Myosin heavy-chain mRNA expression after a single session of heavy-resistance exercise

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ABSTRACT

WILLOUGHBY, D. S., and M. J. NELSON. Myosin heavy-chain mRNA expression after a single session of heavy-resistance exercise. *Med. Sci. Sports Exerc.*, Vol. 34, No. 8, pp. 1262–1269, 2002. **Purpose:** This study determined the effects of a single session of heavy-resistance exercise on myosin heavy-chain (MHC) mRNA expression, along with the expression of the transcription factors Myo-D, myogenin, and Id-1. **Methods:** Four male subjects participated in a control group (CON) and seven in a resistance-exercise group (REX). REX performed one resistance-exercise session employing three sets of 8–10 repetitions at 75–80% one-repetition maximum on the squat, leg press, and leg extension. Vastus lateralis biopsies were obtained pre, post, and at 6 h postexercise. **Results:** In regard to CON, no significant differences were located for any criterion variable ($P > 0.05$). For REX, elevations of 38.19%, 45.61%, and 74.24% ($P < 0.05$), respectively, occurred at 6 h-post for Type I, IIa, and IIx MHC mRNA. Myo-D and myogenin mRNA were elevated 27.28% and 23.58% postexercise ($P < 0.05$), respectively, but no change was observed in Id-1 mRNA. Elevations at 6 h-post of 46.85% and 46.41% ($P < 0.05$), respectively, occurred for Myo-D and myogenin mRNA with no change in Id-1. Myo-D and myogenin protein increased 57.91% and 52.30%, respectively, postexercise and 317.56% and 254.08 at 6 h-post ($P < 0.05$), whereas no change was noted for Id-1. Myofibrillar protein was elevated 84.52% at 6 h-post ($P < 0.05$). Type I and IIa MHC mRNA at 6 h-post were correlated with myogenin mRNA and protein postexercise and 6 h-post, whereas Type IIx at 6 h-post was correlated with Myo-D mRNA and protein postexercise and 6 h-post ($P < 0.05$). **Conclusions:** These results indicate that the mRNA expression of all three MHC isoforms is up-regulated after a single session of heavy-resistance exercise and that Myo-D and myogenin seem to play a role in MHC isoform gene expression. **Key Words:** MYO-D, MYOGENIN, ID-1, TRANSCRIPTION, GENE EXPRESSION

Skeletal muscle myosin heavy-chain (MHC) isoform (Type I, IIa, and IIx) expression has been shown to be regulated at the pretranslational level by myogenic regulatory factors (MRFs) such as Myo-D, myogenin, and Id-1 (7). Myo-D and myogenin are members of a family of basic helix-loop-helix (bHLH) proteins that function as transcription factors due to their inherent properties as DNA-binding proteins and, as a result, initiate transcription by binding as either homo- or heterodimers to the regulatory region within the promoter of the respective MHC genes (4). Previous studies have suggested that Myo-D and myogenin seem to control downstream genes that are involved in establishing and maintaining mature myofiber phenotype. The Myo-D and myogenin proteins regulate both skeletal muscle specification and differentiation. A conserved amphipathic α-helix within the carboxy terminus of Myo-D and myogenin is known to have distinct functions (3). Muscle lineage is established by Myo-D, in part due to the helix of Myo-D facilitating the initiation of endogenous gene expression while the helix of myogenin seems to mediate terminal differentiation (12). The basic region of Myo-D and myogenin, which is just amino-terminal to the HLH domain, is 20–30 amino acids in length and consists of two to three clusters of basic amino acid residues (4). However, in regard to Myo-D, the basic region is not required for heterodimer formation but is required for specific DNA binding and myogenic activity *in vivo* (11). The levels of Myo-D have been shown to be higher in fast-contracting muscles and seem to be associated with the expression of the Type IIx MHC isoform, whereas myogenin levels have been shown to be higher in slower-contracting muscles and seem to be associated with the Type I and IIa MHC isoforms (17).

In regard to Id-1, it is a HLH protein but lacks the basic domain (consisting of two segments capable of forming amphipathic α-helices 12–15 residues each and connected by a nonconserved loop region of varying length) responsible for DNA binding (4). Therefore, Id-1 functions as an inhibitor of DNA binding and antagonizes the actions of Myo-D and myogenin. As a result, Id-1 has been shown to be a putative negative modulator of myogenesis (18). Although the specific role for Id-1 in relation to MHC isoform

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expression is somewhat ubiquitous, there are data that have demonstrated muscle atrophy to occur when Id-1 was overexpressed (14).

The phenotypic expression of MHC is known to be very sensitive to mechanical loading stimuli such as heavy-resistance training. Heavy-resistance training is a powerful modulator of MHC isoform expression and the pretranslational mechanisms regulating MHC mRNA expression are thought to be sensitive to small amounts of resistance training (6). Therefore, it is plausible that changes in MHC isoform mRNA expression as a result of even a single session of heavy-resistance exercise may be a result of concomitant alterations in the expression of the MRFs, Myo-D, myogenin, and Id-1.

