Resistance exercise alters MRF and IGF-I mRNA content in human skeletal muscle

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Psilander, Niklas, Rasmus Damsgaard, and Henriette Pilegaard. Resistance exercise alters MRF and IGF-I mRNA content in human skeletal muscle. J Appl Physiol 95: 1038–1044, 2003. First published April 25, 2003; 10.1152/japplphysiol.00903.2002.—Increasing evidence suggests that the myogenic regulatory factors (MRFs) and IGF-I have important roles in the hypertrophy response observed after mechanical loading. We, therefore, hypothesized that a bout of heavy-resistance training would affect the MRF and IGF-I mRNA levels in human skeletal muscle. Six male subjects completed four sets of 6–12 repetitions on a leg press and knee extensor machine separated by 3 min. Myogenin, MRF4, MyoD, IGF-IEabc (isoforms a, b, and c) and IGF-IEbc (isoform b and c) mRNA levels were determined in the vastus lateralis muscle by RT-PCR before exercise, immediately after, and 1, 2, 6, 24, and 48 h postexercise. Myogenin, MyoD, and MRF4 mRNA levels were elevated (P < 0.005) by 100–400% 0–24 h postexercise. IGF-IEabc mRNA content decreased (P < 0.005) by ~44% after 1 and 6 h of recovery. The IGF-IEbc mRNA level was unaffected. The present study shows that myogenin, MyoD, and MRF4 mRNA levels are transiently elevated in human skeletal muscle after a single bout of heavy-resistance training, supporting the idea that the MRFs may be involved in regulating hypertrophy and/or fiber-type transitions. The results also suggest that IGF-IEa expression may be downregulated at the mRNA level during the initial part of recovery from resistance exercise.

hypertrophy; myogenin; mechanical load; satellite cells

CONVENTIONAL RESISTANCE EXERCISE induces increased synthesis of skeletal muscle proteins, leading to muscle hypertrophy (8, 10, 41). Increased protein synthesis might result from enhanced transcription, more stable mRNA molecules, a higher translation rate, or a combination of these processes. A human study examining the effect of resistance exercise on myosin heavy chain (MHC) and actin expression has indicated that these proteins can be upregulated without an increase in mRNA (37). On the other hand, animal studies examining transcription factors and hormones showed that overloaded rat, mouse, and rabbit muscle undergoing hypertrophy had increased mRNA levels of the myogenic regulatory factors (MRF), Ankrd2 transcription factor, and the hormone IGF-I (3, 19, 40). Thus it seems that resistance training induces both transcriptional and translational changes in the muscle. If translation is a nonspecific process, all genes expressing mRNA will be affected, causing a general increase in protein synthesis. Translational changes may be the basic regulatory mechanism in hypertrophy, with transcription of specific genes such as transcription factors and hormones, as an important additional regulation to further adjust the hypertrophy response. Transcription of specific hormones and transcription factors may also have qualitative effects, thus improving the muscle’s ability to cope with exercise-induced stress.

The signaling pathways leading to transcriptional and translational changes in skeletal muscle in response to resistance exercise are still not fully understood. Baar and colleagues (6) suggest four potential stimuli that may regulate these processes: mechanical load or stress, intracellular calcium, hypoxia, and redox state. These are thought to be first messengers in a signaling cascade in which various transcription factors, hormones, and other regulatory proteins are activated. These proteins, in turn, contribute in controlling the qualitative and quantitative changes occurring in the muscle by regulating gene expression. The MRFs and IGF-I might be such regulatory proteins, because previous studies have reported exercise-induced changes in MRFs and IGF-I expression, at both the mRNA and protein level (1, 7, 9, 15, 16, 23, 29).

The MRFs are a family of skeletal muscle-specific transcription factors that regulate the expression of several skeletal muscle genes (31). The family is composed of four members: myogenin, MRF4, MyoD, and Myf5. These proteins belong to a larger basic helix-loop-helix class of transcription factors that, after dimerization with a ubiquitous E protein, bind to an E-box domain and transactivate downstream muscle genes, such as desmin, troponin I, and myosin light chain (MLC) (21, 22, 38). Because these genes are involved in qualitative and quantitative changes occurring in the muscle after resistance exercise, there might be an increased expression of the MRFs in response to overload. Studies using stretch overload on animals support this theory (9, 23, 24). So far, there has only been one published study examining the effects of resistance exercise on MRF gene expression in...
humans, showing increased mRNA levels of myogenin and MyoD, but these increases were much lower than the ones observed in the animal studies (39). The mRNA response of the myogenic transcription factor MRF4 to resistance exercise has, to our knowledge, never been reported in human muscle.

