Eur J Appl Physiol (2010) 108:771–778
DOI 10.1007/s00421-009-1279-z

ORIGINAL ARTICLE

Myogenic regulatory factor response to resistance exercise volume in skeletal muscle

Micah J. Drummond · Robert K. Conlee · Gary W. Mack · Sterling Sudweeks · G. Bruce Schaalje · Allen C. Parcell

Accepted: 26 October 2009 / Published online: 17 November 2009

Abstract This study examined the impact of resistance exercise volume on myoD and myogenin in rodent quadriceps muscle. Six-month-old male Sprague–Dawley rats (316 ± 2 g) performed either low-volume (LV; 10 sets × 10 contractions) or high-volume (HV; 20 sets × 10 contractions) resistance exercise at 75% one-repetition maximum. Muscles were analyzed for myogenin and myoD mRNA and protein expression 6, 12, 24 and 48 h post-exercise. In red quadriceps (RQ), myogenin mRNA was significantly elevated at 6 h following LV and this response was greater than HV at 6 h, while myogenin protein was significantly increased at 6 and 12 h following LV but only at 12 h following HV ($P < 0.05$). MyoD mRNA was increased at 6 and 12 h following LV and at 12 h following HV, while myoD protein was slightly decreased (LV; $P < 0.05$) or unchanged over time (HV). No changes were detected within the white quadriceps muscle. We conclude that acute resistance exercise can activate myogenin and myoD expression levels in RQ, but when exercise volume is doubled these myogenic responses are not proportional but delayed and blunted possibly because of excessive damage/injury. Further work is needed to determine the consequences of these specific myogenic responses on muscle hypertrophy following high-volume resistance exercise training.

Keywords Myogenin · myoD · Skeletal muscle · Resistance exercise · Rodent

Communicated by Susan Ward.

M. J. Drummond · R. K. Conlee · G. W. Mack · A. C. Parcell (✉)
Department of Exercise Sciences,
Human Performance Research Center,
Brigham Young University, 120-E Richards Bldg.,
Provo, UT 84602, USA
e-mail: allen_parcell@byu.edu

S. Sudweeks
Department of Physiology and Developmental Biology,
Brigham Young University, Provo, UT 84602, USA

G. B. Schaalje
Department of Statistics,
Brigham Young University, Provo, UT 84602, USA

Introduction

Adaptation of an organ system to exercise training is dependent on the magnitude of the exercise stimulus. The exercise stimulus is defined by the intensity of the exercise and the volume of exercise performed. In resistance exercise programs, exercise volume is characterized by a combination of sets of repetitions performed at a given exercise intensity. It has been suggested that exercise volume is important in the design of any resistance exercise interventions in order to optimize increased skeletal muscle size and strength (Kraemer et al. 2002). For example, training programs involving high volume (multiple repetitions) are known to increase strength and muscle size better than lower volume (single set) resistance exercise (Kelly et al. 2007; Rhea et al. 2002). The adaptive response of skeletal muscle to resistance exercise involves a series of molecular signaling pathways that lead to increased expression of contractile proteins (myosin and actin) and an eventual increase in muscle size and strength.

While the exact mechanisms involved in the increased expression of contractile proteins are unclear, certain regulatory factors are evidently associated with increased expression of skeletal muscle contractile proteins. Myogenin

and myoD, members of the myogenic regulatory factor (MRF) family, are known to stimulate myogenesis (Perry and Rudnick 2000) and increase transcription of contractile, cytoskeletal and metabolic genes (Lassar et al. 1989; Li and Capetanaki 1993; Moss et al. 1996; Wentworth et al. 1991; Wheeler et al. 1999). Expression of myogenin and myoD is increased following a single bout of resistance exercise in humans and animals (Kim et al. 2005; Raue et al. 2006; Tamaki et al. 2000; Willoughby and Nelson 2002; Yang et al. 2005) supporting the hypothesis that MRF nuclear transcription factors play some role in skeletal muscle plasticity following resistance exercise.

Although data is lacking on the specific molecular pathways involved in the increased expression of contractile proteins, we assume that myogenin and myoD expression should respond to resistance exercise. Specifically, we hypothesized that myogenin and myoD would respond in proportion to exercise volume during resistance exercise. To test this hypothesis we used a rodent resistance exercise model (Krisan et al. 2004) and evaluated the impact of exercise volume on myogenin and myoD mRNA and protein expression in the exercised skeletal muscle.

Methods

Animals

Seventy-two 6-month-old male Sprague–Dawley (316 ± 2 g; Charles River, Boston MA) rats were used in this study. They were housed in standard cages in a temperature-controlled environment (21–22°C) with a 12-h dark–light cycle. Rats were fed standard rat chow and water ad libitum. All experiments were approved by the Institutional Animal Care and Use Committee and strict adherence to animal care standards was observed.

Experimental design

Rats were randomly assigned to one of three treatment groups: non-exercised time-point control (CON; $n = 24$; body weight (BW) = 310 ± 3 g), low-volume resistance exercise (LV; $n = 24$; BW = 316 ± 3 g) and high-volume resistance exercise (HV; $n = 24$; BW = 320 ± 4 g). Rats from the LV and HV groups were killed 6, 12, 24 and 48 h post-exercise ($n = 6$/time point). Rats in the CON group did not exercise and were killed 6, 12, 24 and 48 h after being harnessed in the resistance exercise apparatus. We chose these post-exercise time points in order to capture peak mRNA and protein expression for our dependent variables (Haddad and Adams 2002; Peters et al. 2003). Myogenin, myoD mRNA and their associated protein were measured at the above mentioned post-exercise time points in the red (RQ) and white (WQ) quadriceps muscle of the right hindlimb from each rat. Data from each group were collected in a randomized order and at approximately the same time of day.

