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ORIGINAL ARTICLE

The influence of eccentric exercise on mRNA expression of skeletal muscle regulators

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Abstract To evaluate change in myostatin, follistatin, MyoD and SGT mRNA gene expression using eccentric exercise to study mechanisms of skeletal muscle hypertrophy. Young (28 ± 5 years) and older (68 ± 6 years) men participated in a bout of maximal single-leg eccentric knee extension on an isokinetic dynamometer at 60°/s: six sets, 12–16 maximal eccentric repetitions. Muscle biopsies of the vastus lateralis were obtained from the dominant leg before exercise and 24 h after exercise. Paired *t* tests were used to compare change (pre versus post-exercise) for normalized gene expression in all variables. Independent *t* tests were performed to test group differences (young vs. older). A probability level of $P \leq 0.05$ was used to determine statistical significance with Bonferroni adjustments. We observed no significant change in myostatin (−0.59 ± 2.1 arbitrary units (AU); $P = 0.42$), follistatin (0.22 ± 3.4; $P = 0.85$), MyoD (0.23 ± 3.1; $P = 0.82$), or SGT (1.2 ± 6.4; $P = 0.58$) mRNA expression in young subjects 24 h after eccentric exercise. Similarly, we did not observe significant change in myostatin (−3.83 ± 8.8; $P = 0.23$), follistatin (−2.66 ± 5.2; $P = 0.17$), MyoD (−0.13 ± 3.1; $P = 0.90$), or SGT (−1.6 ± 3.5; $P = 0.19$) mRNA expression in older subjects. Furthermore, the non-significant changes in mRNA expression were not different between young and older subjects, $P > 0.23$ for all variables. Our data suggests that a single bout of maximal eccentric exercise does not alter myostatin, follistatin, MyoD or SGT mRNA gene expression in young or older subjects.

Keywords Resistance training · Myostatin · Follistatin · MyoD · SGT

Introduction

Skeletal muscle is responsive to a number of growth factors and cytokines produced locally within the muscle tissue, but little data exists describing the effect of age on the expression of muscle regulatory factors (MRFs). Advancing age is characterized by the loss of skeletal muscle, termed sarcopenia. A potential contributor to sarcopenia is the altered function of satellite cells. A reduction in satellite cell proliferation and differentiation has been proposed as a potential source of age-related blunting of the regenerative process (Conboy et al. 2003) and the reduced hypertrophic response to anabolic stimuli in older adults (Grounds 1998). Although the mechanisms of muscle hypertrophy are not clearly defined, the process appears to require satellite cell activation (Carson and Alway 1996). Satellite cells are activated by a variety of local MRFs in response to anabolic stimuli and/or muscle damage and initially express either of the two MRFs: MyoD or Myf-5.

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Previous studies indicate that loading skeletal muscle activates muscle regulators (Kosek et al. 2006; Raue et al. 2006). Loading induces change in mRNA expression of MyoD in humans (Psilander et al. 2003; Raue et al. 2006) and down-regulates levels of muscle inhibitory factors, such as myostatin, which may impair satellite cell activation (Kim et al. 2005). Myostatin, growth differentiating factor-8 (GDF-8), is a catabolic regulator of skeletal muscle mass that is produced in skeletal muscle. Myostatin is complexed with other binding proteins such as small glutamine-rich tetratricopeptide repeat-containing protein (SGT) (Dominique and Gerard 2006) and follistatin. SGT appears to play a role in the regulation of myostatin secretion and activation (Dominique and Gerard 2006; Wang et al. 2003). Myostatin binds to the activin IIb receptor in skeletal muscle and this binding is inhibited by the protein follistatin (Hill et al. 2002). Without inhibitory regulation, myostatin appears to work, in part, by inhibiting myoblast proliferation (Taylor et al. 2001; Thomas et al. 2000) and differentiation (Adams 1998) as well as satellite cell activation (McCroskery et al. 2003). Thus, loading human skeletal muscle initiates a hypertrophic response by reducing myostatin mRNA expression, (Raue et al. 2006) elevating follistatin levels, (Willoughby 2004b) and stimulating an increase in MyoD (Psilander et al. 2003; Raue et al. 2006).

