Effects of Different Intensities of Resistance Exercise on Regulators of Myogenesis

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Abstract

Wilborn, CD, Taylor, LW, Greenwood, M, Kreider, RB, and Willoughby, DS. Effects of different intensities of resistance exercise on regulators of myogenesis. *J Strength Cond Res* 23(8): 2179–2187, 2009—A single bout of high-intensity resistance exercise is capable of activating the expression of various genes in skeletal muscle involved in hypertrophy such as myosin heavy chain (MHC) isoforms, myogenic regulatory factors (MRFs), and growth factors. However, the specific role exercise intensity plays on the expression of these genes is not well defined. The purpose of this study was to investigate the effects of exercise intensity on MHC (type I, IIA, IIX), MRF (Myo-D, myogenin, MRF-4, myf5), and growth factor (insulin-like growth factor [IGF]-1, IGF-1 receptor [IGF-R1], mechano-growth factor [MGF]) mRNA expression. Thirteen male participants (21.5 ± 2.9 years, 86.1 ± 19.5 kg, 69.7 ± 2.7 in.) completed bouts of resistance exercise involving 4 sets of 18–20 repetitions with 60–65% 1 repetition maximum (1RM) and 4 sets of 8–10 repetitions with 80–85% 1RM. Vastus lateralis biopsies were obtained immediately before exercise, and at 30 minutes, 2 hours, and 6 hours after each bout. The levels of mRNA expression were determined using real-time polymerase chain reaction. Data were analyzed using 2×4 multivariate analysis of variance ($p \leq 0.05$). For both intensities, MHC type IIX, IGF-1, IGF-R1, MGF, Myo-D, myogenin, MRF-4, and myf5 mRNA were all significantly increased in response to resistance exercise by 2 hours after exercise, whereas myostatin and the cyclin-dependent kinase inhibitor p27[kip] were decreased at 2 hours after exercise ($p < 0.05$). Resistance exercise between 60–85% 1RM upregulates the mRNA expression of MHC and factors involved in myogenic

activation of satellite cells while concomitantly decreasing expression of myogenic inhibitors.

Introduction

In response to resistance training, skeletal muscle hypertrophy occurs through satellite-cell-mediated mechanisms, whereby protein synthesis is increased and new nuclei are added to maintain the myonuclear domain (15). The increases in protein synthesis and/or activation of muscle stem cells, termed satellite cells (16), that accompany resistance exercise can be stimulated by various signals, including hormones and myogenic regulatory factors (MRFs). Satellite cell activity, which is regulated by various endocrine and autocrine/paracrine mechanisms, is also upregulated in response to resistance exercise (18). Mechano-growth factor (MGF) and insulin-like growth factor I (IGF-I) have been shown to play a role in myogenesis; IGF-1 stimulates satellite cell proliferation and differentiation (11) and, in response to mechanical overload, MGF (a splice variant of IGF-1) stimulates satellite cell activation (12).

Hypertrophic adaptations to resistance training occurring at the cellular level are most likely the result of cumulative effects of transient changes in gene expression after each acute bout of exercise. Resistance exercise has been shown to upregulate the expression of various genes in skeletal muscle, such as myosin heavy chain (MHC) isoforms and MRFs (36), which can invariably induce muscle protein synthesis and result in hypertrophy.

The MRFs (Myo-D, myogenin, MRF-4, myf5) are a family of muscle-specific transcription factors that play a role in muscle hypertrophy by binding to E-boxes in the promoter region of various sarcomeric genes such as myosin heavy chain, myosin light chain, tropomyosin, and troponin-C (6) resulting in transactivation of transcription. Furthermore, the MRFs appear to play a role in myogenic activation by inducing myoblast differentiation, while others factors such as myostatin and the cyclin-dependent kinase inhibitor p27[kip]

appear to negatively regulate hypertrophy by inhibiting satellite cell activation and myoblast proliferation (25).

Myo-D and myogenin have been shown to be significantly elevated 6 hours after a high-intensity resistance exercise bout (36). In addition, Myo-D, myogenin, and MRF-4 mRNA have been shown to be significantly elevated 24 hours following a high-intensity resistance exercise bout, whereas increases in Myo-D, myogenin, and MRF-4 occurred at 8, 8–12, and 2–4 hours after exercise, respectively (40), suggesting that MRF genes are responsive to single bouts of high-intensity resistance exercise and may be involved in regulating hypertrophy and/or myogenesis. However, a single bout of high-intensity resistance exercise has shown no effect on MRF-4 (19) and myf5 (40) mRNA expression 24 hours after exercise. High-intensity resistance exercise has also shown IGF-1 mRNA to be decreased 2.5 hours (14) and 6 hours (28) after exercise, while the IGF receptor 1 (IGF-R1) showed no effect in expression 24 hours after exercise (19). However, MGF has been shown to be significantly elevated 2.5 hours after exercise (14). Additionally, MGF mRNA was shown to be increased 24 hours after exercise, whereas myostatin and p27^{kip} were decreased (18), suggesting resistance exercise to effect the expression of genes related to cell cycle progression.

