ESTRADIOL IN FEMALES MAY NEGATE SKELETAL MUSCLE MYOSTATIN MRNA EXPRESSION AND SERUM MYOSTATIN PROPEPTIDE LEVELS AFTER ECCENTRIC MUSCLE CONTRACTIONS

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ABSTRACT

Eccentric contractions produce a significant degree of inflammation and muscle injury that may increase the expression of myostatin. Due to its anti-oxidant and anti-flammatory effects, circulating 17-β estradiol (E2) may attenuate myostatin expression. Eight males and eight females performed 7 sets of 10 reps of eccentric contractions of the knee extensors at 150% 1-RM. Each female performed the eccentric exercise bout on a day that fell within her mid-luteal phase (d 21-23 of her 28-d cycle). Blood and muscle samples were obtained before and 6 and 24 h after exercise, while additional blood samples were obtained at 48 and 72 h after exercise. Serum E2 and myostatin LAP/propeptide (LAP/pro) levels were determined with ELISA, and myostatin mRNA expression determined using RT-PCR. Data were analyzed with two-way ANOVA and bivariate correlations ($p < 0.05$). Females had greater levels of serum E2 throughout the 72-h sampling period ($p < 0.05$). While males had greater body mass and fat-free mass, neither was correlated to the pre-exercise levels of myostatin mRNA and LAP/pro for either gender ($p > 0.05$). Compared to pre-exercise, males had significant increases ($p < 0.05$) in LAP/propetide and mRNA of 78% and 28%, respectively, at 24 h post-exercise, whereas females underwent respective decreases of 10% and 21%. E2 and LAP/propeptide were correlated at 6 h ($r = -0.804$, $p = 0.016$) and 24 h post-exercise ($r = -0.841$, $p = 0.009$) in males, whereas in females E2 levels were correlated to myostatin mRNA at 6 h ($r = 0.739$, $p = 0.036$) and 24 h ($r = 0.813$, $p = 0.014$) post-exercise and LAP/propeptide at 6 h ($r = 0.713$, $p = 0.047$) and 24 h ($r = 0.735$, $p = 0.038$). In females, myostatin mRNA expression and serum LAP/propeptide levels do not appear to be significantly up-regulated following eccentric exercise, and may be due to higher levels of circulating E2.

KEY WORDS: Estrogen, muscle damage, eccentric contractions, myostatin.

INTRODUCTION

Compared to concentric contractions, eccentric contractions are known to produce a greater degree of mechanical stress injury due to the fact that fewer motor units are recruited. As a result, eccentric contractions involve a smaller active cross-sectional for an equivalent load as with a concentric

contraction (Enoka, 1996). Myostatin (GDF-8) is a stress-responsive and load-sensitive (Carlson et al., 1999) cytokine that is a catabolic regulator of skeletal muscle by activating proteolytic mechanisms, and whose expression in rodents appears to be elevated in response to eccentric muscle contractions. A single bout of eccentric muscle contractions in rodents has been shown to increase myostatin mRNA expression (Peters et al., 2003). However, published studies on the effects of eccentric exercise on myostatin mRNA expression in humans appear to be non-existent. Although the exact mechanisms for such an increase are not well-known, indirect evidence suggests that it may be due to the consequences associated with exercise-induced muscle injury such as oxidative stress (Sacheck et al., 2003) and glucocorticoid receptor signaling (Ma et al., 2003; Willoughby et al., 2003).

Myostatin is a member of the transforming growth factor-β (TGF-β) super-family that is expressed in skeletal muscle and then released into circulation where the release of its propeptide up-regulates downstream response genes involved in muscle proteolysis. Myostatin is produced as a precursor protein that contains a signal sequence, an N-terminal propeptide domain, and a C-terminal domain that is the active ligand for receptor binding. Proteolytic processing between the N-terminal propeptide domain and the C-terminal domain releases mature myostatin. However, the propeptide and mature domains remain non-covalently associated after cleavage, resulting in a latent complex known as latency associated peptide (LAP). Approximately 70% of serum myostatin is bound by its propeptide and inhibited from receptor binding (Hill et al., 2002). At the site of signaling, however, the propeptide moiety is cleaved by serine proteases such as plasmin and cathepsin-D (Hill et al., 2002) and metalloproteinases such as BMP-1 (Wolfman et al., 2003), thereby resulting in active myostatin that can bind to the activin IIb receptor and up-regulate proteolytic signaling cascades in muscle (Lee et al., 2005). However, the exact mechanisms in which myostatin latency is induced in response to exercise are unkown.