Age, gender, exercise mode and intensity likely influence the degree of muscle damage following a loading stimulus. Skeletal muscle loading via high intensity eccentric exercise is known to produce significantly more muscle damage (Clarkson and Hubal 2002) compared to concentric loading, thus leading to greater hypertrophy (Evans 1992; Evans and Cannon 1991; Hortobagyi et al. 1996). In response to muscle injury after loading, satellite cells fuse with damaged and undamaged myofibers as part of the hypertrophic process (Adams 2006). Muscle damage produces a cascade of metabolic events leading to an increase in protein synthesis (Evans 2002). Although muscle damage occurs after a single bout of eccentric exercise, it is unclear whether aged muscle will have impaired protein metabolism compared to young muscle as demonstrated in conventional resistance training studies (Evans 2004).

Skeletal muscle of young men acutely responds to mechanical load by up-regulating expression of mRNA mediators of muscle hypertrophy (Hameed et al. 2004; Willoughby 2004a, b). We, therefore, suspect that there is a blunted response of stimulating factors or a failure to down-regulate the inhibitory factors mediating the hypertrophic response in older men. Our study was primarily designed to detect satellite cell activation 24 h post exercise in young and older men. Indeed, we previously reported lower satellite cell numbers and activation in older compared to young men 24 h after performing maximal eccentric exercise (Dreyer et al. 2006). We selected the time point for muscle biopsy to be taken 24 h after exercise to demonstrate a significant increase in satellite number and activation as previously reported (Jacobs et al. 1995; Schultz et al. 1985). Although we did not power the study to detect significant changes in mRNA gene expression for select skeletal muscle hypertrophic regulators, we believe it is important to report the changes in these hypertrophic regulators 24 h after a maximal bout of eccentric exercise in both young and older men.

In the present study, we sought to determine whether myostatin, follistatin, MyoD and SGT mRNA expression were altered by a single bout of maximal eccentric exercise in healthy young and older men. We hypothesized that myostatin mRNA expression would be reduced in young and there would be a blunted response in older subjects. We further hypothesized that SGT mRNA expression would decrease similarly with myostatin, but that follistatin and MyoD would increase in response to the maximal eccentric exercise bout.

Materials and methods

Selection of study subjects

Subjects were required to provide written informed consent prior to study participation. This study was reviewed and approved by University of Southern California Institutional Review Board (IRB). Ten healthy young (23–35 years) and 11 healthy older (60–75 years) men volunteered to participate and completed a medical history screening, physical examination, and blood tests to ensure an absence of underlying medical conditions or muscle inflammation that could confound results. Subjects were not currently participating in a resistance training program and they had not been previously exposed to eccentric training. Prior to the initial muscle biopsy subjects abstained from recreational activity and did not perform rigorous activity until participating in the maximal eccentric exercise bout. Blood specimens were analyzed by the University of Southern California General Clinical Research Center (GCRC). Subjects with cardiac abnormalities (heart failure, recent myocardial infarction, or angina) resting blood pressure exceeding 180/94 mmHg, active inflammatory conditions, neuropathy, untreated endocrine abnormalities (e.g. diabetes, hypothyroidism), receiving anticoagulation, or inability to perform lower-extremity exercise were excluded from participation. Men in the older group were evaluated with a 12-lead EKG and blood pressure-monitored stress test to achieve at least 85% of their predicted maximal heart rate in order to exclude subjects at risk for cardiac ischemia during maximal eccentric exercise. All procedures involving subjects were conducted at the GCRC except for the exercise testing, which

was performed in the Division of Biokinesiology's Clinical Exercise Research Center.

Maximal eccentric exercise

Single-leg maximal eccentric isokinetic loading was performed on the dominant leg using the KinCom 500 H dynamometer (Chattanooga, Tennessee) at 60°/s. Each subject was familiarized with the equipment immediately prior to the exercise. During the maximal eccentric loading, a shoulder harness, hip restraint, and thigh strap (exercised leg) were used to limit excessive movement and secure the subject to the device. A strap was also placed distally on the dominant leg at the level of the load cell. The load cell was positioned 3 cm proximal to the talocural joint. The exercise protocol consisted of an initial set of 12 maximal repetitions with the subject performing the eccentric component while the investigator performed the concentric component by moving the limb back in the starting position. This was followed by five additional sets of 16 repetitions, for a total of six sets. Each eccentric repetition was separated by the time it took one of the investigators to manually bring the lever arm back to 0° of knee flexion (i.e. starting position). Each of the six sets was separated by 120–180 s. Verbal encouragement was provided by the investigator during each maximal eccentric contraction.