Relative to the increases in protein synthesis associated with hypertrophy, skeletal muscle is largely attributed to the synthesis of the contractile protein myosin (24), with the MHC isoforms comprising approximately 25% of the total muscle protein, thereby making MHC a significant indicator of functional diversity of the muscle fiber. In rodents, high-intensity resistance exercise with as little as 10 contractions elevated the expression of the IIX MHC mRNA by approximately 250% (9). In humans, significant increases in the mRNA expression of the type I, IIA, and IIX MHC isoforms 6 hours after a single bout of high-intensity resistance exercise has been demonstrated (36). Therefore, it is apparent that high-intensity resistance exercise is an effective modulator of MHC mRNA expression and that pretranslational mechanisms are very sensitive to even small amounts of this type of exercise (9).

While the molecular and myogenic responses to single bouts of resistance exercise involving intensities of 80–85% 1 repetition maximum (1RM) have been fairly well established (14,18,28,36,40), the role that varying intensities of resistance exercise play in regulating the expression of genes involved in hypertrophy has not been well delineated. Higher intensities of resistance training with lower volumes are normally considered to be more effective at increasing muscle strength and hypertrophy than lower intensities with higher volume (10). Nevertheless, having a better understanding of the role that differing intensities of resistance exercise have in regulating genes involved in muscle hypertrophy is worthy of consideration. Specifically, it was our intent to determine the effects of different intensities of resistance exercise on markers of myogenesis and the expression of various genes involved in skeletal muscle hypertrophy. Therefore, the purpose of this study was five-fold and was to determine the effects of various intensities of resistance exercise on markers of hypertrophy and myogenesis by investigating the (a) mRNA expression of the MHC isoforms (type I, IIA, IIX); (b) mRNA expression of the MRFs (myo-D, myogenin, MRF-4, myf5); (c) mRNA expression of IGF-1, IGF-R1, and MGF; (d) mRNA expression of myostation and p27kip; and (e) total RNA and DNA content. As such, we hypothesized that either intensity of resistance exercise would have any differential effect on any of the variables assessed.

METHODS

Experimental Approach to the Problem

The specific aim of this study was to investigate the role, if any, that differing intensities of resistance exercise intensity has on markers of myogenesis and the expression of various genes involved in muscle hypertrophy. It is commonly believed that the hypertrophic process is best stimulated with resistance exercise employing higher intensities. Few studies have used lower intensities of resistance exercise to investigate myogenesis. Previous research has shown that the myogenic response may occur quickly after the exercise bout is complete. To investigate this, all participants acted as their own controls in a crossover design. Participants were randomly assigned to participate in bouts of lower-body resistance exercise, separated by 2 weeks, involving 4 sets of 18–20 repetitions with 60–65% 1RM and 4 sets of 8–10 repetitions with 80–85% 1RM. Muscle biopsies were taken at immediately before exercise (PRE), and at 30 minutes (30MPST), 2 hours (2HRPST), and 6 hours (6HRPST) after each resistance exercise session to assess changes in gene expression and myogenic activity.

Subjects

Thirteen recreationally active but nonresistance-trained men (21.5 ± 2.9 years, 86.1 ± 19.5 kg, 177.04 ± 6.85 cm) participated in the study. Only participants considered as either low or moderate risk for cardiovascular disease and with no contraindications to exercise as outlined by the American College of Sports Medicine and/or who had not consumed any nutritional supplements (excluding multivitamins) 1 month prior to the study were allowed to participate. All eligible participants were asked to provide oral and informed written consent based on university-approved 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.

Procedures

Baseline Strength Testing Sessions. Five days prior to each resistance bout, the 1RM of the leg to be used in the upcoming exercise bout was determined using the same isotonic hip/leg sled (Nebula Fitness, Inc., Versailles, OH, USA) and knee

extension (Body Masters, Inc., Rayne, LA, USA) machines used for the resistance exercise bouts.

Resistance Exercise Protocol. Participants performed two separate bouts of resistance exercise separated by 2 weeks during the course of the study. Each participant completed 65% and 85% bout of single-leg resistance exercise. The exercise intensity and leg utilized for the first exercise bout was randomly assigned. On the day of each resistance exercise bout, participants were allowed a light breakfast at 7:00 AM (in which they recorded) and then fasted until they reported to the laboratory for their resistance exercise session at 11 AM.