There is evidence suggesting that females may be less susceptible to the catabolic consequences of eccentric exercise (Carter et al., 2001), and that 17-β estradiol (E2) may give females a protective effect against inflammation and other consequences of exercise-induced muscle injury (Kendall and Eston, 2002). Evidence seems to suggest that, due to the anti-oxidant properties of E2, females have a higher level of anti-oxidant enzyme expression and an improved adaptive response to an altered cellular redox state (Vina et al., 2006). Although the exact

mechanism for this E2-induced attenuation in inflammation and muscle injury in females is unknown, it could possibly be based on the premise that E2-mediated activation has been shown to suppress TGF-β signaling (Mize et al., 2003).

Alternatively, it has been previously shown that sexual dimorphism exists for processed myostatin whereby male rodents had less skeletal muscle levels of myostatin simply by virtue of their larger body and muscle mass (McMahon et al., 2003). However, in humans males have been shown to express greater amounts of myostatin mRNA despite greater amounts of body mass and fat-free mass compared to females compared to females (Kim et al., 2005).

Nonetheless, there appears to be no data in humans regarding the effects of eccentric muscle contractions on myostatin mRNA expression and serum myostatin propeptide levels. Therefore, the purpose of this study was two-fold and was to determine if: 1) body composition in both genders and circulating E2 levels in females were correlated to the levels of serum myostatin propeptide and skeletal muscle myostatin mRNA expression and 2) eccentric exercise had any affect on myostatin gene expression and serum myostatin LAP and propeptide levels in males and females.

METHODS

Participants

Table 1 presents body composition data for the 16 participants at the onset of the study. Specifically, eight males (20.6 ± 1.5 yrs, 1.75 ± 0.11 m, 82.2 ± 12.3 kg) and eight females (21.5 ± 3.2 yrs, 1.60 ± 0.04 m, 57.7 ± 8.1 kg) who were physically active, but untrained (no consistent, structured weight training six months prior to beginning the study) participated in the study. Participants with contraindications to exercise as outlined by the American College of Sports Medicine (2000) were not allowed to participate. All eligible participants signed university-approved informed consent documents and approval was granted by the Institutional Review Board for Human Subjects. Additionally, all experimental procedures involved in the study conformed to the ethical considerations of the Helsinki Code.

Dynamic strength testing

Five days prior to the eccentric exercise bout, maximum dynamic knee extensor strength of the dominant leg was assessed. Prior to testing, each participant underwent a warm-up consisting of a 10-min ride on a bicycle ergometer (Monark 828E, Varberg, Sweden) at a pedal rate of 60 rpm and with

1 kg of flywheel resistance. Maximum dynamic strength using an isotonic knee extension machine (Universal, Cedar Rapids, IA) was assessed using the one-repetition maximum (1-RM) protocol employing a trial-and-error method as previously described (Willoughby et al., 2003). However, 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 5 trials was set for all 1-RM testing sessions throughout the study (Willoughby et al., 2003). All participants were able to obtain their 1-RM within 5 trials and the average (±SD) number of trials for all subjects was 3.25 (±0.58). At the completion of the 1-RM testing session, participants were instructed to continue ingesting a normal mixed diet and to refrain from strenuous physical exercise for the 72 h prior to the eccentric exercise bout.

Body composition testing
Total body mass, percent body fat, fat mass, and fat-free mass were determined immediately prior to the eccentric exercise session. Total body mass (kg) was determined on a standard dual beam balance scale (Detecto, Terre Haute, IN, USA). Percent body fat was determined using hydrostatic weighing. Test-retest reliability of performing these assessments on participants in our laboratory has yielded low mean coefficients of variation and high reliability for the determination of percent body fat (1.86% intraclass, r = 0.93).

