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Effects of blood flow restriction on biomarkers of myogenesis in response to resistance exercise

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Abstract

We investigated the acute myogenic response to resistance exercise with and without blood-flow restriction (BFR). Six men and women (22±1 years) performed unilateral knee extensions at 40% of 1-repetition maximum with BFR applied via pressure cuff inflated to 220 mmHg or without (CNTRL). Muscle biopsies were collected 4h and 24h post-exercise. Addition of BFR increased myoD and c-Met mRNA expression relative to CNTRL. Expression of hepatocyte growth factor (HGF) protein was significantly higher following CNTRL.

Keywords

Blood flow restriction; KAATSU; skeletal muscle; resistance exercise; myogenesis; hepatocyte growth factor

Introduction

Mechanical stimuli—e.g. loading and stretching that occurs during resistance exercise (RE)—is a potent signal for skeletal muscle growth and repair. Extensive evidence suggests that hepatocyte growth factor (HGF) is a key mediator of the skeletal muscle adaptive response following mechanical stimulation. During contraction, mechanical perturbation of the sarcolemma releases HGF from its extracellular tether (Anderson 2000). Free HGF acts in an autocrine/paracrine fashion, interacting with its transmembrane receptor c-Met to initiate migration and proliferation of satellite cells, i.e. myogenic stem cells capable of fusing with and repairing damaged skeletal muscle fibers. The activity of satellite cells is determined by the temporal expression of myogenic regulatory factors (MRFs). Proliferating satellite cells generally express myoD and myf5, whereas satellite cell expression of MRF4 and myogenin indicates terminal differentiation (Perry and Rudnicki 2000). The ability of differentiated satellite cells to repair damaged skeletal muscle fibers or donate myonuclei to existing

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Conflict of interest

The authors declare that they have no conflict of interest.

muscle fibers appears to be crucial for significant skeletal muscle hypertrophy to occur (Petrella et al. 2008). As a result, satellite cell MRF expression is often used as a marker of the acute skeletal muscle myogenic response following exercise (Zanou and Gailly 2013).

Traditionally, high-load contractions have been considered necessary to induce HGF responses to exercise. However, recent evidence suggests that low-load RE performed with blood flow restriction (BFR) is effective for increasing skeletal muscle mass and strength (Slysz et al. 2015). Acute bouts of BFR exercise are known to increase skeletal muscle protein synthesis (Fry et al. 2010) while altering the net balance in transcription of several key myogenic and proteolytic genes (Manini et al. 2011). However, little is known about the effects of BFR exercise on changes in transcription and translation of key factors related to skeletal muscle growth and repair, including HGF, c-Met, myoD, and myf5. Accordingly, the purpose of this study was to determine the effect of BFR on protein and mRNA expression of these myogenic factors following low-load RE. We hypothesized that an acute bout of BFR would increase expression of these myogenic factors relative to free-flow exercise.

Methods

Participants

Six apparently healthy, sedentary adults (3 male, 3 female, 18–30yr, body mass index $<30 \text{ kg}\cdot\text{m}^{-2}$) performed two bouts of unilateral knee extension exercise (with BFR and without BFR [CNTRL]) in a randomized, cross-over design. All participants provided written informed consent using documents approved by the University of Florida (UF) Institutional Review Board. Inclusion and exclusion criteria were published previously (Larkin et al. 2012).

Vital measures and anthropometric characteristics were assessed before familiarizing participants to the experimental protocol and measuring unilateral one-repetition maximum (1RM) on a standard leg extension machine (Paramount Fitness, Los Angeles, CA). Participants were then provided a physical activity monitor (Body Media, Pittsburgh, PA) to wear for seven days to verify exercise habits.

Research Design

Participants were randomly assigned to complete resistance exercise with or without externally-applied vascular restriction. Participants were instructed to refrain from exercise for 48 h before testing. The evening before the exercise bout, participants were admitted to the Clinical Research Unit (CRU) of the UF Clinical and Translational Science Institute. Participants were fed a standard dinner consisting of $12 \text{ kcal}\cdot\text{kg}^{-1}$ with a macronutrient composition of 60% carbohydrate, 20% protein, and 20% fat and a late-night snack. Assessment began the following morning with participants in the fasted state.