Although specific data regarding the turnover of MHC mRNA and protein in human skeletal muscle are limited, animal studies indicate that the increases in mRNA can occur almost immediately upon the respective stimulus and has a half-life of approximately 2–3 d (21). However, the half-life of the corresponding protein products may be on the order of approximately 2–3 wk (2). Recent evidence (1) has shown a mismatch between MHC isoform mRNA and protein expression, suggesting that transition in the phenotypic expression of MHC may account for the differential timing of up- and down-regulation between MHC mRNA and protein. Therefore, due to the slow turnover of MHC protein isoforms in human muscle (1), the MHC protein isoform expression that occurs with prolonged resistance-training programs may not be representative of MHC gene expression known to occur early in a resistance-training program. As a result of this mismatch between MHC mRNA and protein expression during training, little is known about the effects of a single session of heavy-resistance exercise on the expression of MHC isoform mRNA in humans. Also, little is known about the role that Myo-D, myogenin, and Id-1 play in modulating the expression of the MHC isoform genes in response to a single session of heavy-resistance training.

Therefore, the purpose of this study was to determine the changes in mRNA expression of the MHC isoforms while also determining changes in both the mRNA and protein expression of Myo-D, myogenin, and Id-1 after a single session of heavy-resistance exercise in humans. We hypothesized that there would be an up-regulation in Type I, IIa, and IIx MHC gene expression after exercise that would be correlated to a concomitant up-regulation in the mRNA and protein expression of Myo-D and myogenin, whereas the effects on Id-1 would be negligible.

METHODS

Subjects. Eleven apparently healthy male subjects with an average (±SD) age of 23.78 (±3.32) yr, height of 175.67 (±16.01) cm, and body weight of 84.25 (±14.91) kg served as subjects for the study. Subjects were nonsmokers who had abstained from caffeine and alcohol for at least 48 h before all testing and trials, and were not using ergogenic aids (e.g., creatine, anabolic steroids, etc.) at the time of the study. Subjects were instructed to continue ingesting a normal mixed diet throughout the course of the study. Due to the nature of the heavy-resistance exercise session, it was required for all subjects to be physically active rather than sedentary. However, to attempt to ensure possible changes in MHC and MRF expression to the exercise session the subjects were not highly weight trained. As a result, all subjects had previous resistance training experience but had not engaged in a consistent (at least 2 sessions/wk) heavy-resistance–training program for 6 months before the study. Before testing, subjects filled out an informed consent form, medical health history, and physical activity questionnaire. Subjects with contraindications to exercise as outlined by the American College of Sports Medicine (ACSM) were not allowed to participate. All eligible subjects signed university-approved informed consent documents, and approval was granted by the Institutional Review Board for Human Subjects. Additionally, all experimental procedures involved in the study conformed to the ethical considerations of the Helsinki Code. The subjects were explained the purpose of the training program, the protocol to be followed, and the experimental procedures to be used. Seven subjects participated in a single session of heavy-resistance exercise (REX), whereas four subjects served as nonexercised controls (CON). The CON group was included to compare any changes in MHC and MRF expression in the REX group to those of CON. In addition, since multiple biopsies were obtained from the same location that could have possibly resulted in localized muscle damage, we used CON to assess any possible detrimental changes in MHC and MRF.

Testing protocol. Subjects in CON and REX reported to the exercise facility on the first day of testing to determine a one-repetition maximum (1-RM) on the following lifts: 1) squat, 2) leg press, and 3) leg extension. Subjects performed each of the lifts in this order; however, before testing each subject performed a 10-min warm-up, which included walking and/or jogging on a treadmill along with various stretching exercises.

During 1-RM testing, each subject rested 3 min between each successful lift before increasing the weight and repeating the attempt. Testing occurred 10–14 d before the exercise session, and subjects were instructed to refrain from any type of strenuous activity specific to the muscle groups of the lower body during this time. This period was chosen to allow sufficient time for any acute changes in MHC isoform and MRF mRNA and protein expression to return to baseline. As a result, the average number of days between 1-RM testing and the date of the exercise trial was 12.41 (±2.26). This time period should not have been great enough, however, to cause any significant strength decrements to occur (i.e., detraining) between the testing date and exercise trial date (15). Due to the possibility of fatigue as a result of excessive trials (i.e., > 5 trials) during 1-RM testing, based on our previous work, a goal of only five trials was set for the1-RM testing sessions (28,29). All subjects were able to obtain their 1-RM within five trials and the average (±SD) number of trials for all subjects during the 1-RM testing session was 3.58 (±0.64).

Treatment protocol. For both CON and REX, the time of the treatment protocol was matched to the same time of day as the 1-RM testing to eliminate potential diurnal variations (22). The treatment protocol for the REX group involved performing 3 sets of each of the 3 lifts (squat, leg press, and leg extensions) in the same order as during 1-RM testing, but at an intensity of 75–80% of each subject's 1-RM. This intensity allowed subjects to complete between 8 and 10 repetitions per set for each exercise (24). Subjects strictly adhered to a 3-min rest period between sets and between exercises. The total time of the exercise session from the warm-up period to the last repetition was approximately 45 min. The treatment protocol for the CON group involved subjects coming into the laboratory and lying in a supine position for 45 min.

