EFFECTS OF CONCENTRIC AND ECCENTRIC MUSCLE ACTIONS ON SERUM MYOSTATIN AND FOLLISTATIN-LIKE RELATED GENE LEVELS

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ABSTRACT
The present study determined the effects of concentric and eccentric muscle actions on the contents of serum myostatin and follistatin-like related gene (FLRG). Eight untrained males performed one exercise bout with each leg, separated by three weeks. One bout consisted of 7 sets of 10 repetitions of eccentric muscle actions of the knee extensors at 150% of the concentric 1-RM while the other bout consisted of 7 sets of 10 repetitions of concentric muscle actions at 75% 1-RM. The legs used and the bouts performed were randomized. Five days prior to each exercise bout, baseline measurements were taken for muscle strength. For both bouts, a venous blood sample was obtained immediately prior to exercise and again at 6, 24, and 48 hr post-exercise. Data were analyzed with 2 X 4 (bout x test) ANOVA (p < 0.05). Increases in serum myostatin and FLRG occurred with each exercise bout and, excluding 48 hr post-exercise, were significantly correlated to one another (p < 0.05). After eccentric exercise, peak increases of 68% and 50% (p < 0.05) were observed for myostatin and FLRG, respectively. Similar increases of 54% and 44% (p < 0.05) were observed after concentric muscle actions. There was no significant difference in expression of myostatin or FLRG as a function of muscle action type. Our results suggest that a single bout of exercise with either eccentric or concentric muscle actions appear to elicit a similar increase in serum myostatin and FLRG. Therefore, the type of muscle action may not be as much a mitigating factor for increasing serum myostatin and FLRG rather than the muscle action per se.

KEY WORDS: Muscle injury, cytokine, muscle proteolysis, resistance exercise.

INTRODUCTION
Compared to concentric muscle actions, muscle actions involving a significant eccentric (forced-lengthening) component are known to produce a greater degree of muscle injury and force decrement, apparently due to the fact that fewer motor units are recruited during the eccentric phase of muscle action. There are data showing eccentric muscle actions to have 40% less EMG activity compared to concentric muscle actions of the same force (Gibala et al., 1995). This indicates that during an eccentric muscle action a smaller cross-sectional area takes on an equivalent load as that which was handled in the concentric muscle action (Enoka, 1996). We have recently shown that eccentric muscle actions of the knee extensors resulted in greater reductions in dynamic muscle strength while concomitantly producing greater increases in serum cortisol and markers of muscle injury, when compared to concentric muscle actions (Willoughby et al., 2003e). We have also shown eccentric muscle actions to up-regulate the expression of the stress-related genes heat shock protein-72 and several involved in the ATP-dependent ubiquitin proteolytic pathway (ubiquitin, ubiquitin conjugating enzyme, 20S proteasome) (Willoughby et al., 2003b).

The cytokine myostatin (GDF-8) is a catabolic regulator of skeletal muscle via proteolytic and atrophic mechanisms. Myostatin expression appears
responsive to elevated glucocorticoids (Ma et al. 2001; 2003) and muscle immobilization/inactivity (Carlson et al., 1999; Wehling et al., 2000; Willoughby et al., 2003c). Consequently, muscle immobilization increases the expression of myostatin whereas subsequent muscle re-loading results in decreases in myostatin expression (Wehling et al., 2000). Furthermore, inactivity-induced myostatin expression appears to primarily occur in fast-twitch Type 2A and 2B fibers (Carlson et al., 1999).

As of late, research has begun to explore the expression profiles of myostatin in regard to resistance exercise. Employing dynamic muscle actions utilizing a pneumatic lower-body resistance exercise device in humans, Roth et al. (2003) showed decreases in myostatin mRNA expression after 8 wks of lower-body resistance training. However, in rodents Peters et al. (2003) showed increases in myostatin mRNA expression after a single exercise bout involving only eccentric muscle actions. More recently it has been shown that 12 wks of dynamic lower-body heavy resistance training with primarily free-weight training exercises increased skeletal muscle myostatin mRNA and protein, along with serum myostatin and follistatin-like related gene [(FRLG) inhibits myostatin binding with activin IIb receptor] (Willoughby, 2004). Regarding the disparity of results between our data and that of Roth et al. (2003) and Peters et al. (2003), and based on the differences in skeletal muscle injury and serum cortisol that we have also shown to occur with concentric and eccentric actions (Willoughby et al., 2003e), we reasoned that myostatin expression may be preferentially induced in response to only eccentric muscle actions.