Before exercising, participants underwent a blood draw and skeletal muscle biopsy. Participants then performed the appropriate exercise bout according to random assignment. Each bout consisted of 120 repetitions (10 sets of 12 repetitions) of dominant-limb knee extensions performed at 40% of 1RM with 1-minute rest periods between sets. For the BFR

condition, a 5-cm wide lower extremity pressure cuff (Kaatsu-Master Mini, Sato Sports Plaza, Tokyo, Japan) was placed around the most proximal portion of the exercised leg at an initial pressure of 30–40 mmHg (Cook et al. 2013) and inflated to a pressure of 220 mmHg.

Blood and muscle were collected 4h post-exercise, and participants were again provided a standard meal and late-night snack. Blood and muscle samples were repeated the following morning (24h post-exercise) in the fasted state before release from the CRU. After a minimum of three weeks after completion of the first experimental condition, participants returned to complete the other condition. As reported, previously female participants were tested during the mid-follicular phase of their menstrual cycle, and serum estradiol concentrations did not differ between conditions (Larkin et al. 2012).

Biochemical analyses

A percutaneous muscle biopsy was collected from the vastus lateralis as previously described (Buford et al. 2010). Samples at 4h were collected from the initial incision site with an altered needle angle, whereas samples were collected at 24h post-exercise from a new incision site approximately 5 cm from the initial incision. Muscle was aliquoted and placed into cryovials either with or without RNAlater (Ambion, Austin, TX). Cryovials were snap frozen in liquid nitrogen and stored at -80°C .

Muscle mRNA expression was determined using quantitative real-time polymerase chain reaction (Q-PCR) as previously described (Buford et al. 2014). Primer sequences were designed using the Applied Biosystems (ABI) Primer Express 3.0 software and used to construct commercially-synthesized oligonucleotide sense and antisense primers (Integrated DNA Technologies, Coralville, IA) for GADPH (NCBI Accession:J02642), MyoD (NM_002478), Myf5 (NM_005593), c-Met (NM_001127500), and eMHC (NM_002470.2). Q-PCR analysis was performed using the Power SYBR® Green PCR Master Mix (ABI, Warrington, UK), 0.2-nM primers and nuclease-free water in a 25-L reaction. Relative expression was determined using the ABI 7300 real-time PCR system with universal cycling conditions. All samples were examined in triplicate and melt curves performed to verify primer specificity. Sample mRNA expression of target genes was determined relative to the expression of glyceraldehyde-3-phosphate dehydrogenase (GADPH) as it has previously been shown to be a stable control gene for exercise studies (Jemiolo and Trappe 2004).

To quantify protein, 50µg of whole muscle protein extract was separated by gel electrophoresis as previously described (Larkin et al. 2012). Ponceau S staining was used as a loading control (Manini et al. 2011). Blots were probed with the primary antibodies for HGF, MyoD, Myf5, c-Met and eMHC at a dilution of 1:200. Protein was visualized with a DuoLux enhanced chemiluminescence kit (Vectro Laboratories, Burlingame, CA) and detected using a ChemiDoc XRS imager (BioRad, Hercules, CA). Band density was quantified using Image Lab software from BioRad.

Statistical analysis

Skeletal muscle protein data were analyzed by repeated-measures ANCOVA adjusted for baseline protein expression with contrasts applied and are presented as means $\pm$ SE. Because mRNA data were non-normally distributed, fold changes in gene expression were compared

using the nonparametric Wilcoxon test and are presented as medians (Q1, Q3). Differences between conditions were considered significant at $P < 0.05$ (two tailed). Data were analyzed using SPSS 22 for Windows (IBM, Chicago, IL).

Results

Participants had a mean age of 22 ± 1 yr, body mass of 72 ± 7 kg, body mass index of 23.7 ± 1.4 , ABI of 1.05 ± 0.03 , steps per day of 7073 ± 619 , and 1-RM of 44 ± 7 kg. Participants completed all sets and repetitions during each exercise session. mRNA expression of myoD was increased relative to CNTRL 4h post-exercise (fold change 1.69 [1.4, 1.8] vs. 1.14 [1.0, 1.4], $p=0.03$) and c-Met mRNA expression was significantly decreased relative to BFR at 24h post-exercise (fold change 0.85 [0.7, 1.0] vs. 0.52 [0.3, 0.7], $p=0.03$) as shown in Figure 1. Expression of HGF protein was significantly lower following BFR relative to CNTRL (time*condition interaction $p=0.028$) (Figure 2). Relative to CNTRL, the addition of BFR did not alter skeletal muscle protein expression of myf5 (time*condition interaction $p=0.550$), c-Met ($p=0.580$) or myoD ($p=0.179$).