The hormone IGF-I has, in recent years, received increased attention in studies examining exercise-induced muscle hypertrophy. IGF-I increases muscular protein synthesis and stimulates satellite cell proliferation and differentiation in vitro (2, 13). Two isoforms of IGF-I that are expressed and released from skeletal muscle cells have recently been identified (16, 28, 40). One of them, IGF-I Ec, is very similar to the isoform produced by the liver and has probably an endocrine function. This isoform is expressed both in working and nonworking muscle (28). The other isoform, mechano-growth factor (MGF) (also called IGF-I Ec in humans) has a 49 (52 in animals) base long DNA fragment inserted in the E-domain of IGF-I Ea and is mainly expressed in working muscle. This isoform is thought to have an autocrine and paracrine function, stimulating myofibrillar protein synthesis and satellite cell activation (1, 15). Increased MGF (2–864%) and IGF-I (62%) mRNA content has been observed 2.5 and 48 h, respectively, after an acute bout of mechanical loading in humans (7, 16), but a more detailed time course (several time points) of IGF-I mRNA expression during recovery from resistance exercise in humans has never been reported.

The aim of the present study was to test the hypothesis that MRF and IGF-I gene expression is increased in response to resistance exercise. These genes were studied because both IGF-I and the MRFs have been linked to satellite cell activation and because they are potentially important genes in the regulation of hypertrophy. MRF4, myogenin, MyoD, IGF-I Ecabc (isoforms a, b, and c), and IGF-I Ecbc (isoform b and c) mRNA contents were determined in muscle biopsies obtained from the vastus lateralis muscle before, immediately after, and during recovery from a single bout of heavy-resistance training.

METHODS

Subjects. Six subjects with an average age of 23.9 ± 2.2 yr (mean ± SE), height of 183 ± 6.9 cm, and weight of 83 ± 13.5 kg participated in the study. The subjects were habitually physically active without taking part in competitive sports or any form of resistance exercise. They were specifically asked not to participate in any heavy exercise throughout the experimental period. None of the subjects had any medical conditions or disabilities that interfered with his ability to perform the exercises. The subjects were given both oral and written information about the experimental procedures before they gave their written consent. The study conformed with the guidelines in the Declaration of Helsinki and was approved by the Copenhagen and Fredriksberg Ethics Committee.

Dietary intake. An individual food plan was constructed for each subject on the basis of body mass and age (World Health Organization Technical Report Series 724 1985). The subjects received three meals per day plus one evening snack.

Preparatory test. The pretest was completed 2 wk before the study to determine the workload to be used during the first set in the experiment. The test was performed on a leg press and a knee extensor machine, and the load was gradually increased until the subjects could only just complete between six and eight repetitions. The subjects were allowed to rest as much as they wanted between the sets to avoid muscle fatigue.

Exercise protocol. A heavy-resistance training protocol that stimulated the vastus lateralis muscle was used. The exercise protocol contained four sets of 6–8, 6–8, 10–14, and 10–14 repetitions and was first carried out on a leg press machine and then on a knee extensor machine. The intention was to reach total exhaustion in each set. The training started with a 40-kg warm-up on the leg press machine, after which the load from the preparatory test was put on. The load was adjusted during the training so that the predetermined repetitions could be completed. Subjects were encouraged to lower the weights slowly (the eccentric phase) and then push them up as fast as possible (the concentric phase). There was a 90-s rest period between each set and a 3-min rest period between the two machines. The complete exercise protocol (a total of eight sets) was completed in ~20 min.

Muscle biopsies. Muscle biopsies were obtained from the middle portion of the vastus lateralis muscle under local anesthesia by using the percutaneous needle biopsy technique with suction. The samples were quickly dipped on a gauze swab to remove superficial blood and frozen in liquid N2. Samples were stored at −80°C until analyzed. Biopsies were taken 1.5 h before the exercise started (Pre), immediately after the last set of knee extension (0 h), and at 1, 2, 6, 24, and 48 h postexercise. Incisions were made, at minimum, 3 cm apart, and only one biopsy was obtained from each incision site. Attempts were made to extract tissue from approximately the same depth in the muscle each time by using the depth markings on the needle. The Pre, 0-h, and 1-h biopsies were obtained from the left leg, and the 2-, 6-, 24-, and 48-h biopsies were obtained from the right leg. From three of the subjects, an additional muscle biopsy was obtained from the right leg 1 h postexercise. The purpose of this biopsy was to examine if prior biopsies would affect the expression of the genes investigated in the present study.