Therefore, using serum samples collected from our previous study (Willoughby et al., 2003e) the purpose of the present study was to determine the effects of concentric and eccentric muscle actions of the knee extensors on serum levels of myostatin and FLRG. In our previous study we demonstrated that eccentric muscle actions induce more muscle injury and a greater cortisol response than concentric muscle actions. Therefore, the purpose of the present study was to test our hypothesis that eccentric muscle actions would also initiate a greater serum myostatin and FLRG response than concentric muscle actions.

**METHODS**

**Experimental Design**

In the present study, we used remaining blood samples from our previous study (Willoughby et al., 2003e) in which subjects signed university-approved informed consent documents, approval was granted by the Institutional Review Board for Human Subjects, and all experimental procedures conformed to the ethical consideration of the Helsinki Code. However, the specific, more detailed methods and procedures for this study are outlined previously (Willoughby et al., 2003e).

**Subjects**

Eight untrained, recreationally active males were recruited to participate in the study. The subjects were untrained from the standpoint that they had not engaged in consistent weight training for 3 mos. prior to the study; however, all were recreationally active. The eight subjects had a mean age of 21 ± 1 years, height of 1.85 ± 0.05 m, and body mass of 78 ± 8 kg. Before participating each subject completed a medical history questionnaire, was informed of the experimental protocol, and signed a university-approved informed consent form. Subjects with contraindications to exercise as indicated by the American College of Sports Medicine (ACSM, 2000) were not allowed to participate.

**Muscle Strength**

Each subject underwent strength testing to determine the concentric strength of the knee extensors using the standard trial-and-error method of assessing the one repetition maximum (1-RM) on a leg extension/leg curl machine (Universal, Cedar Rapids, IA). Strength tests were performed 5 days prior to and at 6, 24, and 48 hr after each exercise bout. Initial strength tests and each exercise bout were performed on the same apparatus. In order to prevent 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 all 1-RM testing sessions throughout the study (Willoughby, 2004; Willoughby et al., 2003a). All subjects were able to obtain their 1-RM within 5 trials and the average (±SD) number of trials for all subjects over the eight 1-RM testing sessions was 4.21 (±0.75).

**Blood Sampling**

Venous blood samples consisted of approximately 10 mL of blood drawn from the antecubital vein using a vacutainer apparatus immediately prior to each bout and at 6, 24, and 48 hr following each bout. Blood was centrifuged for 10 minutes and serum was extracted and then stored at a temperature of -20°C.

**Exercise Bouts**

Each subject underwent two separate muscle injury-inducing exercise bouts. One bout involved concentric muscle actions only of the knee extensors and the other bout involved eccentric muscle actions only of the knee extensors. Each exercise bout was
separated by three weeks and alternated the leg and type of exercise to avoid the repeated bout effect. For example, if the first exercise session incorporated the left leg and eccentric muscle actions then the subsequent session 3 weeks later utilized the right leg and concentric muscle actions. The type of muscle actions and leg exercised were both randomized to control for order effects. Each exercise bout employed 7 sets of 10 repetitions. However, the eccentric exercise bout involved eccentric muscle actions of the knee extensors using 150% of the concentric 1-RM (Willoughby et al., 2003a; 2003d). In an attempt to standardize for the amount of repetitions across bouts, based on the repetition continuum, a relative intensity of 75% was chosen for the concentric bout. This is based on the premise that 75% 1-RM corresponds to a 10-RM (Heyward, 1998). For the eccentric exercise bout, study investigators raised the weight prior to each repetition, whereas in the concentric exercise bout, study investigators lowered the weight after each repetition. Both bouts began with two warm-up sets of 10 repetitions at 50% of each subject’s 1-RM. For both exercise bouts, each repetition was approximately 2-3 seconds in duration, each repetition was separated by a 15-sec rest interval, and each set was separated by a 3-min rest interval.