Discussion

Numerous studies suggest that BFR exercise may be a viable alternative to high-load exercise for increasing skeletal muscle size and strength. Yet despite a recent expansion in research in this area, the mechanisms underlying BFR-mediated skeletal muscle adaptations remain poorly described. We previously reported that the addition of BFR to low-load exercise upregulated post-exercise gene expression of several markers of angiogenesis, including VEGF and nNOS (Larkin et al. 2012). The present study expands upon our previous report by testing the hypothesis that BFR potentiates post-exercise skeletal muscle expression of molecules involved in early myogenic signaling. The primary findings from this study indicate that the addition of BFR to low-load resistance exercise increased myogenic gene expression but decreased HGF protein expression in the first 24 hours following exercise.

A key finding of this study is the reduced HGF protein response following BFR relative to CNTRL. This finding is perhaps surprising as HGF is one of the most potent known stimuli responsible for inducing skeletal muscle satellite cell activation (Tatsumi 2010), and previous studies indicate that BFR exercise increases satellite cell number (Nielsen et al. 2012). Furthermore, HGF also plays a role in angiogenesis (Nagasaka et al. 2006) which is enhanced following BFR (Larkin et al. 2012). It is possible that the mildly ischemic conditions experienced by the active muscles during BFR exercise contributed to a reduced HGF response. Previously, cultured rat skeletal muscle demonstrated a reduced HGF response under hypoxic conditions compared to normoxia (Flann et al. 2014). As a result, it is possible that BFR may reduce or delay HGF expression secondary to decreased tissue oxygenation.

We also observed a significant increase in myoD mRNA expression at 4h post-exercise following BFR relative to CNTRL, while myf5 mRNA expression was similar between groups at 4h and 24h post-exercise. Previous studies on early MRF expression in response to

acute BFR exercise are equivocal, as Drummond et al. (2008) reported an increase in myoD mRNA expression 3h post-exercise following both BFR and free-flow exercise, while Manini et al. (2011) reported no change in mRNA expression in either condition 8h post-exercise. It is possible that differences between exercise protocols partially explain the differential results between these studies, as the amplitude and the time course of the increase in gene transcription of myoD and myf5 appears to depend on the volume and intensity of exercise (Yang et al. 2005). Our exercise protocol utilized a higher load (40% of 1RM vs. 20% of 1RM for Manini et al. and Drummond et al.) and higher volume (120 repetitions vs. 75 repetitions) exercise protocol. It is also possible that differences between the timing and macronutrient profiles of the meals provided may have influenced these findings though most studies to date on pre-exercise feeding show little or no effect on post-exercise gene expression (Harber et al. 2010, Roberts et al. 2010). Thus substantial evidence would be needed to substantiate this latter hypothesis.

The study has several strengths, including a randomized, cross-over design and the use of a clinical research center to control for potentially confounding factors such as differences in diet and physical activity. Limitations include a small sample size, limited time-frame of follow-up, and evaluation of a limited number of myogenic markers. Other limitations include use of a single cuff pressure for all participants and an exercise protocol not typically used in chronic training. Still, this study adds important information regarding the influence of BFR exercise on HGF-related muscle signaling.

In conclusion, BFR exercise increased myogenic gene expression but decreased HGF protein expression in the first 24 hours following exercise relative to CNTRL. Questions remain about changes in these and other biomarkers of myogenesis for periods longer than 24h post-exercise and in response to longer-term training. Future studies are needed to determine optimal loading and dosage of blood flow restricted exercise to maximize gains in skeletal muscle size and strength.

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Figure 1. Skeletal muscle mRNA expression of myogenic genes after acute low-intensity exercise. Changes in mRNA content are expressed as a fold change from baseline relative to GAPDH at 4h (a) and 24h (b) post exercise. Gray boxes indicate the effects of low intensity exercise (CNTRL), whereas white boxes indicate the effects of adding blood flow restriction (BFR). Boxes indicate medians and quartiles. Bars indicate minimum and maximum. *Significantly different from CNTRL ($p < 0.05$)

Figure 2.

Skeletal muscle expression of hepatocyte growth factor (HGF, a), c-Met (b), myf5 (c), and myoD (d) at baseline, 4h, and 24h after low-load exercise either with blood flow restriction (BFR) or without BFR (CNTRL). Means are adjusted for baseline level of expression. Boxes indicate means and bars indicate SE. Representative blots for each protein and Ponceau staining are provided below. *Indicates significant difference between BFR and CNTRL (time*condition interaction p 0.05)