Resistance exercise apparatus

All rats performed resistance exercise on a squat apparatus as described by Krisan et al. (2004). Briefly, rats were placed in a squat position on a steel plate and harnessed in a vest attached to the squat apparatus. An electrode was attached to the rat's tail and an electrical stimulus (5 mA/0.02 s/10 V) was used to motivate the rats to stand, thus lifting the prescribed weight for each repetition. Rats remained harnessed in the squat apparatus until completion of the resistance exercise protocol.

One-repetition maximum

A one-repetition maximum (1RM) was determined 48–72 h prior to the resistance exercise treatment as follows. Each rat lifted a starting weight of 400 g for 10 repetitions. After a 90-s rest, the weight was increased by 200 g and lifted for 5 repetitions. Following the submaximal lifts, a 1RM was determined by adjusting the weight with increments of 100, 50, 25 and/or 10 g. No more than three 1RM attempts were performed for each rat within this study. Three minutes of rest were given between maximal lifts. The highest weight lifted from squat to standing position was considered the 1RM.

Submaximal resistance exercise

The resistance exercise protocol consisted of 10 sets of 10 repetitions (LV) or 20 sets of 10 repetitions (HV) at 75% of the rodents' predetermined 1RM. We chose 10 and 20 sets for purposes of simulating high-intensity resistance exercise and, therefore, generating an acute myogenic response as measured by myogenin and myoD expression levels. Training with lower volumes of resistance exercise (3 sets of 10 repetitions at 75% 1RM) did not produce muscle hypertrophy using this same protocol (Krisan et al. 2004). Ninety seconds of rest was given between sets and ~2 s between repetitions. After exercise, the rats were placed into their respective cages until the muscles were harvested 6, 12, 24 or 48 h later. The CON group rats were harnessed in the squat apparatus for a length of time equivalent to that of the HV group (~40 min) but did not receive electrical stimulation or perform resistance exercise. We chose not to use a control group that received neither electrical stimulation nor exercise because previous research has indicated that electrical stimulation alone does not influence muscle hypertrophy (Barauna et al. 2005; Tamaki et al. 1992) nor serum creatine kinase levels (Tamaki et al. 1992) in rats.

Sacrifice and tissue harvesting procedure

At the specified time points and same time of day, rats were anesthetized by a combination of ketamine (90 mg/kg body weight) and xylazine (10 mg/kg body weight) via an intraperitoneal injection and samples of the RQ and WQ of the right hindlimb were excised. Muscle samples were immediately frozen in liquid nitrogen. Rats were euthanized with an overdose of sodium pentobarbital followed by opening of the chest cavity. Frozen muscle samples were pulverized with a mortar and pestle while on dry ice. The powdered samples were stored at −80°C until immunoblot and mRNA analysis.

Total protein analysis

Muscle tissue (30–50 mg) was homogenized (5 ml mg^{-1}) on ice for 30 s in a buffer containing 2% sodium dodecyl polyacrylamide (SDS) in 10 mmol L^{-1} Tris–HCL and 0.1 mmol L^{-1} EDTA and a protease inhibitor cocktail (2X, P8340, Sigma–Aldrich, St. Louis, MO). Samples were centrifuged at 13,000 × *g* for 10 min at 4°C and the supernatant collected. Total protein analysis of the homogenate was completed using a micro-bicinchoninic acid procedure (BCA Protein Assay Kit, Pierce, Rockford, IL) according to the manufacturers' directions and absorbance measured on a micro-plate reader (VICTOR3, PerkinElmer Life and Analytical Sciences, Shelton, CT) at a wavelength of 560 nm.

Myosin heavy chain analysis

Myosin heavy chain (MHC) isoform composition was determined in the RQ and WQ of ten randomly selected rodents from the CON and deemed representative of all animals in used in the study. We followed procedures previously published by Reiser et al. (Reiser and Kline 1998).

Immunoblotting

For immunoblot analysis, 20 μg of total protein was loaded onto a 10% (30% bisacrylamide, 1.5 M Tris–HCL pH 8.8, 10% SDS, 5.5 cm high) separating and 4% (30% bisacrylamide, 0.5 M Tris–HCL pH 6.8, 10% SDS, 1.8 cm high) stacking SDS mini gel. A representative sample for each treatment and time point (see representative blot on Figs. 3, 4) and a molecular weight ladder (Precision Plus Protein Standard, Bio-Rad Laboratories, Hercules, CA) were included on each blot. The samples were run in an electrophoresis unit (Mini Proteaan II; Bio-Rad Laboratories, Hercules, CA) at constant 200 V for 40 min. Proteins were transferred to a polyvinylidene difluoride membrane (Bio-Rad Laboratories, Hercules, CA) for 50 min at 350 mA. Blots were blocked in 5% milk dissolved in phosphate-buffered saline with 0.05% Tween 20 (PBST) overnight (4°C; 16 h) and then rinsed in PBST at room temperature (RT). Blots were incubated with a rabbit polyclonal myoD (1:1,000; M-318, Santa Cruz Biotechnology, Santa Cruz, CA) primary antibody and diluted in blocking solution (5% milk in PBST) for 1 h at RT. The blots were then rinsed in PBST solution three times for 10 min each, then incubated for 1 h in secondary antibody [goat anti-rabbit IgG (diluted 1:25,000); Santa Cruz Biotechnology, Santa Cruz, CA] at RT. Blots were rinsed again in PBST three times for 10 min each at RT and chemiluminescent solution (ECL, Santa Cruz Biotechnology, Santa Cruz, CA) was applied to each blot. Blots were exposed to autoradiography film (Hyperfilm ECL, Amersham BioSciences, Piscataway, NJ), and then developed.