Muscle biopsies. Muscle samples (approximately 75 mg) were extracted from the vastus lateralis of the right leg by using a percutaneous needle biopsy combined with suction (28,29). For all subjects in CON and REX, samples were taken midway between the patella and the greater trochanter of the femur at a depth between 1 and 2 cm. Three muscle biopsies were taken per subject and occurred pretreatment (pre), 30 min posttreatment (post), and 6 h posttreatment (6 h-post). Subjects received a local anesthetic (2% Xylocaine with epinephrine), and one incision was made from which all subsequent biopsies were taken. Care was taken to extract muscle tissue from the identical location by using the same incision and depth markings on the needle. Muscle samples were immediately frozen in liquid nitrogen and stored at −70°C for later analysis.

Total RNA isolation. Total cellular RNA was extracted from skeletal muscle biopsies with a monophasic solution of phenol and guanidine isothiocyanate (8,28,29) contained within the TRI-reagent (Sigma Chemical Co., St. Louis, MO). The RNA concentration was determined by optical density (OD) at 260 nm (by using an OD_{260} equivalent to 40 $\mu g \cdot \mu L^{-1}$) (11), and the final concentration was adjusted to 1 $\mu g \cdot \mu L^{-1}$ (28,29). Aliquots (5 μL) of total RNA were then separated with 1% agarose gel electrophoresis, ethidium bromide stained, and monitored under an ultraviolet light to verify RNA integrity and absence of RNA degradation. In line with our previous work, this procedure yielded undegraded RNA, free of DNA and proteins as indicated by prominent 28s and 18s ribosomal RNA bands, as well as an OD_{260}/OD_{280} ratio of approximately 2.0 (9,28,29). The RNA samples were stored at −70°C until later analysis.

Reverse transcription and cDNA synthesis. Two μg of total skeletal muscle RNA were reverse transcribed to synthesize cDNA (28,29). A reverse transcription (RT) reaction mixture [2 μg of cellular RNA, 10x RT buffer (20 mM Tris-HCL, pH 8.3;50 mM KCl; 2.5 mM $MgCl_2$; 100 μg of bovine serum albumin·mL), a dNTP mixture containing 0.2 mM each of dATP, dCTP, dGTP, and dTTP, 0.8 μM MgCl2, 1.0 $\mu \cdot \mu L^{-1}$ of rRNasin (ribonuclease inhibitor), 0.5 $\mu g \cdot \mu L^{-1}$ of oligo$(dT)_{15}$ primer, and 25 $\mu \cdot \mu L^{-1}$ of AMV reverse transcriptase enzyme (Promega, Madison, WI)] was incubated at 42°C for 60 min, heated to 95°C for 10 min, and then quick-chilled on ice. Starting template concentration was standardized by adjusting the RT reactions for all samples to 200 ng before PCR amplification (28,29).

Oligonucleotide primers for PCR. The following 5' sense and 3' antisense oligonucleotide primers were used to isolate the three adult MHC isoforms (Type I, IIa, and IIx) and the MRFs (Myo-D, myogenin, and Id-1): Type I MHC mRNA (5' primer: bases 776–796, 3' primer: bases 1398–1378, GenEMBL AC X06976), Type IIa MHC mRNA (5' primer: bases 1785–1805, 3' primer: bases 2440–2420, GenEMBL AC AF111784), Type IIx MHC mRNA (5' primer: bases 1138–1158, 3' primer: bases 1746–1726, GenEMBL AC AF111785), Myo-D (5' primer: bases 1239–1259, 3' primer: bases 1431–1411, GenEMBL AC X56677), myogenin mRNA (5' primer: bases 1986–2006, 3' primer: bases 2145–2125, GenEMBL AC X62155), and Id-1 mRNA (5' primer: bases 296–316, 3' primer: bases 800–780, GenEMBL AC X77956). We have previously shown these primers to amplify respective PCR fragments of 623, 655, and 609 base pairs (bp) for MHC Type I, IIa, and IIx (28,29), as well as 504, 495, and 486 bp for Myo-D, myogenin, and Id-1, respectively (unpublished observations). In addition, we have tested the specificity of the primers with a diagnostic restriction digest of the PCR products using the EcoR-V restriction endonuclease (28).

The constitutively expressed housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an external reference standard for detecting the relative change in the quantity of mRNA for Type I, IIa, and IIx MHC and Myo-D, myogenin, and Id-1 using PCR (29). For GAPDH mRNA (5' primer: bases 616–636, 3' primer: bases 1189–1169, GenEMBL AC NM 002046), we have previously shown these primers to amplify a PCR fragment of 574 bp (29).

PCR amplification. Two hundred ng of cDNA were added to each of the eight PCR reactions for GAPDH, MHC Type I, IIa, and IIx, Myo-D, myogenin, and Id-1, and an absolute negative control. Specifically, each PCR reaction contained the following mixtures: [10x PCR buffer, 0.2 μM dNTP mixture, 1.0 μM of a cocktail containing both the sense and antisense RNA oligonucleotide primers for GAPDH and IL-6 (Ransom Hill Biosciences, Ramona, CA), 2 mM $MgCL_2$, 1.0 $\mu \cdot \mu L^{-1}$ of Taq DNA polymerase (Sigma), and nuclease-free dH_2O]. Each PCR reaction was amplified with a thermal cycler (Bio Rad, Hercules, CA).