Eccentric exercise bout
Female participants were age-matched with a male and both performed the exercise bout on the same day that corresponded to the point at which each female was in the mid-luteal phase of her 28-d menstrual cycle (d 21-23) reflecting highest E2 levels (Carter et al., 2001). Participants reported to the laboratory for the eccentric exercise bout after an 8-h fast and underwent a warm-up consisting of a 10-min ride on a bicycle ergometer (Monark 828E, Varberg, Sweden) at a pedal rate of 60 rpm and with 1 kg of flywheel resistance and one set of 10 repetitions (reps) at 50% 1-RM of the dynamic knee extension exercise. For the exercise bout, based on previous guidelines (Willoughby et al., 2003), each participant then performed 7 sets of 10 reps at 150% of the 1-RM employing eccentric (forced-lengthening) contractions of the dominant knee extensors. Prior to each rep, participants had to hold their knees extended at an angle of 2.62 rad, when study investigators suddenly released 150% of their 1-RM. Participants were instructed to continue extending their knee against the resistance of the weight even though they were not able to do so. The

principal investigator gave the participants a verbal command when to discontinue each rep. Study investigators manually lifted the weight to the starting position prior to each rep to prevent any concentric loading of the knee extensors of the exercised leg. Each rep lasted ~2-3 s, with 15 s of rest between reps, and the seven sets were each separated by 3 min of rest.

Muscle biopsies and venous blood sampling
Percutaneous muscle biopsies were obtained from the vastus lateralis immediately before and 6 h and 24 h after the eccentric exercise bout. Upon receiving a local anesthetic (2% xylocaine with epinephrine), muscle samples were obtained with the fine needle aspiration procedure using a 16-gauge Tru-Core biopsy needle (Medical Device Technologies, Gainesville, FL). An average (±SD) of 9.54 (2.04) mg of muscle was obtained from the middle portion of the vastus lateralis muscle at the midpoint between the patella and the greater trochanter of the femur at a depth between 1 and 2 cm. For the second and third biopsies, attempts were made to extract tissue from approximately the same location by using the previous biopsy puncture, depth markings on the needle, and a successive puncture that was made approximately 0.5 cm to the former from medial to lateral (Willoughby et al., 2003). After removal, muscle specimens were immediately frozen in liquid nitrogen and then stored at -80°C for later analysis.

Venous blood samples were obtained immediately prior to the eccentric exercise bout from the antecubital vein into a 10 ml collection tube using a standard vacutainer apparatus. Blood samples were also obtained at 6 h and 24 h after exercise. Blood samples were allowed to stand at room temperature for 10 min and then centrifuged at 800 g for 10 min. The serum was removed and frozen at -20°C for later analysis.

Serum estradiol (E2) and myostatin LAP and propeptide quantitation
The concentrations of serum E2 and myostatin LAP and propeptide were determined in duplicate and the average concentrations reported using commercially available enzyme-linked immunoabsorbent assay (ELISA) kits for E2 (Diagnostic Systems Laboratories, Webster, TX) and myostatin LAP/propeptide (BioVendor, Candler, NC). For the myostatin ELISA, the antibody is specific for both LAP and propeptide (LAP/pro). Standard curves were generated for E2 (r^2 = 0.99, p = 0.001) and LAP/pro (r^2 = 0.98, P = 0.001) using specific control antigens contained in each kit. The serum protein concentrations were determined at an optical density

of 450 nm with a microplate reader (Wallac Victor 1420, Perkin Elmer, Boston, MA). Intra-assay coefficients of variation were determined for each duplicate for all subjects and resulted in a coefficient of 1.64% and 1.85%, respectively, for E2 and LAP/pro.