Following analysis, membranes were stripped of primary and secondary antibodies using two 15-min rinses of a stripping buffer (25 mM glycine, pH 2.0 and 1% SDS) followed by two 5-min rinses in PBST at RT. Membranes were blocked in 5% milk in PBST for 1 h, rinsed in PBST, probed with mouse monoclonal myogenin (1:250; F5D, Santa Cruz Biotechnology, Santa Cruz, CA) primary antibody for 2 h, rinsed again in PBST and incubated in secondary antibody (1:25,000; bovine anti-mouse IgG, Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h at RT. Following antibody incubation, myogenin probed membranes were rinsed in PBST, prepared for film development and then exposed in a dark room.

Protein bands were quantified with densitometry (UN-SCAN-IT gel software, version 4.3, Silk Scientific, Orem, UT). Bands were identified at molecular weights of 38 and 36 kDa, for myoD and myogenin, respectively, using a purified protein for myoD [Santa Cruz Biotechnology (sc-4080)] and a cell lysate for myogenin [Santa Cruz Biotechnology (sc-2287)]. In order to compare samples between membranes, band intensity was normalized to the specific time-point control for each protein on the same blot and a percent change of CON (100%) was calculated. Each sample was run in triplicate on separate gels and an average of the three values was taken.

Ribosomal S6 kinase 1 protein expression

S6K1 detection was determined using a phospho-specific rabbit polyclonal antibody against the S6K1 Thr389 phosphorylation site using an antibody dilution of 1:1,000. After band exposure and densitometry (Chemidoc, Bio-Rad), membranes were stripped of primary and secondary antibodies as described above then probed for S6K1 total protein (1:1,000). Phospho-specific and total antibodies were purchased from Cell Signaling (Danvars, MA). Immunoblot data was expressed as a phospho/total protein ratio.

RNA extraction

Total RNA was isolated by homogenizing ~30–40 mg tissue using 1 ml of Tri-reagent and 4 μl of polyacryl carrier (Molecular Research Center, Inc, Cincinnati, Ohio). The RNA was separated into an aqueous phase using 0.2 ml of chloroform and precipitated from the aqueous phase using 0.5 ml of isopropanol. Extracted RNA was washed with 1 ml of 75% ethanol, dried, and then suspended in a known amount (1.5 μl mg^{-1} tissue) of nuclease-free water. RNA was stored at 4°C overnight then RNA was quantified spectrophotometrically (GENESYS 2, Thermospectronic, Rochester, New York) at a wavelength of 260 nm. RNA concentration (μg mg^{-1}) was calculated on the basis of total RNA yield. RNA integrity was assessed by RNA agarose gel electrophoresis followed by visualization of the 18S and 28S ribosomal RNA bands under ultraviolet light.

cDNA synthesis

Total RNA was reverse transcribed into cDNA using a reaction mix of Oligo dT, random primers and nuclease-free water (Invitrogen, Carlsbad, CA). This mix was incubated at 65°C for 5 min then chilled on ice for 1 min. Two microliters of 0.1 M DDT and 4 μl of 5× first strand buffer (250 mM Tris pH 8.3, 375 mM KCl, 15 mM $MgCl_2$) were added to the mixture. Samples were incubated at 42°C for 2 min. This was followed by the addition of 1 μl of reverse transcriptase (Superscript II, Invitrogen, Carlsbad, CA) creating a total reaction tube volume of 20 μl. Samples were heated at 42°C for 50 min followed by termination by heating at 70°C for 15 min. All incubations were conducted using a DNA engine, Peltier thermal cycler (PTC-200, MJ Research, Waltham, MA).

cDNA quantification

cDNA content was quantified using the PicoGreen assay (Invitrogen, Carlsbad, CA) modified to work with a 96-well plate. Briefly, a standard curve was created using a DNA standard from 500 to 0 ng mg^{-1} on a black 96-well plate. Ninty-nine microliters of 1× TE (10 mM Tris–HCl, 1 mM EDTA, pH 7.5) was added to each well. This was followed by the addition of 1 μl of cDNA into each sample well. Finally, 100 μl of PicoGreen reagent was added to each well creating a total volume of 200 μl. Samples and standards were briefly shaken, covered with foil and incubated for 3 min. The measurements were conducted on a micro-plate reader (VICTOR3, PerkinElmer Life and Analytical Sciences, Shelton, CT) and read at 480 nm for excitation and 520 nm for emission.