The amplification profile involved a denaturation step at 95°C for 30 s, primer annealing at 55°C for 30 s, and extension at 72°C for 60 s (28,29). To help control for differences in amplification efficiency during thermocycling, all PCR reactions were prepared from the same stock solution. Also, the number of cycles was optimized at 30 so that the amplified signal was still on the linear portion of a plot with the yield expressed as a function of the absorbance at OD_{260} and the number of cycles for the GAPDH, MHC isoform, Myo-D, and myogenin amplifications (Fig. 1). The specificity of the PCR was demonstrated with an absolute negative control using a separate PCR reaction containing no cDNA.

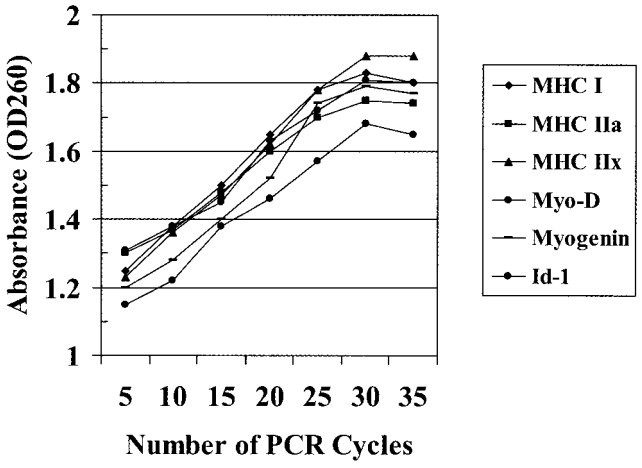

FIGURE 1—A PCR amplification efficiency plot with the yield expressed as a function of the absorbance at OD_{260} and the number of cycles for GAPDH, MHC Type I, IIa, and IIx, Myo-D, and myogenin.

To assess reliability between amplifications, two separate PCR amplifications were performed for each sample to control for systemic differences between samples that could affect amplification efficiencies. Intra-assay coefficients of variation for the two PCR runs for all subjects were performed for all seven PCR reactions and resulted in nonsignificant ($P > 0.05$) coefficients of variation of 2.45%, 2.06%, 3.18%, 2.73%, 1.96%, 2.15%, and 3.28%, respectively, for GAPDH, MHC Type I, IIa, and IIx, and Myo-D, myogenin, and Id-1 mRNA.

Gel electrophoresis and mRNA quantitation. The DNA within each amplified PCR reaction was purified of contaminants such as primer dimers and amplification primers using the Wizard PCR Preps DNA Purification System (Promega, Madison, WI). Aliquots (20 μL) of the purified PCR reaction mixtures were electrophoresed in 1.5% agarose gels in 1X Tris-Acetate-EDTA (TAE) buffer (28,29) to verify positive amplification of target mRNA. The oligonucleotides within the gels were stained with ethidium bromide (present in the TAE buffer at 1 $\mu g \cdot mL^{-1}$) and illuminated with a UV transilluminator. Aliquots of each remaining purified PCR reaction were used to quantify mRNA spectrophotometrically at a wavelength of OD_{260} (9,28,29). The mRNA concentration of Type I, IIa, and IIx MHC, and Myo-D, myogenin, and Id-1 was calculated and normalized relative to GAPDH (ng of target mRNA/ng of GAPDH mRNA) based on a statistical method previously described (23,28,29). It should be noted, however, that this method of PCR quantitation determines relative mRNA concentration only and should not be interpreted as absolute concentration values.

Myo-D, myogenin, and Id-1 quantification. Total muscle protein was isolated from the organic phase of the total RNA isolation using ethanol, isopropanol, and a 95% ethanol solution containing 0.3 M guanidine hydrochloride (28,29). The protein concentrations ($ng \cdot mL^{-1}$) of Myo-D, myogenin, and Id-1 were determined in triplicate and the average concentration reported using a competitive enzyme-linked immunoabsorbent assay (ELISA) using specific rabbit IgG polyclonal antibodies (Santa Cruz Biotech, Santa Cruz, CA). The anti-Myo-D antibody is raised against a peptide with its epitope corresponding to amino acids 1–318, representing full-length Myo-D protein of mouse origin, and does not cross react with myogenin or Id-1. The myogenin antibody is raised against a peptide with its epitope corresponding to amino acids 1–255, representing full-length myogenin protein of rat origin, and does not cross react with Myo-D or Id-1. The anti-Id-1 antibody is raised against a peptide with its epitope, corresponding to amino acids 1–154 representing full-length Id-1 protein of human origin. The secondary antibody immunoglobulin G (IgG) was conjugated to the enzyme horseradish peroxidase (HRP) (ICN Biomedical, Aurora, OH). The control standard was heat shocked human cervical adrenocarcinoma (Hela) cells (StressGen Biotechnology, Vancouver BC, Canada). Protein concentrations were determined at an optical density of 450 nm with a microplate reader (Bio Rad). Intra-assay coefficients of variation were determined for each triplicate for all subjects and revealed nonsignificant coefficients of 2.87%, 3.13%, and 3.62%, respectively, for Myo-D, myogenin, and Id-1.

Myofibrillar protein quantitation. Myofibrillar protein was further isolated from aliquots of the total muscle protein solution by repeated incubations at 50°C in a 1% sodium dodecyl sulfate (SDS) solution (29). Based on our previous work (29), myofibrillar protein content ($mg \cdot mL^{-1}$) was then determined spectrophotometrically based on the Bradford method (5) at a wavelength of 595 nm and using bovine serum albumin as the standard. All assays were performed in triplicate and the average concentrations reported. Also, intra-assay coefficients of variations were determined for each triplicate for all subjects and revealed a nonsignificant ($P > 0.05$) coefficient of variation of 2.83%.