The lower intensity bout also involved the single-leg isotonic hip/leg sled (Nebula Fitness) and single-leg isotonic leg extension (Body Masters) exercises, but consisted of 4 sets of 18–20 repetitions with 60–65% 1RM. Each set was performed over the course of 25–30 seconds and followed by 120 seconds of rest, while 150 seconds of rest (1:5, work:rest ratio) was also allowed between the two exercises.

The higher intensity bout involved the single-leg isotonic hip/leg sled (Nebula Fitness) and single-leg isotonic leg extension (Body Masters) exercises, both consisting of 4 sets of 8–10 repetitions with 80–85% 1RM. Each set was performed over the course of 15–20 seconds and followed by 150 seconds of rest, while 150 seconds of rest was also allowed between the two exercises, as this represents the appropriate work: rest ratio (1:5) for this exercise intensity. Total volume (weight × sets × reps) was calculated for each resistance exercise bout to assess the differences between intensities.

Muscle Biopsies. Percutaneous muscle biopsies (approximately 50–70 mg) were obtained from the middle portion of the vastus lateralis midway point between the patella and the greater trochanter of the femur. For each biopsy, muscle tissue obtained from the same location by using the previous incision and depth markings on the needle for each respective leg. Following the initial biopsy, for each subsequent biopsy the needle was rotated in the clockwise direction from the previous biopsy so the sample was not taken from within the same area of muscle. However, previous research has shown that the multiple biopsy technique does not alter exercise-induced gene expression and provides support for the present biopsy technique (21,36). Muscle samples were immediately frozen in liquid nitrogen and stored at –80°C for future analyses. There were four biopsies taken from each leg at each resistance exercise session occurring immediately before exercise (PRE), and at 30 minutes (30MPST), 2 hours (2HRPST), and 6 hours (6HRPST) following each resistance exercise session.

Dietary Records. The participants' diets were not standardized and participants were asked not to change their dietary habits during the course of the study. However, participants were required to keep dietary records for 24 hours prior to the resistance exercise session. The 24-hour dietary recalls were evaluated with the Food Processor dietary assessment software program (ESHA Research, Salem, OR, USA) to determine the average daily macronutrient consumption of fat, carbohydrate, and protein in the diet prior to exercise.

Skeletal Muscle Total RNA Isolation and Quantitation. Total cellular RNA was extracted from biopsy samples with a monophasic solution of phenol and guanidine isothiocyanate contained within the TRI-reagent (Sigma Chemical Co., St. Louis, MO, USA). The total RNA concentration was determined spectrophotometrically (Helio γ, Thermo Electron, Milford, MA, USA) by optical density (OD) at 260 nm using an OD_{260} equivalent to 40 μg/μL (4), and the final concentration expressed relative to muscle wet-weight. Aliquots of total RNA were then separated with agarose gel electrophoresis and monitored under an ultraviolet light (Chemi-Doc XRS, Bio-Rad, Hercules, CA, USA) to verify RNA integrity and absence of RNA degradation, indicated by prominent 28s and 18s ribosomal RNA bands, as well as an OD_{260}/OD_{280} ratio of approximately 2.0 (4). We observed an average ($\pm$ SD) ratio of 1.92 ($\pm$ 0.034) for all samples. The RNA samples were stored at $-80°C$ until later analysis.

Reverse Transcription and cDNA Synthesis. A total of 2 μg of total skeletal muscle RNA was reverse-transcribed to synthesize cDNA using the iScript cDNA Synthesis Kit (Bio-Rad). Each reverse transcription reaction mixture was incubated at 25°C for 5 minutes, 42°C for 30 minutes, heated to 85°C for 10 minutes, and then quick-chilled on ice. The cDNA concentration was determined by using an OD_{260} equivalent to 50 μg/μL (4), and starting cDNA template concentration was standardized by adjusting all samples to 200 ng prior to amplification (39).

Oligonucleotide Primers for Polymerase Chain Reaction. The mRNA sequences of human skeletal muscle β-actin (NM_001101), type I MHC (X06976), type IIA MHC (AF111784), type IIX MHC (AF111785), Myo-D (X56677), myogenin (X62155), MRF-4 (XM006691), Myf-5 (X14894), IGF-1 (M37483), IGF-R1 (NM_000875), MGF (U40870), myostatin (NM_005259), and p27[kip] (NM_004064) published in the NCBI Entrez Nucleotide database (www.ncdi.nlm. hih.gov) were used to construct polymerase chain reaction (PCR) primers using Beacon Designer software (Bio-Rad), and then commercially synthesized (Integrated DNA Technologies, Coralville, IA, USA). These primers amplify respective fragments of 141, 145, and 148 bp for Type I, IIA, and IIX MHC; 103, 119, 108, and 114 bp for Myo-D, myogenin, MRF-4, and myf5; and 150, 126, 140, 145, and 112 bp for IGF-1, IGF-1R, MGF, myostatin, and p27[kip]. Due to its consideration as a constitutively expressed "housekeeping gene," and the fact that it has been shown to be an appropriate external reference standard in human skeletal muscle using real-time PCR, β-actin was used for detecting the relative change in the quantity of mRNA in response to resistance exercise (22,38). For β-actin, these primers amplify

a PCR fragment of 135 bp. Additionally, we observed β-actin to undergo only a small amount of variation in expression from one sampling point to the next. The overall average variation between all muscle samples was 2.87%, and provides further evidence to suggest β-actin as an appropriate external control for real-time PCR.