Quantitative real-time RT-PCR

Determination of relative mRNA expression was performed by real-time RT-PCR using a MyiQ Single-Color Real-Time PCR Detection System (Bio-Rad, Hercules, CA). GAPDH, myogenin and myoD primers sequences were custom designed to span two exons and to avoid secondary structures (NCBI website). Primers were created using OligoPerfect Designer (Invitrogen, Carlsbad, CA) then purchased from Invitrogen. Myogenin and GAPDH primer sequences have been published previously (Drummond et al. 2008). The forward (F) and reverse (R) primer sequences for myoD were the following: myoD (M84176)-F: CCTACTACAGTGAGGCGTCCCA, R: GCTCCAC TATGCTGCAGAGG. MyoD primers were tested for optimal efficiency (85–100%) then run out on an agarose gel to determine if the appropriate size DNA product was produced.

Ten nanograms of cDNA were analyzed using SYBR Green fluorescence (SuperArray Biosciences Corp., Frederick, MD). The reaction vessel contained 12.5 μl iQ SYBR Green Supermix [100 mM KCL, 40 mM Tris–HCl, pH 8.4, 0.4 mM of each dNTP (dATP, dCTP, dGTP, dTTP), 1 μl iTaq DNA polymerase, 50 units/ml, 0.2× SYBR Green I, 6 mM $MgCl_2$, 20 nM fluorescein, and stabilizers], a known amount of forward and reverse primers and nuclease-free water and 2.0 μl cDNA template. The total volume of the reaction tube was 25 μl. All samples were run in triplicate. An initial cycle for 10 min at 95°C was used to denature the cDNA. This was followed with 40 PCR cycles consisting of a denaturing step at 95°C for 15 s, primer annealing at 60°C for 20 s, and 30 s at 72°C for mRNA extension and elongation. Cycle threshold was determined by calculating the second derivative from the fluorescence curves using the Graph Pad Prism software (Version 4.0, San Diego, CA). Relative fold changes in mRNA were determined by the delta–delta cycle threshold method as described by Livak and Schmittgen (2001) after normalizing to the internal control gene.

GAPDH was used as a control to normalize the mRNA content of the target genes in each sample. GAPDH has been shown to be a reliable internal control within rodent skeletal muscle (Drummond et al. 2008). GAPDH mRNA expression was similar for all tissues, treatment groups, and time points in this study (data not shown).

Statistical analysis

Mean differences due to treatments and times for each muscle type (RQ and WQ) were analyzed using a mixed linear model with 2-fixed factors, treatments (CON, LV and HV) and times (6, 12, 24 and 48 h post-exercise). Both of the fixed factors were between-subject factors. The model was

fitted to the data using the Mixed procedure of SAS (SAS Institute Inc., Cary, NC). Main effects and the interaction of the 2-factors were examined. S6K1 protein expression analysis was determined using a 2-way repeated measure ANOVA (SigmaStat, version 3.5). Results were presented as mean ± SE. The significance level for hypothesis tests was set at alpha = 0.05.

Results

The average 1RM for all the experimental rats was 1,292 ± 21 g (Con, 1,241 ± 35 g; LV, 1,313 ± 36 g; HV, 1,317 ± 35 g). During the exercise session, rats lifted an average of 74.8 ± 0.04% (LV, 74.8 ± 0.05%; HV, 74.8 ± 0.04%) of their 1RM which was 983 ± 19 g (LV, 980 ± 28 g; HV, 985 ± 27 g). Animal body weights immediately before muscle harvesting were Con, 330 ± 3 g; LV, 334 ± 4 g and HV, 332 ± 4 g. There were no significant differences between treatment groups for 1RM, average lifted weight (%) or body weight before harvesting.

Myosin heavy chain composition

Figure 1 illustrates the MHC composition of RQ and WQ muscle homogenates. Red quadriceps skeletal muscle was composed of 10 ± 1% MHC I, 22 ± 1% MHC IIa, 38 ± 2% MHC IIb and 29 ± 1% MHC IIx. White quadriceps skeletal muscle was composed of 69 ± 1% MHC IIb and 31 ± 1% MHC IIx.

S6K1 protein expression

S6K1 has been previously used as a marker of muscle hypertrophy (Baar and Esser 1999) and is rapidly phosphorylated following muscle contraction (Bolster et al. 2003). Therefore, to determine an indirect assessment of muscle activation, S6K1 phosphorylation was measured at 6, 12 and 24 h post-exercise in RQ and WQ muscles of 8 rats. Low-volume ($n = 4$) and HV ($n = 4$) groups within each muscle type were combined in order to increase statistical power. We found that S6K1 phosphorylation (Thr389) in RQ tended to be elevated at 6 h ($P = 0.064$) and was significantly elevated by ~threefold at 12 h compared to CON (Fig. 2; $P < 0.05$). Phosphorylation of S6K1 at 6 and 12 h in RQ were significantly greater than WQ at the same time points ($P < 0.05$). There were no significant changes in S6K1 phosphorylation in the WQ across time.

Red quadriceps skeletal muscle

Both LV and HV resistance exercise increased myogenin mRNA expression ($P < 0.05$) in RQ at 6 h post-exercise by 443 ± 87 and 289 ± 67%, respectively (Fig. 3a). The increase in myogenin mRNA expression at 6 h post-exercise was larger for LV than HV exercise ($P < 0.05$). At 12 h post-exercise, myogenin mRNA expression returned to control levels in LV but remained elevated by 254 ± 67% ($P < 0.05$) in HV. From 24 to 48 h post-exercise, myogenin mRNA expression was similar to control levels for both LV and HV. Myogenin protein expression increased 268 ± 53% ($P < 0.05$) 6 h post-exercise in LV but was not increased at this time point following HV (Fig. 3b). By 12 h post-exercise myogenin protein expression was elevated in LV (268 ± 68%) and HV (282 ± 95%) similarly ($P < 0.05$). Myogenin protein levels returned to control levels by 24 h in both LV and HV and remained at this level through 48 h post-exercise.