Statistical analysis. Statistical analyses were performed using the SPSS 10.0 software package. Separate 2 × 3 (group × test) factorial analysis of variance (ANOVA) procedures were performed for the three testing conditions (pre, post, and 6-h post) including all criterion variables (Type I, IIa, and IIx MHC mRNA, Myo-D, myogenin, and Id-1 mRNA and protein, and myofibrillar protein). Significant main effects were analyzed with separate one-way analysis of variances (ANOVA). Significant differences between testing conditions were determined with a Student Neuman-Keuls *post hoc* procedure. In addition, to protect against Type I error, the observed within-group *F*-ratios were adjusted using the Bonferroni correction factor to account for any inflation in the alpha level. Due to the small number of subjects, the effects of sample size were determined using the partial Eta^2 (H^2) statistic and statistical power was determined. Pair-wise correlations using the Pearson product moment correlation procedure were run on selected criterion variables to determine correlations between variables. A probability level of ≤ 0.05 was adopted throughout.

RESULTS

Weight-training program. As a result of the physical exertion required during the single session of heavy-resistance–training exercise, fatigue precluded some REX subjects from being able to utilize the same training load for all three sets for one or more exercises. As a result, decreases in training load were allowed as long as the average relative intensity was within the range of 75–80% of the subject's 1-RM. Therefore, the average (±SD) relative intensities for the three repetitions for each exercise were 77.39% (±1.77), 75.59% (±4.89), and 76.46% (±3.61) for the squat, leg press, and leg extension exercises, respectively. The average number of repetitions that corresponded to the relative intensities was 9.62 (±1.02), 9.27 (±1.05), and 9.19 (± 0.98), respectively, for the squat, leg press, and leg extension exercises.

MHC isoform mRNA expression. For MHC isoform mRNA expression, results showed significant group × test interactions for MHC Type I ($F(2,24) = 7.27$, $P = 0.003$, power = 0.903, $H^2 = 0.477$), MHC Type IIa ($F(2,24) = 4.46$, $P = 0.023$, power = 0.709, $H^2 = 0.401$), and MHC Type IIx ($F(2,24) = 5.18$, $P = 0.013$, power = 0.776, $H^2 = 0.471$). Significant group effects were noted for Type I ($F(2,24) = 13.19$, $P = 0.001$, power = 0.936, $H^2 = 0.355$), Type IIa ($F(2,24) = 9.08$, $P = 0.006$, power = 0.824, $H^2 = 0.495$), and Type IIx ($F(2,24) = 6.81$, $P = 0.015$, power = 0.707, $H^2 = 0.221$) MHC isoforms. Additionally, significant test effects were also observed for Type I ($F(2,24) = 6.98$, $P = 0.004$, power = 0.891, $H^2 = 0.368$), Type IIa ($F(2,24) = 5.01$, $P = 0.015$, power = 0.762, $H^2 = 0.295$), and Type IIx ($F(2,24) = 6.54$, $P = 0.005$, power = 0.869, $H^2 = 0.353$) MHC isoforms. *Post hoc* results revealed that CON and REX were not significantly different at pre; however, REX was significantly different from CON for all three MHC isoforms at post and 6 h-post. For REX specifically, significant elevations were observed for Type IIx MHC mRNA but not for Type I and IIa at post when compared with pre values. However, at 6 h-post all three isoforms were significantly elevated above both their pre and posttreatment levels (Fig. 2).

Myo-D, myogenin, and Id-1 mRNA expression. For MRF mRNA expression, results showed significant group × test interactions for Myo-D ($F(2,24) = 7.89$, $P = 0.002$, power = 0.926, $H^2 = 0.397$) and myogenin ($F(2,24) = 7.08$, $P = 0.004$, power = 0.895, $H^2 = 0.371$). Significant group effects were noted for Myo-D ($F(2,24) = 22.03$, $P = 0.000$, power = 0.994, $H^2 = 0.479$) and myogenin ($F(2,24) = 21.13$, $P = 0.000$, power = 0.993, $H^2 = 0.468$) mRNA expression but not for Id-1 ($P > 0.05$). Also, significant test effects were observed for Myo-D ($F(2,24) = 9.65$, $P = 0.001$, power = 0.966, $H^2 = 0.446$) and myogenin ($F(2,24) = 14.81$, $P = 0.000$, power = 0.997, $H^2 = 0.552$). *Post hoc* results revealed that CON and REX were not significantly different at pre; however, REX was significantly different from CON for Myo-D and myogenin at post and 6 h-post. For REX specifically, significant elevations were observed that at post both Myo-D and myogenin