Real-Time PCR Amplification and Quantitation. A total of 200 ng of cDNA template was added to iQ SYBR Green Supermix (Bio-Rad) and each PCR reaction was amplified using real-time quantitative PCR (iCycler IQ Real-Time PCR Detection System, Bio-Rad). The amplification profile was run for 40 cycles employing a denaturation step at 95°C for 30 seconds, primer annealing at 58°C for 30 seconds, and extension at 72°C for 30 seconds. Fluorescence was measured after each cycle resulting from the incorporation of SYBR green dye into each amplicon. The expression of mRNA was determined from the ratio of the C_T values relative to β-actin. The specificity of the PCR was demonstrated with an absolute negative control reaction containing no cDNA template, and a single gene product was confirmed using DNA melt curve analysis. Positive amplification of the amplicons was assessed with agarose gel electrophoresis illuminated with UV transillumination (Chemi-Doc XRS, Bio-Rad).

Total DNA Content. Based on our previous guidelines (37), using the remaining homogenate from the original tube used for the total RNA isolation procedure, total DNA was isolated from the remaining interphase of the total RNA isolation procedure with 100% ethanol, 0.1 M sodium citrate, and 8 mM sodium hydroxide. The total DNA concentration was determined spectrophotometrically (Helio γ, Thermo Electron) by optical density (OD) at 260 nm using an OD_{260} equivalent to 50 μg/μL (4), and the final concentration expressed relative to muscle wet-weight.

Statistical Analyses

Statistical analyses were performed by using a repeated-measure two-factor (2 treatment groups × 4 time points] mixed methods multivariate analysis of variance (MANOVA). The MANOVA was performed due to the likelihood of the dependent variables being related to one another. In addition, the use of a MANOVA analysis also reduces the risk of type I errors by controlling for alpha level that could result with the use of repeated analyses of variance (ANOVA). In addition, for all statistical analyses not meeting the sphericity assumption for the within-subjects analyses, a Huynh-Feldt correction factor was applied to the degrees of freedom in order to adjust (increase) the critical F-value to a level that would prevent the likelihood of committing a type I error. Where appropriate, follow-up testing to the MANOVA for each dependent variable was performed using ANOVA. Significant differences in mean values for main effects or interactions were determined using a Tukey post hoc test. Paired samples *t*-tests were run on total volume, thigh mass, exercise intensity, 1RM, and number of repetitions completed. All statistical procedures were performed using SPSS 15.0 software (Chicago, IL) and a probability level of $p \leq 0.05$ was adopted throughout.

RESULTS

Resistance Exercise Sessions

There was no significant difference between the number of repetitions performed between the leg press and the knee extension for 60–65% 1RM ($p = 0.31$). Additionally, there was no significant difference between the number of repetitions performed between the leg press and the knee extension for 80–85% 1RM ($p = 0.14$; Table 1). However, the exercise volume for 60–65% 1RM ($24,066 \pm 605.25$) was significantly greater ($p = 0.001$) than the exercise volume for 80–85% ($16,362.69 \pm 422.35$).

Dietary Analyses

There were no significant differences in protein ($p = 0.36$), fat ($p = 0.76$), carbohydrates ($p = 0.70$), or total calories ($p = 0.16$) between the two resistance exercise bouts. Furthermore, there were no significant differences in protein ($p = 0.32$), fat ($p = 0.10$), carbohydrates ($p = 0.56$), or total calories ($p = 0.78$) between the two exercise bouts for breakfast on each day of testing (Table 2).

MHC Isoform mRNA Expression

No significant Intensity × Time interactions were observed for MHC I mRNA ($p = 0.997$), MHC IIA mRNA ($p = 0.955$), and MHC IIX mRNA ($p = 0.968$). However, significant main effects for time were observed for MHC I mRNA ($p = 0.028$), MHC IIA mRNA ($p = 0.008$), and MHC IIX mRNA ($p = 0.010$, Figure 1). Pairwise comparisons revealed that MHC I expression was significantly greater at 2HPST ($p = 0.032$) compared to PRE. Compared to PRE, MHC IIA was significantly greater at PST ($p = 0.046$), 2HPST ($p = 0.012$), and 6HPST ($p = 0.048$). For MHC IIX, PST ($p = 0.041$) and 2HPST ($p = 0.015$) were significantly different from PRE, while a trend was evident for 6HPST ($p = 0.079$).