Skeletal muscle total RNA isolation

Total cellular RNA was extracted from the homogenate of biopsy samples with a monophasic solution of phenol and guanidine isothiocyanate (Chomczynski and Sacchi, 1987) contained within the TRI-reagent (Sigma Chemical Co., St. Louis, MO). The RNA concentration was determined by optical density (OD) at 260 nm (by using an OD_{260} equivalent to 40 μg/μl) (Current Protocols, 1999), and the final concentration was adjusted to 1 μg/μl. Aliquots (5 μl) of total RNA samples were then separated with 1% agarose gel electrophoresis, ethidium bromide stained, and monitored under an ultraviolet light (Chemi-Doc XRS, Bio-Rad, Hercules, CA) to verify RNA integrity and absence of RNA degradation. We demonstrated that this procedure yielded un-degraded RNA, free of DNA and proteins as indicated by prominent 28s and 18s ribosomal RNA bands (data not shown), as well as an OD_{260}/OD_{280} ratio of approximately 2.0 (Current Protocols, 1999). The RNA samples were stored at - 80°C until later analysis.

Reverse transcription and cDNA synthesis

Two μg of total skeletal muscle RNA were reverse-transcribed to synthesize cDNA using the iSscript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). A reverse transcription reaction mixture [2 μg of cellular RNA, 5x reverse transcription buffer (20 mM Tris-HCL, pH 8.3; 50 mM KCl;2.5 mM $MgCL_2$; 100 μg of bovine serum albumin/ml), a dNTP mixture containing 0.2 mM each of dATP, dCTP, dGTP, and dTTP, 0.8 μM MgCl2, 0.5 μg/μl of oligo(dT)$_{15}$ primer, and 25 u/μg of MMLV RNAase H$^+$ reverse transcriptase enzyme (Bio-Rad, Hercules, CA)] was incubated at 25°C for 5 min, 42°C for 30 min, heated to 85°C for 10 min, and then quick-chilled on ice. The cDNA concentration was determined by using an OD_{260} equivalent to 50 μg/μl (Current Protocols, 1999) and starting PCR template concentration was standardized by adjusting the reactions for all samples to 200 ng prior to amplification (Willoughby et al., 2003).

Oligonucleotide primers for PCR

The mRNA sequences of human skeletal muscle myostatin (GenEMBL NM_005259) and β-actin (GenEMBL NM_001101) published in the NCBI Entrez Nucleotide database (www.ncdi.nlm.hih.gov) were used to construct oligonucleotide PCR primers using Beacon Designer software (Bio-Rad, Hercules, CA). The sense and anti-sense primers were synthesized (Integrated DNA Technologies, Coralville, IA) and used to isolate the mRNA expression of myostatin. These primers amplified a PCR fragment of 141 base pairs (bp) for myostatin. 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 real-time PCR (Mahoney et al., 2004), β-actin was used as an external reference standard for detecting the relative change in the quantity of myostatin mRNA using PCR. For β-actin mRNA, these primers amplified a PCR fragment of 135 bp.

Real-time PCR amplification and quantitation

Two hundred ng of cDNA template were used for each of the PCR reactions for β-actin and myostatin using iQ SYBR Green Supermix (Bio-Rad, Hercules, CA). Specifically, each PCR reaction contained the following mixtures: [10x PCR buffer, 0.2 μM dNTP mixture, 2.0 μM of a cocktail containing both the sense and antisense RNA oligonucleotide primers, 2 mM $MgCL_2$, 1.0 u/μl of hot-start iTaq DNA polymerase, SYBR Green I dye, and nuclease-free dH_2O]. Each PCR reaction was amplified using real-time quantitative PCR (iCycler IQ Real-Time PCR Detection System, Bio Rad, Hercules, CA). The amplification profile was run for 40 cycles employing a denaturation step at 95°C for 30 s, primer annealing at 58°C for 30 s, and extension at 72°C for 30 s. Fluorescence was measured after each cycle resulting from the incorporation of SYBR green dye into the amplified PCR product. To help control for differences in amplification efficiency during thermocycling, all PCR reactions were prepared from the same stock solution and run in duplicate. The specificity of the PCR was demonstrated with an absolute negative control using a separate PCR reaction containing no cDNA and a single gene product was confirmed using DNA melt curve analysis. Additionally, to assess positive amplification of mRNA, aliquots of the PCR reaction mixtures were electrophoresed in 1.5% agarose gels in 1X Tris-Acetate-EDTA (TAE) buffer to verify positive amplification of β-actin and myostatin and the gel stained with ethidium bromide and illuminated with UV transillumination (Chemi-Doc XRS, Bio-Rad, Hercules, CA).