Low-volume resistance exercise significantly increased RQ skeletal muscle myoD mRNA expression 296 ± 53% ($P < 0.05$) by 6 h post-exercise, while myoD mRNA expression remained at control levels 6 h following HV (Fig. 4a). At 12 h post-LV, myoD mRNA expression

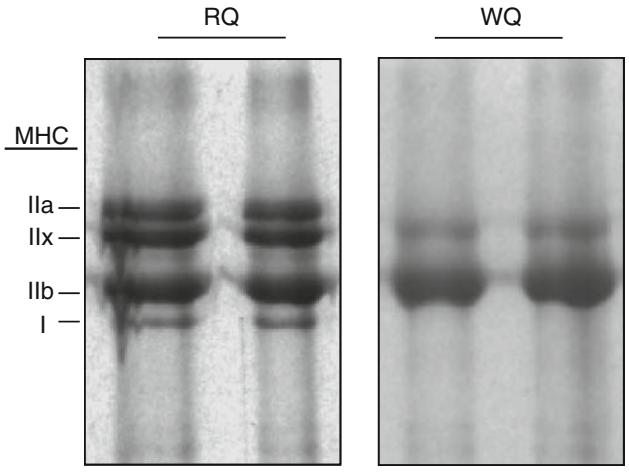

Fig. 1 Representative image for MHC composition in RQ and WQ. Two selected rodent whole muscle homogenates were chosen to represent each muscle type

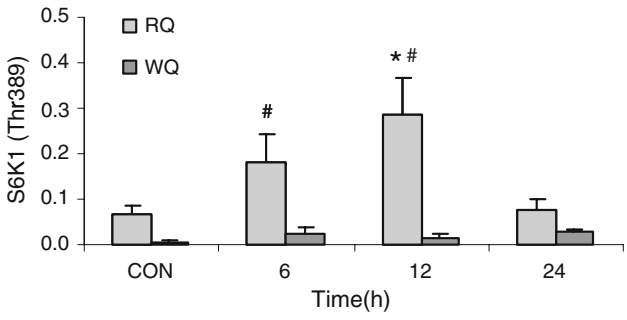

Fig. 2 Data represents ribosomal S6 kinase 1 (S6K1) phosphorylation at Thr389 in RQ ($n = 8$) and WQ ($n = 8$) muscle types. *Significantly different than CON ($P < 0.05$). #Significantly different than WQ at corresponding time point ($P < 0.05$)

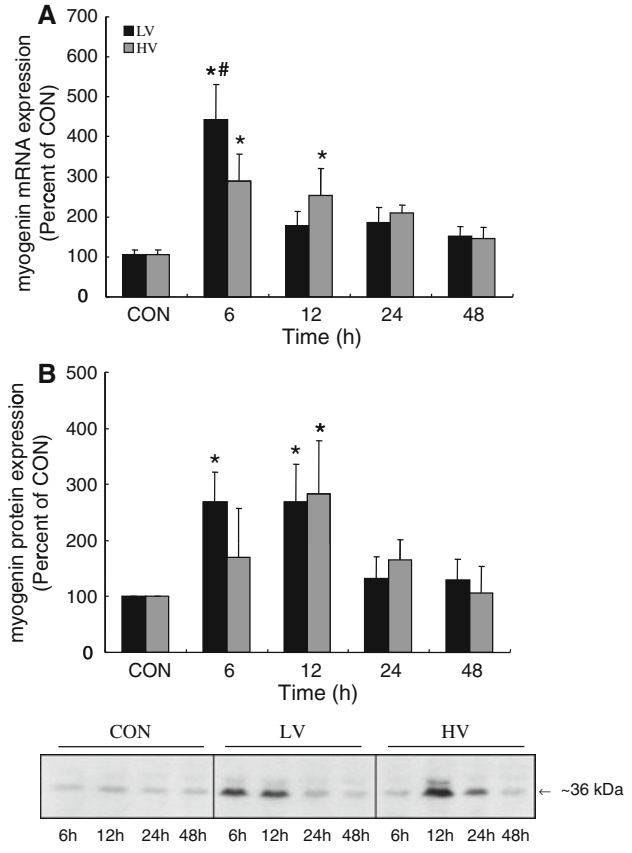

Fig. 3 Myogenin **a** mRNA and **b** protein expression in RQ muscle between LV and HV at 6, 12, 24 and 48 h post-exercise. Values are percent of CON and are expressed as mean ± SE. Representative immunoblots for myogenin protein expression in RQ for CON and after LV and HV resistance exercise are also included. The order of the blots from left to right are CON 6–48 h, LV 6–48 h and HV 6–48 h. *Significantly different from CON ($P < 0.05$). #Significantly different between treatments at corresponding time point ($P < 0.05$)