MRF mRNA Expression

No significant Intensity × Time interactions were observed for Myo-D ($p = 0.973$), myogenin ($p = 0.820$), MRF-4 ($p = 0.901$), and myf5 ($p = 0.721$). However, significant main

TABLE 1. Resistance exercise repetitions.

Bout	Leg press repetitions	Leg extension repetition
60–65%	17.94 ± 0.96	17.71 ± 0.83
80–85%	8.79 ± 0.82	9.27 ± 0.77

Data are mean ± SD.

TABLE 2. Dietary analyses prior to resistance exercise.

Variable	60–65%	80–85%	p Value
24-hour dietary analyses			
Protein (g/day)	74 ± 32	70 ± 30	0.36
Fat (g/day)	70 ± 30	72 ± 38	0.76
Carbohydrates (g/day)	218 ± 73	196 ± 59	0.70
Total calories (kcal/day)	1861 ± 630	1721 ± 628	0.16
Breakfast dietary analyses			
Protein (g)	14 ± 10	15 ± 10	0.32
Fat (g)	14 ± 12	14 ± 12	1.0
Carbohydrates (g)	61 ± 32	59 ± 38	0.56
Total calories (kcal)	420 ± 256	415 ± 286	0.78

Data are mean ± SD.

effects for time were observed for Myo-D ($p = 0.001$), myogenin ($p = 0.001$), MRF-4 ($p = 0.002$), and myf5 ($p = 0.001$; Figure 2). Pairwise comparisons revealed that Myo-D expression was significantly greater at PST ($p = 0.001$), 2HPST ($p = 0.001$), and 6HPST ($p = 0.001$) compared to PRE. Compared to PRE, myogenin was significantly greater at PST ($p = 0.001$), 2HPST ($p = 0.001$), and 6HPST ($p = 0.001$). For MRF-4, PST ($p = 0.021$) 2HPST ($p = 0.004$), and 6HPST ($p = 0.019$) were significantly different from PRE. Compared to PRE, myf5 was significantly greater at PST ($p = 0.001$), 2HPST ($p = 0.001$), and 6HPST ($p = 0.002$).

Skeletal Muscle Growth Factor mRNA Expression

No significant Intensity × Time interactions were observed for IGF-1 ($p = 0.96$), IGF-1R ($p = 0.76$), and MGF ($p = 0.86$; Figure 3). However, significant main effects for time were observed for IGF-1 ($p = 0.02$), IGF-R1 ($p = 0.02$), and MGF ($p = 0.04$), and pairwise comparisons demonstrated that the increases in expression for all three genes at 2HPST to be significantly different from PRE.

Satellite Cell Activation Inhibitor mRNA Expression

No significant Intensity × Time interactions were observed for myostatin ($p = 0.92$) and p27^{kip} ($p = 0.85$, Figure 4). However, significant main effects for time were observed for myostatin ($p = 0.01$) and p27^{kip} ($p = 0.03$), and pairwise comparisons demonstrated that the decreases in expression for the two genes at 6HPST to be significantly different from PRE.
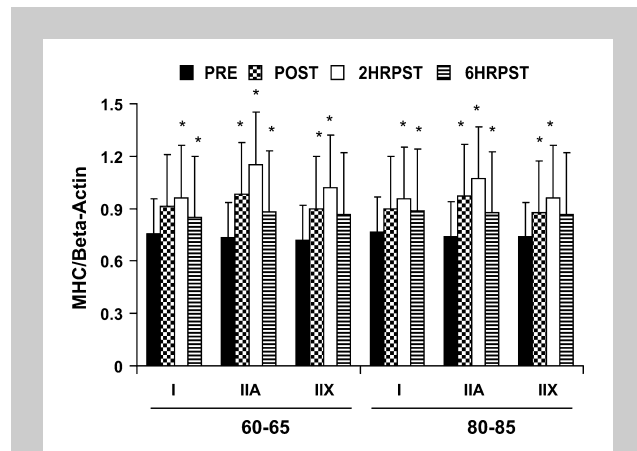