Myostatin mRNA was expressed relative to the expression of β-actin and the change in threshold cycle (ΔC_T) values were used to compare myostatin gene expression between males and females at pre, 6

h, and 24 h post-exercise. To determine reliability between amplifications, the duplicate intra-assay coefficients of variation for the duplicate PCR amplification for all participants were performed and resulted in coefficients of variation of 3.12% and 3.58% for myostatin and β-actin mRNA, respectively. Additionally, the external control standard β-actin displayed only a small amount of variation in expression from one sampling point to the next. The overall average variation from pre-exercise for 6 h and 24 h post-exercise in both males and females was only 2.25% and 2.72%, respectively.

Statistical analysis

Separate two-way (gender x test) factorial ANOVAs were used to determine differences between genders, testing sessions, and interactions. Tukey post-hoc procedures were used to locate significant differences among testing sessions. Bivariate correlations were determined with the Pearson Product Moment Correlation Coefficient. Body composition data at the onset of the study were analyzed by separate independent-group t-tests. A probability level of ≤ 0.05 was adopted throughout.

RESULTS

Body composition

A significant difference was observed between genders for total body mass ($p = 0.01$), fat-free mass ($p = 0.02$), and percent fat ($p = 0.03$); however, no significant difference was observed between genders for fat mass ($p = 0.94$) (Table 1). None of the body composition variables were correlated to the pre-exercise levels of serum LAP/pro or skeletal muscle myostatin mRNA.

Table 1. Pre-exercise body composition values for both genders. Data are means (±SD).

Variable	Males	Females
Total Body Mass (kg)	82.2 (12.3)	57.7 (8.1)**
Fat Mass (kg)	14.7 (6.7)	15.0 (5.9)
Fat-Free Mass (kg)	67.4 (5.8)	42.7 (6.0)*
Body Fat (%)	17.3 (5.8)	25.1 (7.6) *

$* p < 0.05, ** p < 0.01.$

Serum esradiol and myostatin propeptide levels

For E2, no significant interaction was located ($p = 0.853$); however, a significant main effect for gender ($p = 0.001$) demonstrated females to have significantly greater levels than males. However, there was no significant main effect for test ($p = 0.932$) indicating that the levels of E2 were not significantly affected by eccentric exercise (Figure 1).
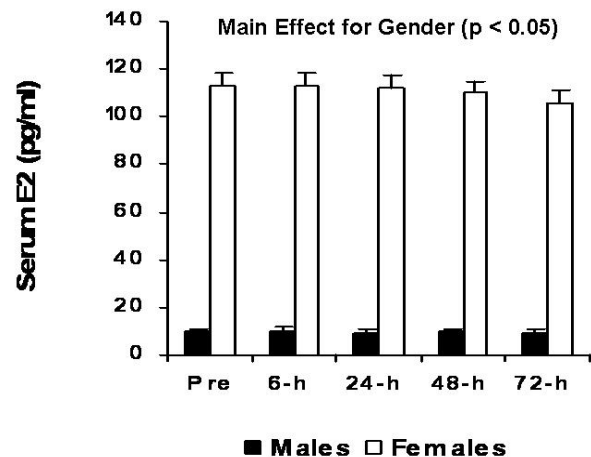

Figure 1. Serum estradiol (E2) levels in males and females. A significant main effect for Gender was observed ($p < 0.05$) suggesting that at each time point the level of E2 in males was lower than females. However, eccentric exercise had no effect on E2 levels for either gender ($p > 0.05$).