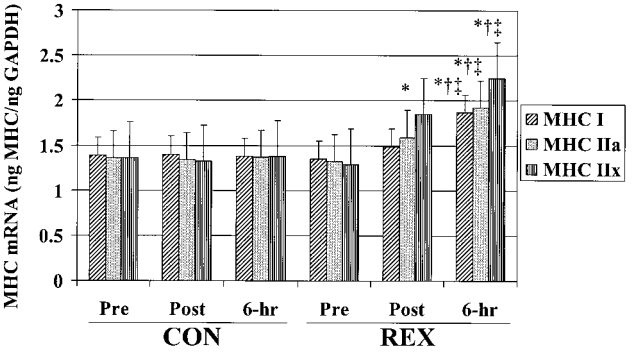

FIGURE 2—The relative quantity of MHC mRNA normalized to the quantity of GAPDH mRNA for CON and REX at the treatments, pre, post, and 6 h-post. * Indicates a significant difference from the corresponding CON value; † indicates a significant difference from pre, and ‡ indicates a significant difference from post ($P < 0.05$).

mRNA were significantly elevated beyond the pre values. In addition, at 6 h-post Myo-D and myogenin mRNA were significantly elevated beyond their corresponding pre and posttreatment values (Fig. 3).

Myo-D, myogenin, and Id-1 protein expression. For MRF protein expression, results did not show any significant group × test interactions for Myo-D, myogenin, or Id-1 ($P > 0.05$). Significant group effects were observed for Myo-D ($F(2,24) = 8.33$, $P = 0.008$, power = 0.794, $H^2 = 0.236$) and myogenin ($F(2,24) = 12.76$, $P = 0.001$, power = 0.931, $H^2 = 0.321$). In addition, significant test effects were noted for Myo-D ($F(2,24) = 3.56$, $P = 0.042$, power = 0.611, $H^2 = 0.209$) and myogenin ($F(2,24) = 3.45$, $P = 0.045$, power = 0.602, $H^2 = 0.205$). *Post hoc* tests revealed no significant differences between CON and REX at pre; however, REX was significantly different from CON at post and 6 h-post for Myo-D and myogenin. For REX, at 6 h-post both Myo-D and myogenin were significantly elevated beyond their corresponding pre and posttreatment values (Fig. 4).

Myofibrillar protein content. For myofibrillar protein content, results did not show a significant group × test interaction ($P > 0.05$). However, significant group ($F(2,24) = 4.58$, $P = 0.019$, power = 0.729, $H^2 = 0.254$) and test

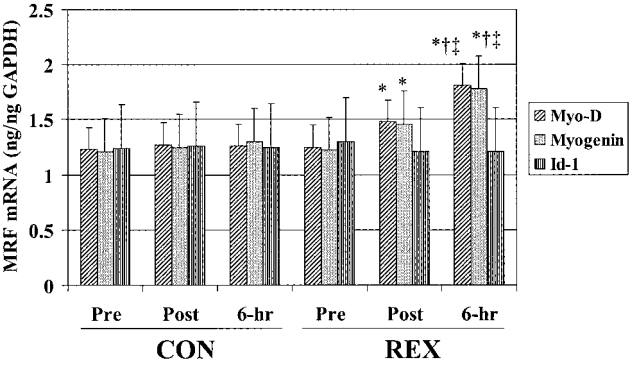

FIGURE 3—The relative quantity of Myo-D, myogenin, and Id-1 mRNA normalized to the quantity of GAPDH mRNA for CON and REX at the treatments, pre, post, and 6 h-post. * Indicates a significant difference from the corresponding CON value; † indicates a significant difference from pre, and ‡ indicates a significant difference from post ($P < 0.05$).

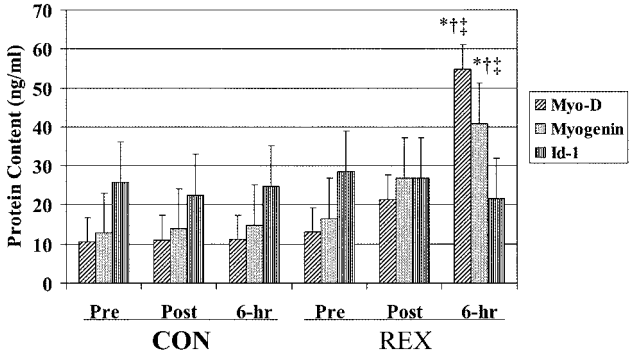

FIGURE 4—The quantity of Myo-D, myogenin, and Id-1 protein (ng·mL^{-1}) for CON and REX at the treatments, pre, post, and 6 h-post. * Indicates a significant difference from the corresponding CON value; † indicates a significant difference from pre, ‡ indicates a significant difference from post ($P < 0.05$).

($F(2,24) = 5.28$, $P = 0.012$, power = 0.791, $H^2 = 0.281$) effects were detected. A *post hoc* test revealed that CON and REX were not significant different for myofibrillar protein content at pre. However, for REX there was a significant elevation at 6 h-post but not at post when compared with pretreatment values.

Correlations between Myo-D, myogenin, and Id-1 mRNA and MHC isoform mRNA. No significant correlations were observed for CON ($P > 0.05$). For REX, correlations between Myo-D mRNA expression and the expression of the three MHC isoforms at either the 30 min-post or 6 h-post time points, Myo-D mRNA expression at 30 min-post was found to be significantly correlated with MHC IIx mRNA at 30 min-post (r = 0.859, $P = 0.028$). Additionally, Myo-D mRNA expression at 6 h-post was significantly correlated with MHC IIx mRNA at 6 h-post (r = 0.867, $P = 0.025$). A significant correlation was located between the myogenin mRNA expression occurring at 30 min-post and Type I (r = 0.967, $P = 0.002$) and IIa (r = 0.934, $P = 0.006$) MHC mRNA expression occurring at 6 h-post. Significant correlations were also located between Id-1 mRNA expression occurring at 30 min-post and Type I (r = −0.875, $P = 0.022$) and IIa (r = −0.858, $P = 0.029$) MHC mRNA at 6 h-post.