Figure 1. Myosin heavy chain (MHC) isoform mRNA expression in response to resistance exercise bouts with 60–65% and 80–85% 1 repetition maximum. Compared to immediately before exercise, MHC expression was significantly greater at 2 hours (2HRPST). MHC IIA was significantly greater at 30 minutes (PST), 2HRPST, and 6 hours (6HRPST) after exercise. MHC IIX was significantly greater at PST and 2HPST. *Significant main effect for time ($p < 0.05$).
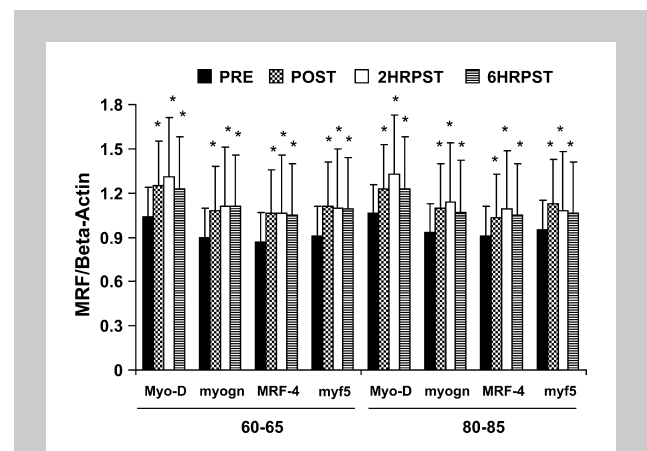

Figure 2. Myogenic regulatory factors (MRF) mRNA expression in response to resistance exercise bouts with 60–65% and 80–85% 1 repetition maximum. Compared to immediately before exercise, Myo-D expression was significantly greater at 30 minutes (PST), 2 hours (2HRPST), and 6 hours (6HRPST) after exercise. Myogenin, MRF-4, and myf5 were significantly greater at PST, 2HPST, and 6HPST. *Significant main effect for time ($p < 0.05$).
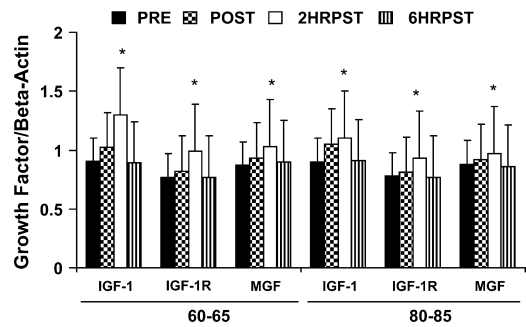
Figure 3. Growth factor mRNA expression in response to resistance exercise bouts with 60–65% and 80–85% 1 repetition maximum. Compared to immediately before exercise, IGF-1, IGF-R1, and MGF were all significantly increased at 2 hours after exercise. *Significant main effects for time ($p < 0.05$).
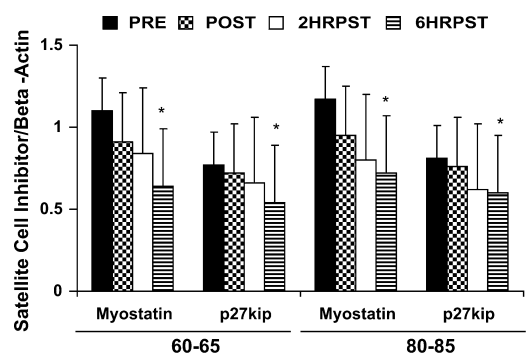

Figure 4. Satellite cell activation inhibitor mRNA expression in response to resistance exercise bouts with 60–65% and 80–85% 1 repetition maximum. Compared to immediately before exercise, myostatin and p27^{kip} were significantly decreased at 6 hours after exercise. *Significant main effects for time ($p < 0.05$).
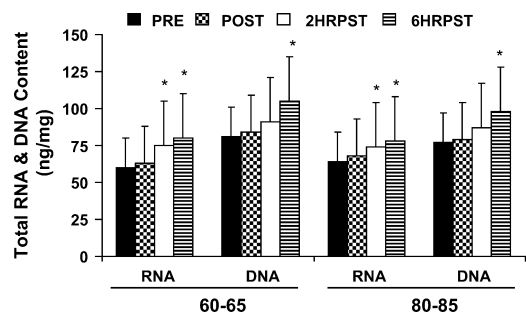

Figure 5. Total RNA and DNA content in response to resistance exercise bouts with 60–65% and 80–85% 1 repetition maximum. Compared to immediately before exercise, RNA significantly increased at 2 hours (2HRPST) and 6 hours (6HRPST) after exercise and DNA significantly increased at 6HPST. *Significant main effects for time ($p < 0.05$).

Total RNA and DNA Content

No significant Intensity × Time interaction was observed for total RNA ($p = 0.88$) or DNA content ($p = 0.79$, Figure 5). However, a significant main effect for time was observed for RNA ($p = 0.01$) and DNA ($p = 0.02$). Pairwise comparisons demonstrated that the increase in RNA content at 2HPST and 6HPST and DNA content at 6HPST to be significantly different from PRE.