Muscle biopsies

Two muscle biopsies of the vastus lateralis were obtained from the dominant leg. The first biopsy (pre-exercise) served to generate baseline measures for variables of interest. The pre-exercise biopsy was conducted 11–15 days prior to the second muscle biopsy (post-exercise) to ensure any inflammation from the first biopsy would not influence the second biopsy procedure. The second biopsy was performed 24 h after the subjects completed a single bout of unilateral maximal eccentric exercise of the dominant quadriceps muscle group. All subjects were instructed to refrain from exercise for 24 h before and 72 h after each muscle biopsy.

Biopsies were obtained from the mid-portion of the vastus lateralis muscle approximately 18 cm proximal to the patella, approximating the midline of the quadriceps muscle group. The post-exercise biopsy was performed at a distance of 2–4 cm proximal or distal (randomly assigned) to the pre-exercise biopsy site. Biopsies were performed using a sterilized 5-mm Stille muscle biopsy needle (Micrins Surgical, Lake Forest, Illinois, USA) using applied suction. Approximately 100–175 mg of muscle tissue was obtained from each biopsy.

Muscle biopsy specimen processing

Muscle tissue samples were immediately rinsed with pre-chilled normal saline and dissected free from connective and adipose tissue. The muscle tissue was flash-frozen in liquid nitrogen and stored at −80°C until being processed for analysis. Each muscle biopsy sample was assigned a subject identification number to minimize bias.

Analysis of RNA expression

RNA extraction

Total RNA was extracted from muscle using a commercially available kit (Qiagen Kit). The manufacturer's instructions were followed using the RNeasy mini protocol for isolation of total RNA from muscle tissue. Briefly, approximately 10 mg of muscle was removed from the freezer and immediately samples were lysed in a guanidine–isothiocyanate-containing lysis buffer (Buffer RLT) and β-mercaptoethanol (β-ME). The tissue was homogenized using a polytron homogenizer (Kinematica Polytron PT1200C, Switzerland). After dilution of the lysate, the sample was treated with proteinase K for 10 min at 55°C before pelleting debris by centrifugation. Ethanol was added to the cleared lysate and RNA was bound to the RNeasy silica-gel membrane. DNase and any contaminants were washed away with simple wash spins (Buffers RW1 and RPE), and total RNA was eluted in RNase-free water. The concentration and purity of the RNA was determined using a UV spectrophotometer (Beckman Coulter DU 640, Fullerton, CA, USA) by measuring absorbance at 260 and 280 nm.

Reverse transcription and cDNA synthesis

Five hundred nanograms of total skeletal muscle RNA was reverse transcribed to synthesize cDNA using Taqman reverse transcription reagents (Applied Biosystems, Branchburg, NJ, USA). A reverse transcription (RT) reaction master mixture for one reaction was 50 µl total volume (0.5 µg of cellular RNA; 5 µl 10× reverse transcription buffer; 11 µl MgCL2; 10 µl deoxynucleotide 5′-triphosphate (dNTPs); 2.51 µl Random Hexamer; 1.25 µl RNase inhibitor; DEPC H20 to a final volume of 50 µl) and was incubated at 25°C for 10 min, 48°C for 30 min, 95°C for 5 min, and 4°C infinite (reaction done in the Mycycler, Bio-Rad, Hercules, CA, USA). We assumed 100% conversion of RNA to cDNA and based on this assumption we amplified 500 ng of cDNA.

Oligonucleotide primers for PCR

Oligonucleotide primers were used to amplify the mRNA expression of myostatin, follistatin, MyoD, and SGT. Primer sequences were designed using Primer3 program (Rozen and Skaletsky 2000). The primer sequences for the specific target mRNAs and melt curve temperatures are shown in Table 1 (Marx et al. 2002). Melt curves were determined for each primer set to ensure amplification of pure PCR products.

PCR amplification

A quantitative real time PCR (qRT-PCR) method was applied in the present study using 18s ribosomal RNA as an internal standard to determine relative expression levels of mRNAs for myostatin, follistatin, MyoD, and SGT. A total of 10 ng of cDNA were added to each of the 20 µl PCR reaction for myostatin, follistatin, MyoD, SGT, 18s rRNA. Specifically, each PCR reaction contained the following mixture: 10 µl 2.5× iQSYBRgreen supermix (BioRad); 7 ul RNase free water, 10 ng cDNA and 10 pmol of each primer (F and R) of interest. Each PCR reaction was amplified using BioRad iCycler iQ thermal cycler (BioRad). Thermal cycling conditions were as specified by the manufacturer: the amplification profile involved the denaturation step at 95°C for 3 min, and 60 cycles as follows: 95°C for 15 s and ramp to 56°C for 1 min, followed by a predetermined melt temperature for 10 s (Table 1).

