study (Fig. 2). Using a similar EMS training protocol in rats, we had previously observed that MGF mRNA was increased at very early time points (e.g., 6–12 h) after contractile activity (25). In the present study, the muscles from the SCI subjects exhibited an approximately twofold increase in MGF mRNA 24 h after the second bout of EMS-induced resistance exercise. This contrasts to the muscles of the AB subjects in whom no MGF response was

evident at this time point. One possibility for this difference might be that some cellular transduction and signaling mechanisms, which mediate the MGF response, may have an increased sensitivity in the muscles of the SCI subjects.

Myogenic markers. In rats, increased loading has been shown to result in the proliferation and differentiation of satellite cells (reviewed in Refs. 24, 42). There is an increasing body of evidence that indicates that these myogenic processes are an obligatory component of the compensatory hypertrophic response (2, 21). In the present study, we used the mRNA for cyclin D1 as a marker of the intent of some cell population within muscle to enter the cell cycle. In contrast to our previous studies conducted in rats, we did not detect statistically significant increases in the expression of cyclin D1 mRNA in the muscles from either the AB or SCI subjects (25). It is possible that the failure to detect a significant cyclin D1 response was related to the limitation imposed by selecting a single time point for sample collection. In our rodent studies, we have found that the cyclin D1 response is delayed compared with other measures. Our laboratory has previously reported that one of the earliest responses to increased muscle loading is marked increase in myogenin mRNA (2, 3, 25). Myogenin is a member of the myogenic regulatory factor family and, as such, is important for the expression of muscle-specific proteins (24, 42). In the present study, the expression and/or accumulation of myogenin mRNA was increased in both the AB (~3-fold) and SCI (~2-fold) subjects 24 h after the second EMS-induced resistance exercise bout (Fig. 3). In fully innervated skeletal muscle, increased expres-

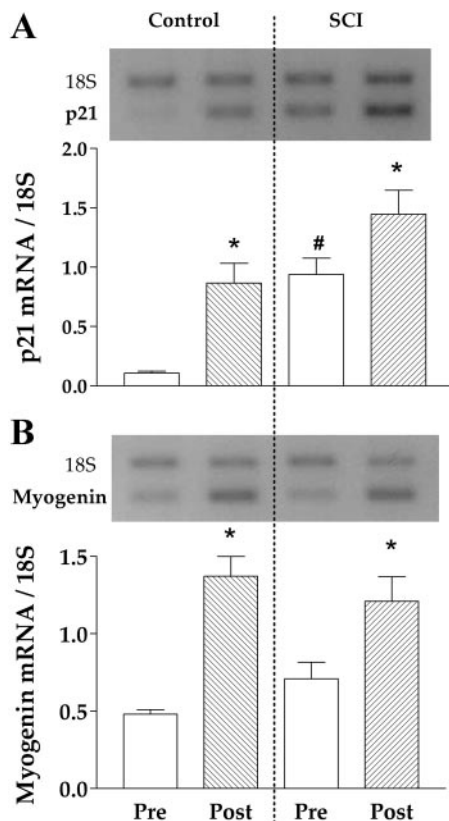


Fig. 3. Effects of EMS on the mRNA for p21 and myogenin in AB and SCI subjects. *A*: mRNA for p21 was significantly higher in SCI than AB muscles before exercise. Compared with Pre values, EMS resulted in significant increases in p21 mRNA in both AB and SCI muscles. *B*: mRNA for myogenin was increased significantly Post in the muscles from AB (2.9-fold) and SCI (2-fold) subjects. Above each plot is a representative gel image showing both 18S and target mRNA PCR products for each group. Values are means \pm SE. * P < 0.05 vs. Pre. # P < 0.05 vs. Control-Pre.

sion of myogenin is thought to indicate that a population of myogenic cells is differentiating (3, 23, 27, 34, 42, 43, 46). In support of this, the increase in myogenin mRNA in the present study was highly correlated with the mRNA for p21, a general cell-type marker of differentiation, in both AB and SCI subjects (Fig. 5). This result suggests that the exercise-induced response of the muscles in both the AB and SCI subjects included the differentiation of some class of myogenic precursor