RNA extraction. Total RNA was isolated from ~25 mg of muscle tissue by a modified guanidine thiocyanate-phenol-chloroform extraction method, adapted from Chomczynski and Sacchi (12), as previously described (34).

RT. RT of the RNA samples was performed by using the Superscript II RNase H system (Invitrogen, Carlsbad, CA), according to the manufacturer’s instructions, as previously described (32). Eleven microliters of total RNA were reverse transcribed, and the RT product was diluted to a total of 200 μl. The samples were stored in −20°C for later PCR analysis.

Real-time PCR. The probes and primers used (Table 1) were constructed from human-specific sequence databases (Entrez-NIH and Ensemble, Sanger Institute, using gene bank numbers NM 000618 (IGF-I Ea), HUMGFB (IGF-I Eb), XM001688 (Myogenin), XM 006691 (MRF4), XM 036341 (MyoD)), and applying DNA analysis computer software (Primer Express, Applied Biosystems). All self-designed probes were 5’TAMRA labeled. For each of the genes, a Blast Search revealed that sequence homology was obtained only for the target gene. The primer and probes were optimized to determine optimal primer concentration, probe concentration, and efficiency of amplification. The expected sizes of the PCR products were initially verified by separation on an agarose gel by electrophoresis. The primers and probes for IGF-I Eb were designed to detect and am-
The heavy-resistance exercise induced a transient increase in myogenin, MyoD, and MRF4 mRNA during recovery (Fig. 1). Myogenin showed the most marked change, with a 440% increase (P < 0.05) in the mRNA content 6 h postexercise. MyoD increased with 110% immediately postexercise (0 h), and MRF4 was elevated by 120% (P < 0.05) 2 h postexercise. MRF4 also tended to be higher (100%; P < 0.09) than Pre 24 h postexercise.

**IGF-I.** IGF-Iac mRNA was reduced by 44% (P < 0.05) at 1 and 6 h postexercise compared with the Pre sample (Fig. 2). This decrease was absent when the IGF-Ia isoform was excluded (see IGF-Iac in Fig. 2), indicating that the observed decrease was due to a decrease in IGF-Iac. As expected, the levels measured during the real-time PCR indicated that more IGF-Ia mRNA content was present than IGF-Iac mRNA, although direct comparisons between the absolute levels of isoform a and isoform c cannot be performed based on the present results.

**Effects of the repeated biopsies on MRF and IGF-I mRNA content.** The prior biopsies in the left leg did not seem to affect the mRNA expression of the MRF and IGF-I genes. The fold change observed for the left leg (where prior biopsies had been taken) was, on average, 1.04 ± 0.25 for MRF4, 1.29 ± 0.21 for myogenin, 1.59 ± 0.58 for MyoD, 1.01 ± 0.17 for IGF-Iac, and 1.03 ± 0.33 for IGF-Iabc of the values obtained for the right leg (where no biopsies had been taken). This shows that there were no consistent differences between the two legs, indicating that the repeated biopsies were not a source of error that could explain the changes in gene expression observed in the present study.

**DISCUSSION**

**MRF.** The present data demonstrate that a bout of heavy-resistance training increases the mRNA content of myogenin, MyoD, and MRF4 in human skeletal muscle, indicating that these genes may be involved in regulating hypertrophy and/or fiber-type transition. The myogenin, MyoD, and MRF4 mRNA levels were elevated by 100–400% 0–24 h postexercise, which is in accordance with two animal studies that observed a 100–500% increase in MRF mRNA content 6–72 h after stretch overload in adult quail muscle (23, 24).

The MRFs regulate the transcription of several skeletal muscle-specific genes, such as MLC, troponin I, and desmin (21, 22, 38). These proteins are building blocks of larger protein complexes, and it may, therefore, be speculated that one or more of these proteins are rate limiting when muscle fibers synthesize new structural and contractile elements. Skeletal muscle may thus, to some extent, be able to change the synthesis rate of more complex structures by regulating transcription of the building blocks. The MRFs may, therefore, contribute to regulating hypertrophy by con-