A melting curve analysis was generated by the following amplification profile: 95°C for 3 min, and 60 cycles as follows: 95°C for 15 s and ramp to 56°C for 1 min, followed by an increase in set-point temperature by 0.5°C every 5 s for each sample. A single melt peak observed for each sample was used to validate that only a single product was present. To help control for differences in amplification efficiency during thermocycling, all PCR reactions were prepared from the master mix stock solution.

Table 1 Primer set sequences used for real-time PCR

Target mRNA	PCR primer sequence 5′→3″	Melt curve temperature (°C)
MyoD	F: AGC ACT ACA GCG GCG ACT R: AGG CAG TCT AGG CTC GAC AC	82
Myostatin	F: CTC TAA CCT TCC CAG GAC CA R: CCC ATC CAA AAG CT CAA AA	82
Follistatin	F: AAG ACC GAA CTG AGC AAG GA R: TTT TTC CCA GGT CCA CAG TA	84
SGT	F: TTG GGG TGA CGG TAG AAG AC R: GTT GAG CTC GAT GGC TTT TC	77
[a]18s rRNA	F: CGG CTA CCA CAT CCA AGG AA R: TGC TGG CAC CAG ACT TGC CTC	82

[a] 18s rRNA sequence (Marx et al. 2002)

mRNA quantification

mRNA expression levels were normalized to the 18s rRNA control gene after determining that there was no change in 18s RNA in response to the eccentric exercise bout. Consistent with our experimental design, we used RNA from young and older male control patients' skeletal muscle that was isolated both pre- and post-exercise to determine the most reliable housekeeping gene for normalization of the data. The real time RT-PCR analysis, performed in triplicate three independent times, for the housekeeping genes GAPDH, histone H3.3, laminin and β2- microglobulin (β_2M), showed unexpected and inconsistent fluctuations in mRNA expression, except for 18s rRNA (Supplemental Figure 1). Due to the large and inconsistent fluctuations of most of these genes, we could not employ geometric averaging (Vandesompele et al. 2002) to normalize myostatin gene expression in these samples with any confidence. Exercise appeared to alter gene expression in each of these housekeeping genes; therefore we did not use these controls to normalize the data. Our pilot data demonstrates that 18s rRNA remained stable in younger and older male samples before and after maximal eccentric resistance exercise and, therefore, could be used to confidently determine relative myostatin expression levels in skeletal muscle.

The average cycle threshold (C_T) value for 18s rRNA in young subjects pre-exercise was analyzed to be 20.5 ± 0.676 AU and post-exercise was 20.595 ± 0.872 AU. For old subjects, pre-exercise 18s rRNA was calculated as 22.93 ± 1.66 AU and post-exercise was 22.17 ± 1.44 AU. Pre- and post-data were determined by averaging the eight wells for each sample and then normalizing the data to 18s rRNA. All pre-exercise samples were then averaged to determine the average mRNA expression prior to exercise. This process was repeated to determine post exercise mRNA expression.

Statistical analysis

Statistical analyses were performed using SPSS, version 14.0 (SPSS Inc., Chicago, IL, USA). Paired *t* tests were used to compare changes (pre- vs. post-exercise) for normalized gene expression in all variables. Independent *t* tests were performed to test group differences (young vs. older). A probability level of $P \leq 0.05$ was used to determine statistical significance with Bonferroni adjustments.