Serum Protein Quantitation
The binding affinity of the anti-myostatin and anti-FLRG antibodies with the serum samples was qualitatively verified with dot blotting (Figures 1b and 2b) using an immuno-blotting protocol (Immuno-Blot Colorimetric Assay Kit, Bio-Rad, Hercules, CA) and our previous guidelines (Willoughby and Taylor, 2004). Briefly, equal aliquots of serum (1.0 µl) were blotted onto nitrocellulose membranes, blocked with non-fat dry milk in TBS buffer, incubated with human-specific polyclonal antibodies (diluted to 5µg·ml⁻¹) against mature myostatin raised against a peptide mapping at the amino terminus of GDF-8 (sc-6885) and FLRG raised against a peptide mapping within an internal region of FLRG (sc-21302) (Santa Cruz Biotechnology, Santa Cruz, CA) and our previous guidelines (Willoughby and Taylor, 2004). Briefly, equal aliquots of serum (1.0 µl) were blotted onto nitrocellulose membranes, blocked with non-fat dry milk in TBS buffer, incubated with human-specific polyclonal antibodies (diluted to 5µg·ml⁻¹) against mature myostatin raised against a peptide mapping at the amino terminus of GDF-8 (sc-6885) and FLRG raised against a peptide mapping within an internal region of FLRG (sc-21302) (Santa Cruz Biotech, Santa Cruz, CA), incubated with a secondary IgG antibody conjugated to biotinylated streptavidin alkaline phosphatase, and the color developed with BCIP (5 bromo-chloro-3-indolyl phosphate) and NBT (nitro blue tetrazolium). The blot was then illuminated with white light transillumination (Chemi-Doc, Bio-Rad, Hercules, CA).

Based on previous procedures (Willoughby, 2004), the serum myostatin and FLRG concentrations were then quantified in duplicate and the average concentrations reported using an ELISA (Figures 1a and 2a). This incorporated the same human-specific polyclonal myostatin and FLRG antibodies used for immuno-blotting as primary/capture antibodies. The secondary antibody immunoglobulin-G (IgG) was conjugated to the enzyme horseradish peroxidase (ICN Biomedical, Aurora, OH). A standard curve was generated for myostatin (r² = 0.93, p = 0.004) using a specific control peptide for myostatin (sc-6885-P, Santa Cruz Biotechnology, Santa Cruz, CA) and FLRG (r² = 0.91, p = 0.006) using a specific control peptide for FLRG (sc-21302-P, Santa Cruz Biotechnology, Santa Cruz, CA). Serum myostatin and FLRG concentrations were determined at an optical density of 450 nm with a microplate reader (Bio-Rad, Hercules, CA) and expressed relative to changes in plasma volume (Dill and Costill, 1974). An intra-assay coefficient of variation was determined for each duplicate for all subjects and resulted in coefficient of 3% and 4% for myostatin and FLRG, respectively.

Statistical Analysis
Statistical analyses were performed by utilizing separate 2 x 4 [Bout (eccentric, concentric) x Test (pre-exercise and 6, 24, 48 hr post-exercise)] factorial analyses of variance (ANOVA) with repeated measures for each criterion variable. Significant between-group differences were determined using the Student Neuman-Keuls Post Hoc Test. Bivariate correlations were performed between serum myostatin, FLRG, and the cortisol data from our previous study (Willoughby et al., 2003e) using the Pearson Product Moment Correlation Coefficient. All statistical procedures were performed using SPSS 11.0 software and a probability level of ≤ 0.05 was adopted throughout.

RESULTS

Serum Myostatin Content
Neither the eccentric or concentric exercise bouts were significantly different from one another for serum myostatin (p > 0.05); however, for both types of muscle actions it was shown that the increases in myostatin that occurred at 24 hr post-exercise were significantly greater (p = 0.021) than baseline and those that occurred at 6 and 48 hr post-exercise (Figure 1a).

Serum FRLG Content
For serum FRLG results showed the eccentric and concentric muscle actions to not be significantly different from one another (p > 0.05); however, for both types of muscle actions it was shown that the
Figure 1. (A) From ELISA, a quantitative representation of the means (±SD) for the levels of serum myostatin prior to and 6-hr, 24-hr, and 48-hr after eccentric (ECC) and concentric (CON) muscle contractions. * significantly different from pre-exercise value, † significantly different from 6-hr value (p < 0.05). (B) From immuno-blotting, a qualitative representation of serum myostatin and anti-myostatin antibody reactivity in one participant after prior to exercise and 6-hr, 24-hr, and 48-hr after ECC and CON contractions.

Increases in FRLG that occurred at 24 hr post-exercise were significantly greater (p < 0.031) than baseline and those that occurred at 6 and 48 hr post-exercise (Figure 2a).