DISCUSSION

The results of the current study support our hypothesis, as the primary purpose of this study was to determine the effects of different intensities of resistance exercise on markers of myogenesis and the expression of various genes involved in skeletal muscle hypertrophy. As such, both intensities of resistance exercise resulted in similar increases in the mRNA expression of the MHC isoforms (type I, IIA, and IIX), MRFs (Myo-D, myogenin, MRF-4, and myf5), IGF-1, IGF-R, and MGF mRNA, as well as increases in total RNA and DNA. In addition, there were significant decreases in both myostatin and p27^{kip} that were no different between the two intensities. Therefore, these data demonstrate that resistance exercise performed in the intensity range of 60–85% modulates the mRNA expression of MHC and factors involved in myogenic activation of satellite cells.

Increases in total RNA, the majority of which is ribosomal, represents an increase in the machinery that is necessary for translation of mRNA into protein. Muscle hypertrophy involves both an increase in total RNA concentration and an increased transcriptional activity for MHC (13). Therefore, an increase in total RNA per unit muscle mass is indicative of a higher anabolic potential (7). In the present study, we showed an increase in total RNA over the course of 6 hours after exercise concomitant with upregulation in the expression of genes involved in muscle hypertrophy and myogenesis. Resistance exercise using neuromuscular electrical stimulation rather than dynamic exercise has been shown to not increase total RNA until 72 hours after exercise (7). Consequently, resistance exercise has been shown to increase myofibrillar protein synthesis without concomitant increases in total RNA and mRNA concentration (35); however, this study involved elderly participants and only the leg extension exercise, thereby making comparison to the present study difficult.

In the present study, we demonstrated that type I, type IIA, and type IIX, MHC mRNA expression was upregulated within 30 minutes following the 60–65% 1RM and 80–85% 1RM resistance exercise bouts, and that the response was not dependant on exercise intensity. The present findings for 80-85 are in agreement with our previous work (36) in which we explored the effects of a single session of high-intensity resistance exercise on MHC mRNA expression and demonstrated that 3 sets of 8–10 repetitions at 75–80% 1RM on the squat, leg press, and leg extension exercise resulted in elevations Type I, IIA, and IIX MHC mRNA expression. These

collective findings demonstrate that resistance exercise is a modulator of MHC isoforms.

In our previous study (36), we also showed that Myo-D and myogenin mRNA expression were upregulated at 6 hours after a single bout of high-intensity resistance exercise. It has also been shown that the mRNA levels of myogenin, Myo-D, and MRF-4 were significantly upregulated over the course of 6 hours after a single bout of 4 sets of 6–12 repetitions on a leg press and knee extension machine exercises (28). In response to 3 sets of 10 repetitions at 70% 1RM of knee extensions, Myo-D, myogenin, and MRF-4 were increased from 4 to 8 hours after exercise (40). In women, a single-resistance exercise bout has been shown to increase Myo-D, myogenin, MRF-4, and myf5 4 hours after exercise (29). In the present study, we have demonstrated that a single bout of resistance exercise, regardless of the intensity, is sufficient to upregulate Myo-D, myogenin, MRF-4, and myf5 mRNA expression over the course of 6 hours after exercise. The importance of these findings is that the MRFs, by serving as transcription factors, conceivably play an important role in the regulation of MHC gene expression. Furthermore, since the MRFs regulate myogenesis and promote the development of muscle into mature skeletal muscle fibers, given the relatively early 4- to 6-hour time point in which mRNA expression was upregulated in these studies, it is likely the upregulation noted in MRF mRNA came primarily from postmitotic nuclei (29).

Activation of skeletal muscle satellite cells, defined as entry to the cell cycle from a quiescent state, add nuclei to muscle fibers and is essential for normal growth and for regeneration of tissue damaged by injury or stress (17). The cell cycle is regulated by a number of CDK inhibitors (e.g., p21^{cip}, p27^{kip}, p57^{kip}), and p27^{kip} is typically considered an inhibitor of cell cycle initiation (G1) and is often regarded as a general marker of differentiation. However, in addition to promoting withdrawal of actively proliferating cells, p27^{kip} specifically suppress cell cycle initiation and/or progression. Proliferation of satellite cells may occur in response to heavy resistance exercise. In humans, satellite cell proliferation appears to be involved in replacing cells damaged by training, so that there is no significant increase in the net number of muscle fibers (17). Once activated, satellite cells re-enter the cell cycle and express myogenic regulatory factors (MRFs) (Myo-D, myogenin, MRF-4, and myf5). Myo-D and myf5 have been suggested to have a functional role during satellite cell activation (31). Myf5 is also thought to cooperate with Myo-D to regulate determination of myoblasts (37). This is important, as myf5 expression indicates satellite cell activation and that satellite cells are committed to the myogenic lineage (5). In contrast to myf5 and Myo-D, myogenin and MRF-4 have differentiation functions (8). Myogenin and MRF-4 likely regulate contractile protein target genes (23,41) that include genes involved in fast and slow fiber differentiation (27). Myogenin has been found to accumulate in type I fibers and Myo-D in type II fibers (36). Moreover, a study conducted by Mozdziak et al. (26)

indicated that myogenin and Myo-D mRNA levels are associated more with alterations in MHC isoform composition than changes in muscle mass. In addition, we have previously demonstrated myogenin to be associated with type I and IIA MHC mRNA expression, whereas Myo-D was associated with type IIX MHC mRNA expression (36).