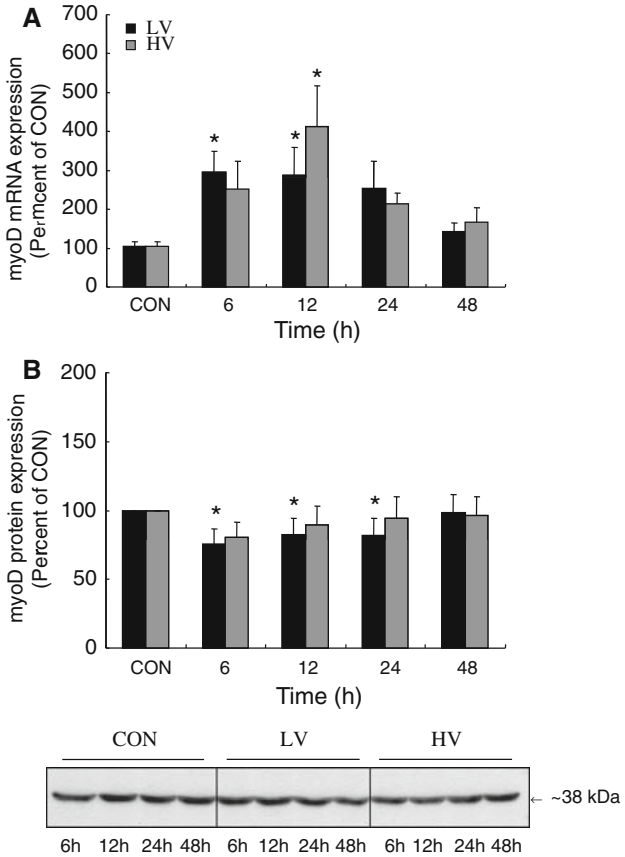

Fig. 4 MyoD **a** mRNA and **b** protein expression in RQ muscle between LV and HV at 6, 12, 24 and 48 h post-exercise. Values are percent of CON and are expressed as mean ± SE. Representative immunoblots for myoD protein expression in RQ for CON and after LV and HV resistance exercise are also included. The order of the blots from left to right are CON 6–48 h, LV 6–48 h and HV 6–48 h. *Significantly different from CON ($P < 0.05$)

remained elevated by 288 ± 71% ($P < 0.05$), while at this same time point, HV increased myoD mRNA expression by 412 ± 105% ($P < 0.05$). The increase in myoD mRNA expression at 12 h post-exercise was similar for LV and HV exercise. By 24 h post-exercise, myoD mRNA expression returned to control levels for LV and HV and remained at these levels through 48 h post-exercise. MyoD protein expression was unchanged post-HV (Fig. 4b). In contrast, MyoD protein expression was slightly reduced compared to control levels ($P < 0.05$) at 6–24 h post-LV. MyoD protein expression was not significantly different at each time point for LV and HV.

White quadriceps skeletal muscle

Myogenin and myoD mRNA and protein expression in WQ skeletal muscle was similar to the control throughout the 6–48 h post-exercise period for LV or HV (data not shown).

Discussion

The rodent model of resistance exercise used in this study increased myogenin and myoD mRNA and myogenin protein expression in active skeletal muscle within 6–12 h post-exercise. The important novel observations of this study were that the MRF response to resistance exercise, characterized by myogenin and myoD expression, was limited to the RQ muscle group and was not proportional to the volume of resistance exercise. In fact, HV resistance exercise appeared to blunt and delay myogenin mRNA and protein expression in the RQ muscle. A similar delay in myoD mRNA expression was seen with HV resistance exercise in RQ muscle. The MRF response following LV resistance exercise indicates muscle remodeling occurred after a single bout of resistance exercise. However, when the exercise volume is doubled (HV), we show a delayed and blunted myoD and myogenin

response that may be reflective of excessive damage/injury.

This unique rodent resistance exercise model elicited a MRF response in RQ but not WQ. It is possible that the lack of MRF response in WQ following resistance exercise was due to insufficient activation of this muscle group. As indicated in Fig. 1, there are clear differences in contractile protein composition between the two muscle types with WQ muscle expressing primarily MHC IIb isoforms. Krisan et al. (2004) trained rats for 12 weeks at 75% 1RM (3 sets × 10 repetitions per session, 3 times a week) using a similar rodent resistance exercise model and reported that the majority of the metabolic adaptations occurred in the RQ rather than the WQ muscle. Additionally, the experimental rats in this study may not have attained a "true" 1RM and, therefore, performed resistance exercise less than 75% 1RM. This would explain the ability of the animals to successfully complete 20 repetitions at 75% 1RM in the HV treatment group which from a human perspective is difficult, if not impossible, to undergo. This remains a possibility since the above mentioned study (Krisan et al. 2004) did not identify muscular hypertrophy in the quadriceps muscles following training. To indirectly assess whether the RQ was recruited to a higher degree than the WQ following our exercise model, we measured ribosomal S6 kinase 1 (S6K1) phosphorylation (Thr389) which has been reported to be a key marker of skeletal muscle growth (Baar and Esser 1999). We report that S6K1 phosphorylation (Thr389) was significantly elevated in the RQ but not in the WQ (Fig. 2; $P < 0.05$) thus supporting that our exercise model primarily targets the RQ and not the WQ. Perhaps, the frequency of resistance exercise sessions may need to be 'stacked' (separated by 3 h) in order to activate the WQ as demonstrated in rats by Coffey et al. (2007). In that study, S6K1 phosphorylation was increased in the WQ following three 'stacked' exercise sessions. Nonetheless, we suspect that the overload used in our study imposed by the rodent resistance exercise model recruits primarily the red aspect of the quadriceps group.