Correlations Between Myostatin, FRLG, and Cortisol

The levels of serum cortisol associated with the

Figure 2. (A) From ELISA, a quantitative representation of the means (±SD) for the levels of serum FLRG prior to and 6-hr, 24-hr, and 48-hr after eccentric (ECC) and concentric (CON) muscle contractions. * significantly different from pre-exercise value, † significantly different from 6-hr value (p < 0.05). (B) From immuno-blotting, a qualitative representation of serum FLRG and anti-FRLG antibody reactivity in one participant after prior to exercise and 6-hr, 24-hr, and 48-hr after ECC and CON contractions.
Table 1. Mean (±SD) data for selected dependent variables in response to concentric and eccentric contractions for the three times of recovery. Adapted from Willoughby et al. (2003e). Reprinted with permission from the Journal of Exercise Physiology online.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pre-Exercise</th>
<th>6 hr</th>
<th>24 hr</th>
<th>48 hr</th>
<th>Peak % Change</th>
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<tr>
<td><strong>Strength (kg)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CON</td>
<td>44.4 (6.8)</td>
<td>41.6 (7.9)</td>
<td>39.1 (4.9)</td>
<td>42.7 (5.2)</td>
<td>-11.7</td>
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<tr>
<td>ECC</td>
<td>43.2 (7.0)</td>
<td>40.5 (6.6)</td>
<td>30.0 (5.4) †</td>
<td>40.6 (4.2)</td>
<td>-30.2</td>
</tr>
<tr>
<td><strong>SORE (Bout x Test, p &lt; 0.05)</strong></td>
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<tr>
<td>CON</td>
<td>2.1 (1.7)</td>
<td>2.4 (1.0)</td>
<td>5.7 (9) †</td>
<td>2.3 (7)</td>
<td>173.1</td>
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<tr>
<td>ECC</td>
<td>2.0 (1.1)</td>
<td>3.6 (2.1)</td>
<td>8.3 (1.1) †</td>
<td>3.7 (9) ‡</td>
<td>306.8</td>
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<tr>
<td><strong>sTnI (ng·ml⁻¹)</strong></td>
<td></td>
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</tr>
<tr>
<td>CON</td>
<td>69.2 (25.0)</td>
<td>78.3 (38.2)</td>
<td>91.0 (42.1) †</td>
<td>82.4 (42.5)</td>
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<td>ECC</td>
<td>71.3 (26.5)</td>
<td>89.6 (43.2)</td>
<td>128.5 (40.4) †</td>
<td>98.3 (34.3) ‡</td>
<td>80.1</td>
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<tr>
<td><strong>CK (U/ml)</strong></td>
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<tr>
<td>CON</td>
<td>93.0 (30.0)</td>
<td>152.6 (46.4)</td>
<td>226.5 (25.9) †</td>
<td>138.1 (21.2)</td>
<td>143.4</td>
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<tr>
<td>ECC</td>
<td>104.6 (26.8)</td>
<td>248.7 (65.5) *</td>
<td>342.8 (65.5) †</td>
<td>198.0 (61.2) ‡</td>
<td>227.7</td>
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<tr>
<td><strong>CORT (ug·dl⁻¹)</strong></td>
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<tr>
<td>CON</td>
<td>13.2 (1.8)</td>
<td>15.0 (2.4)</td>
<td>17.2 (2.9)</td>
<td>15.3 (2.8)</td>
<td>30.1</td>
</tr>
<tr>
<td>ECC</td>
<td>13.1 (1.9)</td>
<td>17.6 (1.3)</td>
<td>21.5 (3.2)</td>
<td>16.4 (2.8)</td>
<td>63.4</td>
</tr>
</tbody>
</table>

Abbreviations: CON = concentric, ECC = eccentric.
* significantly (p < 0.05) different at 6 hr post-exercise.
† significantly (p < 0.05) different at 24 hr post-exercise.
‡ significantly (p < 0.05) different at 48 hr post-exercise.

Concentric and eccentric exercise bouts were not shown to be correlated to serum myostatin or FLRG at any of the time points measured (p > 0.05). However, for the concentric exercise bout, serum myostatin and FLRG were shown to be significantly correlated at pre-exercise (r = 0.873, p = 0.046), 6-hr post-exercise (r = 0.918, p = 0.028), and 24-hr post-exercise (r = 0.871, p = 0.047). For the eccentric exercise bout, serum myostatin and FLRG were also shown to be significantly correlated at pre-exercise (r = 0.892, p = 0.042), 6-hr post-exercise (r = 0.893, p = 0.042), and 24-hr post-exercise (r = 0.903, p = 0.036).

DISCUSSION

Selected muscle injury markers from our previous study (Willoughby et al., 2003e) can be seen in Table 1 (reprinted with permission from the Journal of Exercise Physiology online). In this study, for eccentric muscle actions we observed significant decrements in dynamic muscle strength after eccentric muscle actions. We also observed significantly greater increases in serum cortisol and concentration of muscle injury markers creatine kinase and skeletal muscle troponin-I, as well as perceived soreness when compared to concentric muscle actions. Therefore, it is apparent that eccentric muscle actions resulted in a greater magnitude of muscle injury than concentric muscle actions.