Correlations between Myo-D, myogenin, and Id-1 protein and MHC isoform mRNA. No significant correlations were detected for CON ($P > 0.05$). However, a significant correlation was located between the expression of Myo-D at 6 h-post and Type IIx MHC mRNA at 6 h-post (r = 0.893, $P = 0.017$). In relation to myogenin expression occurring at 6 h-post, significant correlations were detected between Type I (r = 0.954, $P = 0.003$) and IIa (r = 0.919, $P = 0.010$) MHC mRNA expression at 6 h-post. No significant correlations were located between Id-1 protein expression and any of the MHC mRNA isoforms ($P > 0.05$).

Correlations between myofibrillar protein. No significant correlations were revealed for CON ($P > 0.05$). However, significant correlations were located between myofibrillar protein content at 6 h-post and the 6 h-post values for Type I (r = 0.821, $P = 0.021$), Type IIa (r = 0.875, $P = 0.022$), and Type IIx (r = 0.917, $P = 0.10$) MHC mRNA, Myo-D mRNA (r = 0.839, $P = 0.037$) and myogenin mRNA (r = 0.807, $P = 0.048$).

DISCUSSION

The results of this study indicate that a single session of heavy-resistance exercise is effective in up-regulating the mRNA expression of all three MHC isoforms by 6 h postexercise, and that the increased Type I and IIa MHC expression is correlated to the up-regulation in the mRNA and protein expression of myogenin, whereas Type IIx MHC mRNA is correlated with the mRNA and protein expression of Myo-D. In regard any concern that multiple biopsies from the same location may have produced localized muscle damage, thereby negatively affecting the MHC and MRF expression profiles, results from CON were all statistically unchanged throughout the three sampling points. Therefore, our multiple biopsy procedure did not appear to negatively affect our results.

The expression of MHC mRNA expression in humans (28,29) and rodents (6) in response to prolonged whole-body exercise training has been shown to occur in an intensity-dependent manner. Although specific data regarding the turnover of MHC mRNA and protein in human skeletal muscle are limited, animal studies indicate that the increases in mRNA can occur almost immediately upon the respective stimulus and has a half-life of approximately 2–3 d (21). However, little is known about the MHC isoform mRNA expression in humans in response to a single session of heavy-resistance exercise. There are data from rodents, however, which have demonstrated that a heavy-resistance exercise protocol produced a rapid elevation in the Type IIx MHC mRNA isoform after only two training sessions and that elevations occurred in a dose-responsive manner with as few as 10 contractions per exercise session (6). It should be noted, however, that increases in the mRNA template available for translation and protein synthesis in muscle as a result of heavy-resistance training can be a reflection of alterations in transcriptional efficiency, transcriptional capacity, and/or mRNA stability (26).

Our results indicate the Type IIx mRNA isoform to be the most responsive to the heavy-resistance exercise session, when compared with the Type I and IIa isoforms, by undergoing significant elevations of ~27% by 30 min postexercise and ~75% at 6 h postexercise. The ~27% increase in the mRNA expression of Myo-D at 30 min and ~47% increases at 6 h postexercise was correlated with the mRNA expression of Type IIx MHC at 30 min and 6 h-post. Also, the ~318% increase in protein expression of Myo-D at 6 h postexercise was correlated to the mRNA expression of MHC IIx at 6 h postexercise. Our results also indicate, however, that the mRNA expression of the Type I and IIa MHC isoforms were not significantly elevated until 6 h-post exercise, at which point the increases of ~38% and ~46%, respectively, for the Type I and IIa MHC isoforms were correlated to the ~24% increase in the mRNA expression of myogenin occurring at 30 min postexercise and the ~254%

increase in myogenin protein expression occurring at 6 h postexercise.

It appears that the expression of the Type IIx MHC gene may be governed by a pretranslational mechanism relative to Myo-D expression and may also be more responsive to a single session of heavy-resistance exercise than the Type I and IIa MHC genes. The more rapid response in Type IIx MHC mRNA expression observed in this study may be explained by the fact that domains have been identified on the Myo-D protein that are necessary to initiate transcription from endogenous skeletal muscle genes (10). These domains (one is rich in cysteine and histidine residues and resides between the acidic activation domain and the bHLH domain and the other resides in the carboxy terminus of the protein) appear to mediate chromatin remodeling at the target locus, resulting in greater accessibility of other transcription factors to the locus (12). Myogenin lacks these chromatin-remodeling domains and has been shown to be 10-fold less efficient than Myo-D at activating transcription from an endogenous gene (12). Therefore, the possible increased transcription efficiency of Myo-D seems to suggest that the activity of the Type IIx gene might operate as a default gene (13) in response to a single session of heavy-resistance exercise. The MHC IIx gene is very sensitive to heavy-resistance exercise involving differing muscle contractions; therefore, the MHC IIx gene may have the ability to either preferentially increase or decrease its expression as a result of a single session of heavy-resistance exercise based on the expression of Myo-D.