The increased exercise volume associated with HV appears to blunt and delay the MRF response. Although not measured in the current study, it is a reasonable assumption that the novel squat exercise resulted in some damage in the hind limb muscles of the exercised rodents as well as an associated inflammatory response. Exercise-induced muscle damage is a powerful stimulator of myoD and myogenin mRNA and protein expression in human (Bamman et al. 2004; McKay et al. 2008; Yang et al. 2005) and rat (Hentzen et al. 2006; Okada et al. 2008; Peters et al. 2003) skeletal muscle; however, we propose that "excessive" muscle damage and the resultant inflammatory response may acutely impact muscle MRF gene and protein expression patterns. For instance, in C2C12 cells, Chandran et al. (2007) observed that stretch caused attenuation in the acute MRF response and was associated with an increase in tumor necrosis factor alpha. In another study, chemotoxic injury to rodent skeletal muscle (Sakuma et al. 2005) resulted in a reduction in myogenin and myoD protein levels 1–6 days after the injection. Therefore, the observed MRF response following resistance exercise in HV may reflect the competing input from a stimulatory exercise stimulus and an inhibiting stress response associated with muscle damage or injury. It is unclear if the blunted/delayed myogenic response in HV is simply an acute response to unaccustomed exercise or an early sign of "overtraining". Thus, it remains to be determined if repeated bout of exercise using HV resistance exercise would minimize the inhibitory stresses and restore the myogenic response to that of LV or further exasperate the positive muscle growth signals.

Following resistance exercise, myoD protein expression has been reported to be unchanged (Tamaki et al. 2000) in rats and unchanged (Bamman et al. 2004) or increased (Willoughby and Nelson 2002) in humans. In our study myoD protein expression was decreased slightly (LV) or remained unchanged (HV) within 48 h after resistance exercise. Whether the decline (~20%) in myoD protein expression is a physiological adaptation to this exercise volume is uncertain. A point of interest is that myoD protein did not increase following an apparently robust mRNA response (~300–400%). This may be because modulations of myoD protein expression occurred outside of our sampling times since we are essentially evaluating "snapshots" of protein expression. However, as mentioned previously, the current exercise model may not have been a suitable load to recruit the WQ muscle. From a metabolic standpoint, 100 (LV) or even 200 (HV) contractions may mimic endurance exercise rather than resistance exercise. This is supported by Kadi et al. in which a single bout of endurance exercise in humans elevated myogenin, but not myoD, protein expression in myonuclei (Kadi et al. 2004). Thus, the exercise intensity may not have been sufficient to elevate myoD mRNA to the appropriate threshold that would impact protein expression levels in the RQ. It is unknown if myoD mRNA and protein expression patterns in a fast-twitch muscle type such as the WQ behave similarly.

In conclusion, we report that a single bout of resistance exercise has the ability to activate myogenin and myoD exclusively in RQ muscle. However, the MRF response was not proportional to the exercise volume. Indeed, HV resistance exercise appeared to blunt and delay the MRF response. We suggest that the attenuated response in HV represents an interaction between the adaptive response to exercise and the cellular responses to muscle damage associated with high-tension, high-volume contraction stimuli. These data indicate that with chronic exercise training the

MRF response should have a more positive influence on the muscle adaptation to resistance exercise and that higher volumes of this type of exercise will diminish the myogenic response.

Acknowledgments We thank Nathan Bexfield, Garrett Welch, Chris Allinger, Don Reeder and Dr. Ben Yaspelkis III for their technical assistance. We are grateful to Drs. William Winder and Pat Vehrs for their review of the research. This research was funded by the Mary Lou Fulton Fund in the College of Health and Human Performance and the Douglas Smith Memorial Endowment in the Department of Exercise Sciences at Brigham Young University.

References

Baar K, Esser K (1999) Phosphorylation of p70(S6k) correlates with increased skeletal muscle mass following resistance exercise. Am J Physiol 276:C120–C127

Bamman MM, Ragan RC, Kim JS, Cross JM, Hill VJ, Tuggle SC, Allman RM (2004) Myogenic protein expression before and after resistance loading in 26- and 64-year-old men and women. J Appl Physiol 97:1329–1337

Barauna VG, Junior ML, Costa Rosa LF, Casarini DE, Krieger JE, Oliveira EM (2005) Cardiovascular adaptations in rats submitted to a resistance-training model. Clin Exp Pharmacol Physiol 32:249–254

Bolster DR, Kubica N, Crozier SJ, Williamson DL, Farrell PA, Kimball SR, Jefferson LS (2003) Immediate response of mammalian target of rapamycin (mTOR)-mediated signalling following acute resistance exercise in rat skeletal muscle. J Physiol 553:213–220

Chandran R, Knobloch TJ, Anghelina M, Agarwal S (2007) Biomechanical signals upregulate myogenic gene induction in the presence or absence of inflammation. Am J Physiol Cell Physiol 293:C267–C276

Coffey VG, Reeder DW, Lancaster GI, Yeo WK, Febbraio MA, Yaspelkis BB 3rd, Hawley JA (2007) Effect of high-frequency resistance exercise on adaptive responses in skeletal muscle. Med Sci Sports Exerc 39:2135–2144

Drummond MJ, Glynn EL, Lujan HL, Dicarlo SE, Rasmussen BB (2008) Gene and protein expression associated with protein synthesis and breakdown in paraplegic skeletal muscle. Muscle Nerve 37:505–513