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**Table 1. Primers and probes used for real-time PCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Probe</th>
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<tbody>
<tr>
<td>MYOG</td>
<td>5′-GGCTCTCTGAGTGCAGGT-3′</td>
<td>5′-AGTGCAAGTTTTGCGACCT-3′</td>
<td>5′-CAGTGAGTTCAAGGGCAACCCCA-3′</td>
</tr>
<tr>
<td>MYOD</td>
<td>5′-CGCCACAGGAACAGCTACGTC-3′</td>
<td>5′-CAGTGGAAAGAGGACATAG-3′</td>
<td>5′-CGGTTTGTATTGTTGGAAGTGA-3′</td>
</tr>
<tr>
<td>MRF4</td>
<td>5′-GCGTCCCTCTGCGCTGTC-3′</td>
<td>5′-GAAGGGGAGCGGCAGCAGC-3′</td>
<td>5′-TGCAACAGATACTGTCGAGCT-3′</td>
</tr>
<tr>
<td>IGF-Iabc</td>
<td>5′-GACCTGCTGTGGTGGGTG-3′</td>
<td>5′-TGCACAGATGCTGTCGGACCT-3′</td>
<td>5′-CAGTGAGTTCAAGGGCAACCCCA-3′</td>
</tr>
<tr>
<td>IGF-Ib</td>
<td>5′-CCGTCCCTCTGCGCTGTC-3′</td>
<td>5′-GAAGGGGAGCGGCAGCAGC-3′</td>
<td>5′-TGCAACAGATACTGTCGAGCT-3′</td>
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**MYOG, myogenin; MYOD, myogenic differentiation antigen; MRF4, myogenic regulatory factor 4; IGF-Iabc, IGF-I isoform a, b, and c.**

**Statistical analysis.** Results are presented as means ± SE. The mRNA contents were normalized to GAPDH, and the samples were expressed relative to the corresponding control (Pre) sample, which was set to 1. A one-way ANOVA for repeated measures was used to determine whether there were any significant differences over time among the samples. If a significant difference was present, a Bonferroni t-test was used to locate the difference. Differences were considered significant at P < 0.05.
trolling transcription of genes such as MLC, troponin, and desmin. In line with such a possibility is a correlation between changes in muscle mass and MRF expression found in three studies (3, 4, 17). Hespel and colleagues (17) showed that the MRF4 protein content in human muscle was closely correlated with the concomitant change in average muscle-fiber size after 10 wk of resistance exercise. In rats, aging was associated with a 50% reduction in muscle to body weight, and this decline was similar to the decline in mRNA levels of the MRF-regulated genes desmin and MLC (4). In addition, hypertrophy was attenuated in old rats compared with young rats, and the hypertrophied muscle from the old rats had significantly lower levels of MRF than the muscle from the young rats (3).

The MRFs are known to be of importance when myoblasts proliferate and differentiate (30). This im-

Fig. 1. Myogenin (A), myogenic regulatory factor 4 (MRF4; B), and MyoD (C) mRNA content in the vastus lateralis muscle, during recovery from a heavy-resistance training bout, presented as fold changes relative to the Pre value, which was set to 1.0. Data are normalized to GAPDH mRNA. Muscle biopsies were obtained before (Pre), immediately after exercise (0 h), and 1, 2, 6, 24, and 48 h into recovery. Values are means ± SE (n = 6). A one-way ANOVA for repeated measures was used. *Significantly different from Pre (P < 0.05).

Fig. 2. IGF-Iabc (including isoforms a, b, and c; A) and IGF-Iabc (including isoform b and c; B) mRNA content in the vastus lateralis muscle, during recovery from a heavy-resistance training bout, presented as fold changes relative to the Pre value, which was set to 1.0. Data are normalized to GAPDH mRNA. Muscle biopsies were obtained before (Pre), immediately after exercise (0 h), and 1, 2, 6, 24, and 48 h into recovery. Values are means ± SE (n = 6). A one-way ANOVA for repeated measures was used. *Significantly different from Pre (P < 0.05).
plies that these transcription factors are involved in the stretch-induced activation and subsequent differentia-
tion of satellite cells, and it has been speculated that myogenin regulates the expression of a protein that is required for satellite cell fusion (23). Satellite cell proliferation, differentiation, and fusion with muscle fibers are necessary if the hypertrophy response is to continue during longer periods of mechanical over-
load (23, 34), and the MRFs might, therefore, be in-
volved in regulating prolonged muscle growth. Several
studies have shown that muscle injury induces satel-

tite cell activation and MRF expression (20, 26, 33).

Although recent studies examining human muscle af-

erter high-force eccentric muscle contractions do not show any signs of muscle injury, even though delayed onset of muscle soreness was observed (25, 42), it cannot be ruled out that the training protocol used in the present study may have resulted in muscle injury because it contained a high number of eccentric muscle contractions (14). The possibility, therefore, exists that the changes observed in MRF mRNA in the present study may originate from activated satellite cells re-

generating damaged muscle fibers.