Results

Characteristics of the study groups

Subjects in each group had similar physical characteristics, blood counts, and chemistries (Table 2). Adequate muscle tissue was not available from muscle biopsies for three

Table 2 Subject characteristics and blood chemistries

Measure	Young men ($n = 10$)	Older men ($n = 10$)	P value
Age (years)	28 ± 5	68 ± 6	<0.001
Weight (kg)	78 ± 13	82 ± 19	0.06
BMI	25 ± 3	26 ± 5	0.22
LDH (U/l)	157 ± 24.9	163 ± 19.4	0.28
CK (U/l)	211 ± 99	148 ± 73	0.14
Testosterone (ng/dl)	535 ± 101	434 ± 123	0.12

Data are presented as Mean ± SD. Mean values from six of ten subjects

BMI Body mass index (kg/m^2), *LDH* lactate dehydrogenase, *CK* creatine kinase

Table 3 Muscle fiber type characteristics

Measure	Young men ($n = 10$)	Old men ($n = 9$)
Type I (%)	52 ± 9	66 ± 16*
Type I CSA (μm)	6,431 ± 1,638	5,804 ± 1,357
Type II (%)	48 ± 9	34 ± 16*
Type II CSA (μm)	6,257 ± 1,569	4,714 ± 1,195*

Data are presented as Mean ± SD. Data for percent fiber types are generated from pre-exercise biopsy (Dreyer et al. 2006)

* Significantly different than young ($P \leq 0.02$)

subjects in the young group. Thus, a total of 18 subjects were included in the analyses. In our previous publication there was a trend for lower testosterone levels in older subjects as well as fiber type specific atrophy of Type II fibers compared to young subjects (Table 3) (Dreyer et al. 2006). There was no evidence of baseline muscle damage based on CK or LDH values in either young or older subjects (Dreyer et al. 2006). Older subjects had a significantly higher follistatin mRNA expression at baseline compared to young subjects (8.23 and 5.49 AU, respectively; $P = 0.015$). Baseline mRNA expression were not significantly different between young and older subjects for MyoD ($P = 0.27$), myostatin ($P = 0.06$) and SGT ($P = 0.73$) (Fig. 1).

Changes in skeletal muscle mRNA expression

In older subjects, the maximal eccentric exercise bout did not significantly change myostatin (−3.83 ± 8.8 AU, $P = 0.23$); follistatin (−2.66 ± 5.2 AU, $P = 0.17$); MyoD (−0.13 ± 3.1 AU, $P = 0.90$); and SGT (−1.6 ± 3.5 AU, $P = 0.19$) mRNA expression (post–pre values) (Fig. 1). Similarly, in young subjects myostatin (−0.59 ± 2.1 AU, $P = 0.42$); follistatin (0.22 ± 3.4 AU, $P = 0.85$); MyoD (0.23 ± 3.1 AU, $P = 0.82$); and SGT (1.2 ± 6.4 AU, $P = 0.58$) mRNA expression did not significantly change after the maximal eccentric exercise bout (Fig. 1). Lastly, there were no significant differences in change (post–pre values) between young and older groups for myostatin ($P = 0.23$), follistatin ($P = 0.99$), MyoD ($P = 0.58$), and