We have recently shown 12 wk of heavy-resistance training (85–90% 1-RM) to have no change on Type IIx MHC mRNA expression while the level of Type IIx MHC protein was decreased (29). Most skeletal muscle fibers contain either a single mRNA and isoprotein or a mixed Type I/IIa or IIa/IIx MHC phenotype with coexistence of mRNAs and isoproteins (1). Heavy-resistance training (12 wk) has been shown to decrease the Type IIa/IIx MHC phenotype, resulting in a decrease in the Type IIx MHC isoform while concomitantly increasing the Type IIa MHC isoform (27). The results from the present study demonstrate an increase in Type IIx MHC mRNA expression at 6 h-post that may conceivably be explained on the premise that the Type IIx MHC gene may be up-regulated in response to a single bout of heavy-resistance exercise; however, with continued resistance training, Type IIx MHC gene expression is down-regulated as muscle fibers attempt to undergo alterations toward the Type IIa/IIx and/or Type IIa MHC phenotype.

In regard to the expression of Type I MHC mRNA and its correlation with myogenin, the Type I MHC gene is regulated, at least in part, by a muscle-specific enhancer located within the *cis*-acting regulatory sequence that is known to interact with muscle-specific nuclear proteins. However, the Type I MHC gene has been shown to be unresponsive to Myo-D, and a Myo-D binding site in the gene's promoter is not required for transcriptional activity. As a result, Myo-D and Myo-D related processes do not seem to be required for Type I MHC gene expression (25). Our results seem to indicate this lack of association between the Type I MHC gene and Myo-D because we did not observe a significant correlation between Type I MHC mRNA and Myo-D protein expression.

Because the Id-1 protein lacks the basic domain responsible for DNA binding, Id-1 heterodimers have a low affinity for DNA binding and will inhibit HLH-dependent transcription (7). Therefore, high levels of Id-1 seem to inhibit transcription (7) of the MHC isoforms and may be involved in the mechanisms that induce muscle atrophy (14). Our results demonstrate that the ~11% decrease in Id-1 mRNA expression occurring at 30 min postexercise was correlated to the increase in Type I and IIa MHC mRNA expression occurring at 6 h postexercise. Based on the premise that we observed no increase in either Id-1 mRNA or protein and no correlation with Type IIx MHC mRNA, it appears that the expression of Id-1 in response to a single session of heavy-resistance exercise is regulated at the pretranslational level and may be attenuated by increases in only Type I and IIa MHC mRNA expression. As such, in agreement with previous results (18) from hypothyroidism and low-frequency stimulation, our present results indicate that a single session of heavy-resistance exercise has no marked effects on either the mRNA or protein expression of Id-1.

It has been established that short-term (i.e., < 2 wk) programs of heavy-resistance training increases myofibrillar protein synthesis. There are recent data from humans demonstrating an 88% and 121% increase in the rate of MHC and mixed muscle protein synthesis, respectively, after only two weeks of heavy-resistance training (16). Also, 2 wk of heavy-resistance training have also been shown to increase the rate of mixed muscle protein synthesis by ~150% (30). Even though we did not determine the rate of synthesis in response to a heavy-resistance–training program, our results do demonstrate an ~85% increase in the content of myofibrillar protein at 6 h-post that was correlated to the increases in mRNA expression for all three MHC isoforms occurring at 6 h-post. As a result, this increase in myofibrillar protein is likely to be contingent upon the increased expression of all three MHC mRNA isoforms occurring up to 6 h-post after a single session of heavy-resistance exercise.

At this time, it is unknown whether the increased expression of Myo-D and myogenin occurring at 6 h-post will have an effect on muscle mass or functional capacity (e.g., strength and endurance). There are data from rodents demonstrating increases in muscle regulatory factor 4 (MRF4) and decreases in myogenin after 2 d of treatment that altered muscle mass by increasing the load with a passive stretch model (19). However, 4 wk of functional overload was shown to have no effect on Myo-D and myogenin expression even though muscle mass was significantly increased (20). As a result, previous research has concluded that Myo-D and myogenin mRNA levels are likely to be more associated with alterations in MHC isoform composition rather than alteration in muscle mass (20). Even though our exercise protocol did not allow us to determine effects on muscle mass, the results of the present study demonstrate a correlation between the increase in myofibrillar protein content at 6 h-post and the increased mRNA expression of

Myo-D and myogenin at 6 h-post. Therefore, based on the present results it is conceivable to assume that our observed increase in myofibrillar protein in response to a single session of heavy-resistance exercise is likely due to increases in the expression of the Type I, IIa, and IIx MHC genes, predicated on the related increases in the mRNA and protein expression of Myo-D and myogenin.

In summary, our results indicate that a single session of heavy-resistance exercise is effective in up-regulating the Type I, IIa, and IIx MHC genes and that the increased expression of the MHC mRNA isoforms is correlated to increases in the mRNA and protein expression of Myo-D and myogenin. However, it should be pointed out that there may not be a simple relationship between the expression of the MHC mRNA isoforms and that of Myo-D and myogenin in response to a single session of heavy-resistance exercise, but a more complex network that involves the integration and interaction of such factors as the ubiquitously expressed HLH factors known as E-proteins or signal transduction proteins such as MEF2, NFAT, and calcineurin.

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