It is suggested that IGF-I increases the proliferation of satellite cells once they are activated (2). IGF-I increases muscular protein synthesis and stimulates satellite cell proliferation and differentiation in vitro (11). The discovery of two IGF-I isoforms, MGF and IGF-I, has suggested that MGF initiates satellite cell activation and proliferation while IGF-I promotes differentiation of proliferating satellite cells (40). Our present findings support the premise that resistance exercise is capable of upregulating factors specific to satellite cell activation such as IGF-1 and MGF. Adams and Haddad (1) reported a positive relationship between IGF-1 protein and total DNA content in muscle during resistance exercise overload due to satellite cell proliferation stimulated by the locally produced IGF-1. Previous studies (14) have shown increased MGF and IGF-I mRNA content 2.5 and 48 hours, respectively, after a single bout of resistance exercise. These findings are in agreement with our present findings in that we observed significant increases in IGF-1, IGF-1R, and MGF mRNA expression at 2 hours after exercise. However, this is in contrast to findings in which there was no change in IGF-I mRNA levels (28).

Satellite cells are negatively regulated by a protein called myostatin, which inhibits myoblast proliferation and differentiation in developing muscle and plays a limiting role in growth/repair/regeneration of differentiated adult muscle by inhibiting satellite cell activation (34). Myostatin may therefore play a key role in the inhibition of hypertrophic processes. The inhibition of differentiation by myostatin is regulated by inhibiting Myo-D expression, and it has been recognized that Myo-D functions as the muscle transcription factor on myostatin expression. Myo-D binds to the myostatin promoter and stimulates its activity, suggesting that direct up-regulation of myostatin expression is due to its ability to control myoblast cell cycle withdrawal and differentiation (3).

Increased levels of myostatin upregulate CDK inhibitors such as p27^{kip} and thereby prevent the differentiation of satellite cells (25). Single bouts of resistance exercise have been shown to suppress myostatin mRNA expression at 4 hours after exercise (20,29) in response to 3 sets of 10 repetitions at 70% 1RM using the leg extension exercise. These studies have demonstrated that resistance loading in humans causes decreases in myostatin mRNA expression (18,20,29). Our present study showed that a single bout of resistance exercise is capable of downregulating myostatin and p27^{kip} mRNA expression at 6 hours after exercise. A single bout of resistance exercise has been show to up-regulate the mRNA expression of myostatin, which impairs satellite cell proliferation and differentiation, and p27^{kip}, which inhibits early (G1-S) cell cycle progression (18). Our

present findings agree in that myostatin and p27^{kip} mRNA were decreased by 6HPST and MGF mRNA was upregulated at 2HPST.

There is evidence suggesting that muscle hypertrophy may require the stimulation of satellite cell proliferation and fusion with myofibers to maintain some finite ratio between muscle fiber size (e.g., cytoplasmic volume) and myonucleus number (30). Increases in myonuclear number and cellular volume are proportional, such that the myonuclear domain size (defined as the volume of cytoplasm per myonucleus) of the muscle fiber remains constant. The satellite cell and myonuclear numbers increase sequentially (32) in a pattern consistent with the premise that the new myonuclei arise from the incorporation of proliferating satellite cells. Because an increase in myonuclear number expands the quantity of DNA available for protein production, the additional myonuclei may facilitate skeletal muscle hypertrophy. Because mature myofibers nuclei are thought to be mitotically inactive (33), increased DNA content in skeletal muscle cells suggests activation of satellite cells. In the present study, we observed increases in total DNA content that occurred concomitantly with increases in the MRFs and IGF-1 and decreases in myostatin and p27^{kip}. This increase in DNA content, along with alteration in the observed markers of myogenesis, suggest that resistance exercise, regardless of intensity, promotes myogenic activation of satellite cells.

PRACTICAL APPLICATIONS

It has been concluded that resistance exercise with an intensity ranging from 60–85% 1RM is effective at modulating the expression of genes involved in hypertrophy and myogenesis in skeletal muscle. Furthermore, the current study has supported the notion that resistance exercise within this intensity range can induce significant changes in the hypertrophic and myogenic regulatory network likely involved in the muscle hypertrophy typically observed with resistance training programs. This gives us insight into the potential role of exercise intensity and volume as it pertains to the adaptive response of exercise. Exercise intensity is the basis for prescription in many resistance training programs. Therefore, the current study provides further insight into how resistance exercise impacts the hypertrophic and myogenic responses.

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