For myostatin propeptide, a significant interaction was located ($p = 0.012$) suggesting males to have a greater differential response to eccentric exercise. Post-hoc analysis indicated that at 24-h post-exercise males underwent an exercise-induced increase in myostatin propeptide, whereas females experienced a decrease ($p < 0.05$) (Figure 2).
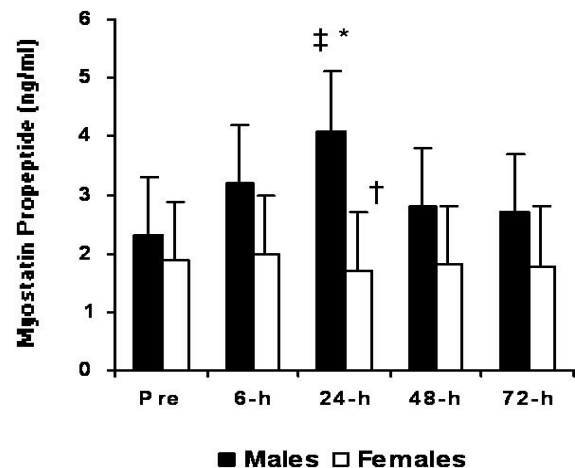

Figure 2. Serum myostatin propeptide levels in males and females produced a significant interaction ($p < 0.05$). In males, eccentric exercise resulted in a significant increase in myostatin (*), whereas females underwent a significant decrease (†). ‡ Significant Gender x Test Interaction ($p < 0.05$), * Significantly > Pre ($p < 0.05$), † Significantly < Pre ($p < 0.05$).

Myostatin mRNA expression

A significant interaction was observed for myostatin mRNA ($p = 0.013$) indicating males to have a greater differential response to eccentric exercise.

Post-hoc analysis showed that males underwent increases at 6 h and 24 h post-exercise that were significantly greater (p < 0.05) than females who underwent a significant decrease at 24 h post-exercise (Figure 3).
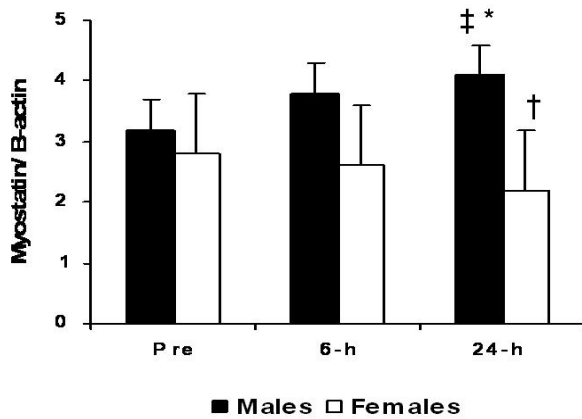

Figure 3. Myostatin mRNA expression in males and females produced a significant interaction (p < 0.05), and is presented as ΔC_T values relative to β-actin at each time point. Relative to pre-exercise, in males eccentric exercise resulted in a significant increase in myostatin mRNA (*), whereas females underwent a significant decrease (†). ‡ Significant Gender x Test Interaction (p <0.05), * Significantly > Pre (p < 0.05), † Significantly < Pre (p < 0.05).

Correlations between serum E2, myostatin mRNA, and LAP/propeptide
In males, serum E2 and LAP/pro were correlated at 6 h (r = -0.804, p = 0.016) and 24 h post-exercise (r = -0.841, p = 0.009). In females serum E2 levels were correlated to myostatin mRNA at 6 h (r =0.739, p = 0.036) and 24 h (r = 0.813, p = 0.014) after exercise and LAP/pro at 6 h (r = 0.713, p = 0.047) and 24 h (r = 0.735, p = 0.038) post-exercise.

DISCUSSION

The present results seem to suggest that the levels of serum LAP/pro and skeletal muscle myostatin mRNA expression are not different between males and females and do not appear to be related to body composition as has previously been suggested in rodents (McMahon, 2003). This study presents data demonstrating skeletal muscle myostatin mRNA expression and serum LAP/propeptide levels to be increased in males as a result of eccentric exercise, but that this response is attenuated in females. In males, these results are additionally highlighted by the exercise-induced increases we observed in serum LAP/pro. Myostatin circulates as a latent complex non-covalently bound to its propeptide. Once

released from the propeptide, myostatin becomes activated and can bind to the activin IIB receptor, thereby activating myostatin signaling in skeletal muscle (Lee et al., 2005). However, the mechanisms involved in the cleavage of the latent complex *in vivo*, thereby activating serum myostatin, are unknown at this time.