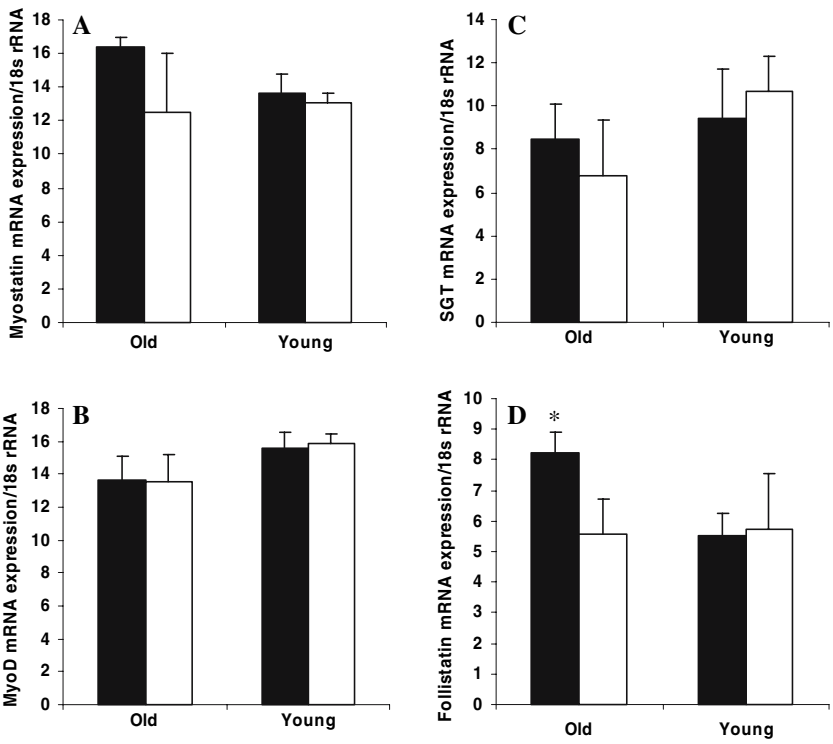

Fig. 1 Relative RT-PCR results for Myostatin (**a**), MyoD (**b**), SGT (**c**), and Follistatin (**d**) mRNA expression using 18s ribosomal RNA as an internal standard. The histograms represent pre-exercise (*shaded box*) and post-exercise (*not shaded*) mRNA gene expression for old and young subjects. There were no significant changes after exercise and no significant differences between groups. There were significant differences in pre-exercise follistatin mRNA expression between young and old subjects ($P = 0.015$). Values are means ± SE. *Significantly different from young ($P = 0.015$)

SGT ($P = 0.26$) mRNA expression between the young and older subjects.

Discussion

The results from the present study demonstrate that 24 h after a single bout of maximal eccentric knee extension exercise, there were no significant changes in mRNA gene expression of the specific muscle regulators myostatin, follistatin, MyoD, and SGT. It is important to investigate the influence of these muscle regulators as recent data suggests that they play a role in the mechanism(s) of muscle hypertrophy. Although the markers of muscle hypertrophy we evaluated did not change, other studies have indicated altered gene expression with exercise (Coffey et al. 2006; Touchberry et al. 2006).

Average myostatin mRNA expression was variable among subjects, but older subjects demonstrated higher, non-significant baseline myostatin mRNA expression. Indeed, increased expression of the myostatin gene is associated with the reduction in fat-free mass in older adults at risk for sarcopenia (Yarasheski et al. 2002). Although speculative, age-related changes in myofiber size and type may be associated with baseline differences in myostatin between young and older subjects. Muscle fiber types presented in this report were analyzed in a previous study (Dreyer et al. 2006) (Table 3), and these data indicate that the cross-sectional area (CSA) of Type I and II muscle fibers in older subjects were reduced compared to the CSA of young subjects. Likewise, the percentages of Type II muscle fibers in older subjects were significantly less than that of young subjects, suggesting age-related denervation atrophy. Lastly, older subjects may not be as active, thus leading to a decrease in muscle mass and associated elevation in myostatin mRNA expression.

Skeletal muscle appears to be more responsive to mechanical loading in young men compared to older men (Kim et al. 2005). In contrast, our data, although non-significant, indicate that changes in muscle regulators were more evident in the older subjects. In both young and older subjects, we observed a trend of reduced myostatin mRNA expression 24 h after the maximal eccentric exercise bout, suggesting that eccentric exercise may alter gene expression. Prior work involving resistance exercise in humans and/or mice have shown inconsistent results. Immobilization increases the expression of myostatin in mice, whereas re-loading muscle results in decreases in myostatin expression (Wehling et al. 2000). Similarly, Roth et al. (2003) analyzed young and older men and women's myostatin expression after 8 weeks of lower body resistance training and reported that myostatin mRNA expression was reduced. In contrast, Willoughby (2004a) demonstrated increases in myostatin mRNA expression following resistance exercise. These inconsistent findings may be due to differences in muscle sampling time-points, exercise mode, intensity, and duration making comparisons between studies difficult.

Elevated MyoD mRNA expression is expected after maximal exercise suggesting that muscle damage is present and that satellite cells are activated, thus leading to protein synthesis and muscle hypertrophy. Our results demonstrate that MyoD mRNA expression did not significantly change after one bout of maximal eccentric exercise in both young and older subjects; however, young subjects appeared to have an increase in MyoD mRNA expression while older subjects had a decrease. These findings are consistent with other studies which indicate that one exercise bout does not significantly influence MyoD mRNA expression in older subjects (Hameed et al. 2003). The absence of change in MyoD could be due to age-related de-sensitization to mechanical loading or fiber-type specific responses. Indeed, previous reports have determined that the myogenic response to a single bout of exercise is impaired in muscle of older animals (Tamaki et al. 2000).

An important limitation and possible explanation for the current finding is the use of a single 24 h post-exercise muscle biopsy to evaluate the responses to exercise induced mRNA MyoD expression. A previous study reported that transcriptional activation of MyoD occurs primarily during the initial hours (4–8 h) of recovery (Pilegaard et al. 2000; Yang et al. 2005). Because these proteins may experience post-translational modifications, mRNA gene expression from a single 24 h muscle biopsy may not accurately represent initial post bout levels of the functional proteins. Alternatively, repeated bouts of exercise may be necessary to induce transcriptional activity of muscle regulatory genes. Multiple testing parameters may be needed to ensure that protein synthesis occurs after eccentric exercise.