Several studies have shown that the MRFs are up-
regulated, not only during hypertrophy but also during atrophy (5, 36). Why are the MRFs induced during both atrophy and hypertrophy? There are two possibilities that might explain this paradox. First, if the MRFs do not regulate muscle mass, the reason for the observed upregulation during hypertrophy and atrophy could be that they regulate other processes coinciding with changes in muscle mass. One such process is fiber-type transition. MyoD and myogenin have been suggested to be implicated in regulating muscle-fiber type, as myogenin has been found to accumulate in slow-twitch fibers and MyoD in fast-twitch fibers (18, 36). More-

over, a study conducted by Modzdziai et al. (29) indi-

cates that myogenin and MyoD mRNA levels are asso-
ciated more with alterations in MHC isoform composi-
tion than changes in muscle mass. Second, the increase in MRF during unloading might be an attempt from the muscle to offset muscle wasting. This possibility is supported by the findings that aged rats have a higher rate of atrophy and a lower increase in MRF mRNA and protein levels during unloading than young rats (5). The authors of the latter study suggest that this failure to accumulate adequate amounts of MRF pro-

tein may play a role in the accelerated muscle loss seen in senescent rats during unloading.

IGF-I. The present study shows a 44% decrease in IGF-IEabc mRNA levels after resistance exercise. This is in contrast to the recent findings, with no change in IGF-IEa 2.5 h after mechanical loading (16) and a 62% increase in IGF-I 48 h after resistance exercise (7). However, it is important to note that the increase in the latter study (7) was observed much later in recov-

ery than the decline found in the present study: 1 and 6 h postexercise. Bamman et al. (7) did not separate the different IGF-I isoforms. This was completed to some extent in the present study in which the mRNA content of IGF-IEabc and IGF-IEbc was examined. Thus IGF-

IEabc includes the IGF-I isoforms, IGF-IEa (exons 1, 3, 4, and 6), IGF-IEb (exons 1, 3, 4, 5), and IGF-IEc (exons 1, 3, 4, a small part of 5 and 6), whereas IGF-IEbc includes the b and c isoforms. IGF-IEa is the most abundant isoform in both liver and muscle, whereas IGF-IEc is expressed at a much lower level (11, 16, 35, 40). Several animal studies have examined the impact of muscle overload on IGF-I isoform expression (15, 28, 40), but there has, to our knowledge, only been one human study in which this has been examined (16). That study examined the expression of the MGF (IGF-

IEc) and IGF-IEa isoforms before and 2.5 h after a single bout of high-resistance weight-lifting exercise, with MGF being upregulated by 2–865% and the IGF-

IEa isoform remaining unchanged. This is in contrast to the present findings in which a decrease in IGF-

IEabc mRNA levels and no change in IGF-IEbc levels were observed. The fact that IGF-IEbc remained un-

changed indicates that the decrease in IGF-IEabc was caused by a decrease in IGF-IEa. These different re-

sults might be due to individual responses (see para-

graph below), the use of different training protocols, and/or differences in sampling time points (2.5 h vs. 0, 1, 2, 6, 24, and 48 h). In addition, the expression pattern of the IGF-IEb isoform in human muscle is still unclear.

Because the various IGF-I isoforms originate from the same gene, the possibility exists that the ratio between the isoforms depends on how the IGF-I mRNA precursor is spliced. Certain kinds of signals might alter the splicing, reducing the production of one iso-

form while increasing the formation of another. If re-

sistance exercise induces such signals, this might, for example, lead to a decreased production of IGF-IEa and an increased production of IGF-IEc. Thus changes in the individual IGF-I mRNA isoforms may occur without changes in transcription of the IGF-I gene.

Individual response. The mRNA content measured during recovery from exercise was highly variable among subjects, with some subjects showing a marked increase and some showing no change. This might indicate individual variations in gene response to a given resistance exercise protocol, which is supported by the results obtained in the study by Hameed et al. (16). In addition, the mean increase in muscle-fiber area has been shown to differ among subjects after 12 wk of heavy-resistance training (27). The explanation for these individual responses could be individual dif-

ferences in the gene expression pattern, depending on the inherited organization of the genome, so that resis-
tance exercise induces a more marked expression of genes important for hypertrophy in some individuals than in others.

In conclusion, the present study shows that resis-
tance exercise induces a transient increase in mRNA levels of myogenin, MyoD, and MRF4 in human skel-

etal muscle. This implies that these transcription fac-

tors are regulated at the transcriptional level and that they might be involved in the regulation of fiber-type transitions and/or hypertrophy. In addition, the present data suggest that one bout of heavy-resistance
training reduces the mRNA content of the IGF-IeA isoform during the initial part of recovery.

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DISCLOSURES

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