Several studies have demonstrated that resistance loading in humans causes decreases in myostatin mRNA expression (Roth et al., 2003; Wehling et al., 2000). It has also been shown that an acute bout of dynamic resistance exercise decreased myostatin mRNA expression to a similar degree in both males and females (Kim et al., 2005). However, no published data in humans apparently exists demonstrating the effects of eccentric muscle contractions on myostatin mRNA expression. One study with rodents demonstrated that a single bout of 30 eccentric muscle contractions increased myostatin mRNA expression after only 30 min, peaked after 12 h, and returned to baseline levels 24 h after exercise (Peters et al., 2003). We have herein demonstrated in human males that eccentric exercise caused myostatin mRNA expression to increase 19% and 28% above pre-exercise values, respectively, at 6 h and 24 h post-exercise, whereas in females myostatin mRNA underwent respective decreases of 7% and 21% at 6 h and 24 h post-exercise (Figure 3). As a result of eccentric exercise, in males we also observed increases from pre-exercise in LAP/pro of 39% and 78%, respectively, at 6 h and 24 h post-exercise. However, females underwent a decrease in LAP/pro of 10% at 24 h post-exercise (Figure 2).

In males, the increases in LAP/pro were correlated to the low levels of serum E2 at 6 h (r = -0.804) and 24 h post-exercise (r = -0.841). However, in females the elevated serum E2 levels were correlated to the decreases in myostatin mRNA at 6 h (r =0.739) and 24 h (r = 0.813) post-exercise and LAP/pro at 6 h (r = 0.713) and 24 h (r = 0.735) post-exercise. The females were exercised during their mid-luteal phase (d 22.4 ±1.2 of their 28-d cycle), and as expected, we observed females to have E2 levels that were 1,053% higher than males. Therefore, if E2 attenuates myostatin signaling and the release of mature myostatin from the latent complex our data may suggest that the apparent exercise-induced decrease in myostatin mRNA expression and myostatin propeptide observed in females may be due to their greater levels of circulating E2. However, because myostatin proteins undergo post-translational modifications, myostatin mRNA levels may not accurately represent circulating levels of myostatin (McMahon *et al.*, 2003). It should be emphasized, however, that

the antibody used in the serum myostatin ELISA is specific for both unbound propeptide and that associated with the LAP. Therefore, making a specific distinction between the two must be made with caution.

In rodents, no differences in the steady-state skeletal muscle levels of myostatin mRNA or the latent form of myostatin have been observed; however, 50% greater levels of processed myostatin were observed in females, who weighed 40% less than males (McMahon et al., 2003). These data suggest gender-related differences in transcription and translation of myostatin than processed myostatin; however, the mechanism is unkown.

However, previous data with human (Kim *et al*., 2005) showed males to be expressing approximately 85% greater amount of pre-exercise myostatin mRNA than females, even though males had 80% greater body mass and 65% greater fat-free mass than females. Our results are similar where, at pre-exercise, we observed males to be expressing 88% more myostatin mRNA and 82% more myostatin propeptide than females, even though males had 70% more body mass and 63% more fat-free mass (Table 1). Therefore, while our results and those of Kim et al. (2005) suggest no sexual dimorphism in myostatin mRNA expression based on the issue of difference between body mass and muscle mass between genders, they do seem to suggest, however, that the myostatin gene in males to be transcriptionally regulated in response to eccentric exercise. Furthermore, our results also suggest a possible gender-specific mechanism induced by eccentric exercise that could possibly facilitate the cleavage of myostatin propeptide from the latent complex, which may be attenuated in the presence of elevated circulating E2.