Haddad F, Adams GR (2002) Selected contribution: acute cellular and molecular responses to resistance exercise. J Appl Physiol 93:394–403

Hentzen ER, Lahey M, Peters D, Mathew L, Barash IA, Friden J, Lieber RL (2006) Stress-dependent and -independent expression of the myogenic regulatory factors and the MARP genes after eccentric contractions in rats. J Physiol 570:157–167

Kadi F, Johansson F, Johansson R, Sjostrom M, Henriksson J (2004) Effects of one bout of endurance exercise on the expression of myogenin in human quadriceps muscle. Histochem Cell Biol 121:329–334

Kelly SB, Brown LE, Coburn JW, Zinder SM, Gardner LM, Nguyen D (2007) The effect of single versus multiple Setson strength. J Strength Cond Res 21:1003–1006

Kim JS, Kosek DJ, Petrella JK, Cross JM, Bamman MM (2005) Resting and load-induced levels of myogenic gene transcripts differ between older adults with demonstrable sarcopenia and young men and women. J Appl Physiol 99:2149–2158

Kraemer WJ, Adams K, Cafarelli E, Dudley GA, Dooly C, Feigenbaum MS, Fleck SJ, Franklin B, Fry AC, Hoffman JR, Newton RU, Potteiger J, Stone MH, Ratamess NA, Triplett-McBride T (2002) American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. Med Sci Sports Exerc 34:364–380

Krisan AD, Collins DE, Crain AM, Kwong CC, Singh MK, Bernard JR, Yaspelkis BB III (2004) Resistance training enhances components of the insulin signaling cascade in normal and high-fat-fed rodent skeletal muscle. J Appl Physiol 96:1691–1700

Lassar AB, Buskin JN, Lockshon D, Davis RL, Apone S, Hauschka SD, Weintraub H (1989) MyoD is a sequence-specific DNA binding protein requiring a region of myc homology to bind to the muscle creatine kinase enhancer. Cell 58:823–831

Li H, Capetanaki Y (1993) Regulation of the mouse desmin gene: transactivated by MyoD, myogenin, MRF4 and Myf5. Nucleic Acids Res 21:335–343

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25:402–408

McKay BR, O'Reilly CE, Phillips SM, Tarnopolsky MA, Parise G (2008) Co-expression of IGF-1 family members with myogenic regulatory factors following acute damaging muscle-lengthening contractions in humans. J Physiol 586:5549–5560

Moss JB, Olson EN, Schwartz RJ (1996) The myogenic regulatory factor MRF4 represses the cardiac alpha-actin promoter through a negative-acting N-terminal protein domain. J Biol Chem 271:31688–31694

Okada A, Ono Y, Nagatomi R, Kishimoto KN, Itoi E (2008) Decreased muscle atrophy F-box (MAFbx) expression in regenerating muscle after muscle-damaging exercise. Muscle Nerve 38:1246–1253

Perry RL, Rudnick MA (2000) Molecular mechanisms regulating myogenic determination and differentiation. Front Biosci 5:D750–D767

Peters D, Barash IA, Burdi M, Yuan PS, Mathew L, Friden J, Lieber RL (2003) Asynchronous functional, cellular and transcriptional changes after a bout of eccentric exercise in the rat. J Physiol 553:947–957

Raue U, Slivka D, Jemiolo B, Hollon C, Trappe S (2006) Myogenic gene expression at rest and after a bout of resistance exercise in young (18–30 year) and old (80–89 year) women. J Appl Physiol 101:53–59

Reiser PJ, Kline WO (1998) Electrophoretic separation and quantitation of cardiac myosin heavy chain isoforms in eight mammalian species. Am J Physiol 274:H1048–H1053

Rhea MR, Alvar BA, Ball SD, Burkett LN (2002) Three sets of weight training superior to 1 set with equal intensity for eliciting strength. J Strength Cond Res 16:525–529

Sakuma K, Nakao R, Aoi W, Inashima S, Fujikawa T, Hirata M, Sano M, Yasuhara M (2005) Cyclosporin A treatment upregulates Id1 and Smad3 expression and delays skeletal muscle regeneration. Acta Neuropathol (Berl) 110:269–280

Tamaki T, Uchiyama S, Nakano S (1992) A weight-lifting exercise model for inducing hypertrophy in the hindlimb muscles of rats. Med Sci Sports Exerc 24:881–886

Tamaki T, Uchiyama S, Uchiyama Y, Akatsuka A, Yoshimura S, Roy RR, Edgerton VR (2000) Limited myogenic response to a single bout of weight-lifting exercise in old rats. Am J Physiol Cell Physiol 278:C1143–C1152

Wentworth BM, Donoghue M, Engert JC, Berglund EB, Rosenthal N (1991) Paired MyoD-binding sites regulate myosin light chain gene expression. Proc Natl Acad Sci USA 88:1242–1246

Wheeler MT, Snyder EC, Patterson MN, Swoap SJ (1999) An E-box within the MHC IIB gene is bound by MyoD and is required for gene expression in fast muscle. Am J Physiol 276:C1069–C1078

Willoughby DS, Nelson MJ (2002) Myosin heavy-chain mRNA expression after a single session of heavy-resistance exercise. Med Sci Sports Exerc 34:1262–1269

Yang Y, Creer A, Jemiolo B, Trappe S (2005) Time course of myogenic and metabolic gene expression in response to acute exercise in human skeletal muscle. J Appl Physiol 98:1745–1752