The present results suggest that a single bout of exercise with either eccentric or concentric muscle actions appears to elicit a similar increase in serum myostatin and FLRG. Herein we show both serum myostatin and FLRG to increase after each exercise bout. For the eccentric bout, peak increases of 68% and 50%, respectively, are shown for myostatin and FLRG. For the concentric bout, respective peak increases of 54% and 44% for myostatin and FLRG are demonstrated. Furthermore, for both types of muscle actions our present results demonstrate that myostatin and FLRG are positively correlated to one another prior to each exercise bout and also at the 6-hr and 24-hr post exercise time points (p < 0.05). This suggests that the increases in serum FLRG after a single exercise bout may not be necessarily contingent upon the type of muscle actions but rather the increase in serum myostatin that occurs with muscle contractions associated with resistance exercise.

Both serum myostatin and FLRG increase after 12 wks of dynamic weight training in which muscle strength and size and myofibrillar protein content were significantly elevated, and the body likely in primarily an anabolic state (Willoughby, 2004). Since serum myostatin is inhibited from binding to the activin IIb receptor by FLRG (Hill et al., 2002), FLRG likely plays a role in reducing myostatin signaling within skeletal muscle. Therefore, based on our present and previous results...
We submit that in young, apparently healthy males participating in resistance exercise the increases in serum FLRG that accompany increased serum myostatin may serve to inhibit myostatin signaling and muscle catabolism that could conceivably accompany heavy resistance exercise.

In addition to serum myostatin, it has also been shown that 12 wks of dynamic weight training resulted in significant elevations in serum cortisol after selected training sessions (Willoughby, 2004). Since the regulatory region within the promoter of the myostatin gene contains enhancers responsive to glucocorticoids (Ma et al., 2001), the expression of myostatin mRNA in adult skeletal muscle fibers may operate by way of a glucocorticoid receptor mediated mechanism to induce muscle proteolysis (Ma et al., 2003). Studies in which serum glucocorticoids were significantly elevated from such extenuating conditions as glucocorticoid infusion (Ma et al., 2001; 2003) and sepsis and thermal injury (Lang et al., 2001) have shown marked increases in myostatin expression along with subsequent muscle proteolysis; however, in the present study elevations in serum cortisol were within the normal physiologic range and only occurred for short time periods. Increases in serum cortisol are associated with high-intensity exercise training (Borst et al., 2002) and are also known to up-regulate the glucocorticoid receptor (Czerwinski and Hickson, 1990). We have previously shown eccentric muscle actions to result in significant increases in serum cortisol (Willoughby et al., 2003d; 2003e) and increased expression of the glucocorticoid receptor (Willoughby et al., 2003d); although, in the present study the levels of serum myostatin does not appear to be preferentially affected by the cortisol response that occurred for either the eccentric or concentric exercise bout. Furthermore, the myostatin responses associated with both types of muscle actions were not significantly correlated (p > 0.05) to serum cortisol levels. Therefore, it is plausible that the cortisol response associated with either a single eccentric or concentric exercise bout may not be a primary factor responsible for instigating the observed increases in serum myostatin.

However, fast-twitch (Type II) muscle fibers have been shown to be more susceptible to eccentric contraction-induced muscle injury (Vijayan et al., 2001), and that myostatin is primarilly expressed in Type II muscle fibers (Carlson et al., 1999). Incidentally, the human quadrecips femoris contains a high percentage of Type II muscle fibers, with the vastus lateralis containing approximately 57% Type II muscle fibers (Hakkinen et al., 2001). Therefore, it is reasonable to assume that the observed increase is serum myostatin may be more dependent on fiber type than based on the type of muscle action and/or increases in serum cortisol.

CONCLUSIONS

In light of our previous study (Willoughby et al., 2003e) in which we concluded that eccentric muscle actions have the capability to produce a greater severity of muscle injury and decrement in muscle strength than concentric muscle actions, our present results suggest that a single bout of exercise with either eccentric or concentric muscle actions appear to elicit a similar increase in serum myostatin and FLRG. Therefore, we conclude that the type of muscle actions may not be as much a mitigating factor for increasing serum myostatin and FLRG rather than simply the muscle action, per se, that is associated with resistance exercise.

REFERENCES


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KEY POINTS

- Eccentric muscle actions do not preferentially increase serum myostatin.
- Increases in serum myostatin in response to eccentric muscle actions are associated with increase in serum FLRG.
- Increases in serum myostatin and FLRG in response to eccentric muscle actions are not correlated to serum cortisol.

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