Eccentric exercise commonly results in muscle damage. The inflammatory response to exercise-induced muscle damage is characterized by the production of inflammation-responsive cytokines (e.g., IL-6, TNF-alpha, etc.). Current evidence suggests that inflammatory responses to muscle damage from eccentric exercise to be gender-dependent (Kendall and Eston, 2002). However, there is evidence to suggest that the levels of the cytokine myostatin may be inversely related to the production of the cytokine, IL-6 (Ramakrishnan et al., 2005). We have previously shown eccentric contractions to result in greater increases in serum IL-6 then concentric contractions in men (Willoughby et al., 2003). After eccentric exercise, we have also demonstrated increases in circulating IL-6 (Willoughby et al., 2003), whereas in mice increases in muscle IL-6 precedes the disruption of myofibers (Tomiya et al., 2004). Exogenous administration of E2 in post-menopausal women has been shown to reduce IL-6 levels (Eilertsen et al., 2005; Rachon et al., 2006), suggesting that E2 may possess the ability to counteract the release and subsequent inflammatory effects of IL-6. As a result of the anti-oxidant properties of E2, females are known to have an enhanced ability to combat oxidative stress, indicated by a higher level of anti-oxidant enzyme expression and activity, an improved ability to normalize perturbations in intracellular redox state (Vina et al., 2006), and a reduction in inflammatory markers (Sunday et al., 2006).

Increased levels of E2 are known to be associated with the increased expression of the estrogen receptor (ER), and the cellular actions of E2 can be initiated at the cell membrane. It is well known that the ER interacts with the estrogen response elements in target gene promoters directly regulating their transcription (Sukovich et al., 1994). Therefore, we may have observed an ER-mediated inhibition in myostatin signaling. The opposing effect of E2 and myostatin expression in females is conceivable due to the known inhibitory cross-talk between E2 and myostatin, where E2 signaling has been shown to suppress myostatin signaling (Matsuda et al., 2001). The decreased myostatin mRNA expression and subsequent decrease in myostatin propeptide in females in response to eccentric exercise may likely be due to an inhibiting in myostatin signaling.

The data we present herein is the first in humans to demonstrate eccentric exercise-related effects on myostatin mRNA expression and serum LAP/pro levels and suggests that eccentric exercise up-regulates myostatin mRNA expression and possible increases in active myostatin in males, whereas the response in females is apparently attenuated. Our results are partially supported by data from human breast cancer cells showing that treatment with anti-estrogens led to a release of mature TGF-β from the latent complex (Benson and Baum, 1996), and that in human kidney carcinoma cells, E2-mediated activation was shown to suppress TGF-β signaling (Mize et al., 2003). Because of the observed difference in circulating E2 between males and females, and since the regulation of myostatin by its propeptide is highly similar to TGF-β, it is not unreasonable to assume that an E2-related mechanism may exist for the attenuated response in myostatin propeptide we observed to occur in females.

CONCLUSIONS

Our results seem to suggest that the levels of serum LAP/prop and skeletal muscle myostatin mRNA expression were not different at pre-exercise and do not appear to be related to differences in body composition between genders. Eccentric exercise is capable of up-regulating myostatin mRNA in males, likely through activation in myostatin signaling. However, myostatin mRNA expression in females is attenuated due to an apparent inhibition in myostatin signaling, and appears to be related to the presence of a higher level of circulating E2, rather than differences in body mass. Even though males and females had similar baseline levels of skeletal muscle myostatin mRNA and serum myostatin propeptide, females underwent decreases in myostatin mRNA expression and serum LAP/pro levels after eccentric muscle contractions that were correlated to their circulating levels of E2. Therefore, our present results suggest that due to their higher level of E2, females seem to be less susceptible to the mechanism by which eccentric exercise may up-regulate myostatin signaling and mRNA expression in males.

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KEY POINTS

- The pre-exercise levels of myostatin mRNA and propeptide were not significantly different between genders, and even though the total body mass and fat-free mass of males were significantly greater than females, neither was correlated to myostatin mRNA or LAP/propeptide.

- Myostatin mRNA expression in females is less than in males 24 h after a single bout of eccentric exercise.

- Myostatin LAP/propeptide levels in females are lower in females than in males 24 h after a single bout of eccentric exercise, thereby suggesting a gender-specific mechanism in which females may be less responsive to eccentric exercise than males.

- Myostatin mRNA expression in females is attenuated, possibly due to inhibition in myostatin signaling, and appears to be more related to the presence of a higher level of circulating E2 rather than body composition.

- Due to their higher level of E2, females seem to be less susceptible to the mechanism by which eccentric exercise apparently up-regulates myostatin mRNA expression in males.

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