Follistatin blocks myostatin's ability to bind to the activin IIb receptor sight. Without follistatin or other potential myostatin inhibitors, myostatin binds to the activin IIb receptor, and appears to work, in part, to inhibit myoblast proliferation (Taylor et al. 2001; Thomas et al. 2000) and differentiation (Adams 1998) as well as satellite cell activation (McCroskery et al. 2003). Baseline follistatin may be elevated in older subjects to counteract increases in myostatin mRNA expression associated with muscle fiber atrophy in advanced age. It is possible that age-related differences in follistatin are indicative of a mechanism in aging muscle to prevent sarcopenia. In fact, our data supports this contention as we report baseline values for follistatin mRNA expression being greater in older compared to young subjects. However, we do not have a plausible explanation as to why follistatin levels decreased in older subjects following maximal eccentric exercise. We hypothesized that

follistatin would have increased after eccentric exercise because prior evidence suggests that after resistance training, follistatin-like related gene (FLRG) increases (Willoughby 2004a, b). Resistance training appears to induce changes in FLRG; however, one possible limitation in our study may be that a single maximal eccentric exercise bout is not sufficient to increase follistatin levels in both young and older subjects or perhaps the timing of the 24 h post exercise muscle biopsy is not optimal to demonstrate change in follistatin expression.

Previous findings indicated that SGT functions to mediate myostatin secretion and activation in skeletal muscle cells during myogenesis (Wang et al. 2003), reflecting myostatin's biological activity in skeletal muscle cells. We expected myostatin and SGT to have a direct relationship; however, this pattern only existed within the older subjects. Since SGT is suspected to mediate myostatin's ability to bind to cell surface receptors with an inhibitory role in muscle cell development (Wang et al. 2003), it is not clear why young subjects had decreased myostatin mRNA expression and increased SGT mRNA expression. Future research is necessary to clarify the interaction between SGT and myostatin in skeletal muscle cells.

Our negative findings may reflect methodological limitations. Housekeeping genes are not completely stable due to outside influences or disruption to the muscle cells and it is essential to normalize data to a consistent housekeeping gene that acts as an internal standard. Recent research has demonstrated that the expression of housekeeping genes may be altered due to differences in exercise (Jemiolo and Trappe 2004), and age (Mahoney et al. 2004), suggesting that normalizing methods such as geometric averaging (Vandesompele et al. 2002) to determine stable housekeeping genes may not be the best method to normalize relative gene expression patterns under these circumstances. This does not appear to be an important consideration when applying geometric averaging in tissues that have not been exposed or introduced to stresses (Barber et al. 2005; Vandesompele et al. 2002) such as maximal eccentric exercise. We determined that 18s rRNA was the least susceptible to change after one exercise bout in both young and older subjects; however, other studies have determined that when comparing GAPDH to 18s rRNA, β-actin, and β-2M expression, GAPDH is the most stable housekeeping gene in response to exercise (Jemiolo and Trappe 2004). This discrepancy may have led to our non-significant findings and potentially our data could have been confounded by other variables which affect 18s rRNA that were not considered. However, our experimental design is unique in that we are attempting to quantify specific changes in mRNA expression levels following maximal eccentric resistance training. Lastly, our sample size was initially powered to detect changes in satellite cell number; however, the sample size may not have been appropriate to detect a significant difference in myostatin, MyoD, follistatin and SGT mRNA expression.

In summary, the findings of the present study demonstrate that an acute maximal eccentric exercise bout was not sufficient to induce significant changes in mRNA gene expression for myostatin, MyoD, follistatin and SGT, but showed a significant elevation in baseline follistatin for older compared to young adults. The non-significant decrease in myostatin mRNA expression after one bout of eccentric exercise supports previous literature (Roth et al. 2003; Walker et al. 2004). The molecular mechanisms of skeletal muscle hypertrophy have considerable clinical importance and understanding these mechanisms may help individuals suffering from muscle wasting conditions due to age or illness, or perhaps even healthy athletes with a desire to enhance muscle strength and mass. Further work is necessary to elucidate the significance of these muscle regulators and their association with skeletal muscle hypertrophy.

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