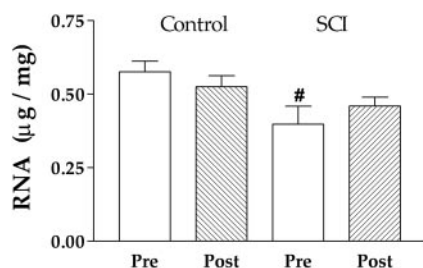


Fig. 4. Compared with the AB subjects, the concentration of RNA was significantly lower in the muscles from SCI subjects before the EMS-induced exercise bouts. This difference was no longer significant after the exercise bouts. * P < 0.05 vs. Control-Pre.

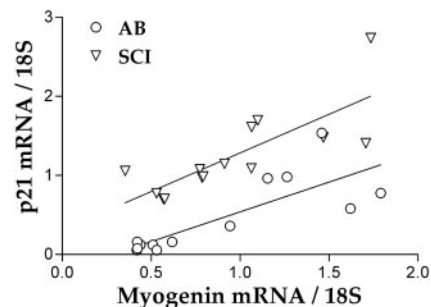


Fig. 5. Relationship between the levels of myogenin and p21 mRNA. There was a strong correlation between the amount of myogenin and p21 mRNA in the muscle samples from both the AB ($r = 0.80$, $P = 0.009$) and SCI ($r = 0.79$, $P = 0.009$) subjects.

cell. This result, correlation between the increase in p21 and myogenin, is similar to that seen in our laboratory's previous rodent studies (2, 3, 25).

Hespele et al. (29) found that VL myogenin protein levels were unchanged after 2 wk of cast immobilization and after 3 wk of knee-extension rehabilitation exercise. In that study, myogenin and myogenic regulatory factor-4 protein levels were increased after 10 wk of progressive exercise rehabilitation, a time point when significant muscle hypertrophy was evident.

Total RNA. The majority of the RNA present in muscle cells consists of ribosomal RNA. Therefore, the concentration of RNA in skeletal muscle provides an indication of the synthetic potential of the cells that make up that tissue. It should be noted that potential differences in translational efficiency would not necessarily be reflected in this measurement. The concentration of RNA in the muscles of SCI subjects before the exercise bouts was significantly lower than that in the muscles of the AB subjects. The postexercise SCI RNA concentration was not statistically different from the preexercise value; however, it was no longer significantly different from that of the AB subjects. Decreased anabolic potential of the SCI muscles is not a particularly surprising finding. However, the possibility that this parameter might begin to respond after just two exercise bouts suggests that the anabolic potential of the muscles from long-term SCI subjects may be capable of recovery, given appropriate stimuli.

In summary, the primary divergence in the observed molecular level responses between the AB and SCI subjects was a potentially exaggerated response in the abundance of MGF mRNA in the muscles of the SCI subjects. In this context, it should be noted that the actual duty cycle of the contractile activity in this study was 7.5 min per training bout. This would most likely represent a relatively small proportion of the total daily contractile activity, albeit at higher forces, for the muscles of the AB subjects, but an essentially infinite increase (i.e., from nothing to something) for the muscles of the SCI subjects, and thus a greater loading-induced response would not be unexpected. In this respect, it is probably more surprising that the various additional indicators did not demonstrate a differential

responses. Taken together, the results of this study suggest that, at the molecular level, the muscles of long-term SCI subjects appear to respond to increased contractile activity in a very similar fashion to the muscles of AB subjects.

The authors thank the subjects for participation in this study and Anqi Qin for technical assistance with the molecular analysis.

Funding was provided, in part, by the Foundation for Physical Therapy (to C. S. Bickel) and National Institute of Child Health and Human Development Grants HD-37439-S and HD-39676 (to G. A. Dudley).

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