Load-mediated downregulation of myostatin mRNA is not sufficient to promote myofiber hypertrophy in humans: a cluster analysis

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Kim J-s, Petrella JK, Cross JM, Bamman MM. Load-mediated downregulation of myostatin mRNA is not sufficient to promote myofiber hypertrophy in humans: a cluster analysis. J Appl Physiol 103: 1488–1495, 2007. First published August 2, 2007; doi:10.1152/japplphysiol.01194.2006.—Myostatin is a potent inhibitor of myogenesis; thus differential expression might be expected across individuals varying in responsiveness to myogenic stimuli. We hypothesized that myostatin would be differentially regulated across humans with markedly different hypertrophic responses to resistance training (RT; 16 wk). Targets were assessed in muscle biopsies at baseline (T1) and 24 h after the first (T2) and last (T3) loading bouts in previously untrained subjects statistically clustered based on mean myofiber hypertrophy as extreme (Xtr; n = 17, 2,475 \( \mu \)m\(^2\), modest (n = 32, 1,111 \( \mu \)m\(^2\), and nonresponders (n = 17, –16 \( \mu \)m\(^2\)). We assessed protein levels of latent full-length myostatin protein complex and its propeptide; mRNA levels of myostatin, cyclin D1, p21\(^{\text{cip1}}\), p27\(^{\text{kip1}}\), and activin receptor IIB; and serum myostatin protein concentration. Total RNA concentration increased by T3 in nonresponders (37%) and modest responders (40%), while it increased acutely (T2) only in Xtr (26%), remaining elevated at T3 (40%). Myostatin mRNA decreased at T2 (~44%) and remained suppressed at T3 (~52%), but not differentially across clusters. Cyclin D1 mRNA increased robustly by T2 (38%) and T3 (74%). The increase at T2 was driven by Xtr (62%, P < 0.001), and Xtr had the largest elevation at T3 (82%, P < 0.001). No effects were found for other target transcripts. Myostatin protein complex increased 44% by T3 (P < 0.001), but not differentially by cluster. Myostatin protein complex propeptide and circulating myostatin were not influenced by RT or cluster. Overall, we found no compelling evidence that myostatin is differentially regulated in humans demonstrating robust RT-mediated myofiber hypertrophy vs. those more resistant to growth.

Resistance training induces hypertrophy of both type I and type II myofibers, with the magnitude of growth typically ~1.5-fold greater in type II vs. type I myofibers (22). However, there is a high degree of interindividual variability regarding the hypertrophic adaptation (22, 34). Long-term resistance training reduces myostatin mRNA levels (13, 40), and, as we found in humans, a single bout of resistance loading markedly inhibits myostatin mRNA expression (20), suggesting a tight coupling to mechanical load. This finding was recently confirmed in rodent muscle after acute resistance exercise (15).

Myostatin protein is synthesized exclusively in skeletal muscle as a 375-amino acid (AA) precursor protein or latent complex (MSTN\(_{\text{CMPLX}}\)), which apparently circulates as a homodimer with the disulfide linkage occurring within the 109- AA COOH-terminal bioactive peptide at residue 339 (accession no. 014793) (25). When glycosylated, a single glycosylation site exists at residue 71, which falls within the propeptide (MSTN\(_{\text{PRO}}\)). The latent complex is processed by two proteolytic cleavage steps: first, removal of the NH\(_2\)-terminal 23-AA signal sequence (necessary for signal transduction in exiting the cell); and second, cleavage to release the 109- AA COOH-terminal bioactive peptide (12 kDa), leaving behind the 243-AA MSTN\(_{\text{PRO}}\) (28-kDa nonglycosylated). Studies of myostatin protein levels in humans and animals are very difficult to deduce based on antibody specificities and differences in interpretation (14, 29, 42, 50, 54, 55). For example, whether the 26-kDa protein reported by some is in fact myostatin in any form has been questioned (57). Assessment of the full-length latent MSTN\(_{\text{CMPLX}}\) provides an index of myostatin expression, while the concentration of either MSTN\(_{\text{PRO}}\) or active myostatin should serve as a useful indicator of myostatin activation, since the ratio between MSTN\(_{\text{PRO}}\) and active myostatin is 1:1, at least in serum (16). In this work, we quantified muscle myo-
statin mRNA, muscle protein lysate concentrations of the full-length MSTNCMPLX and MSTNPRO by immunoblotting, and serum myostatin concentrations by ELISA. In our hands, under reducing SDS-PAGE, the MSTNCMPLX monomer migrates to 50 kDa, while MSTNPRO migrates to 28 kDa in nonglycosylated form.

Because resistance loading suppresses myostatin mRNA expression, we tested the hypothesis that myostatin would be differentially regulated across humans with markedly different myofiber hypertrophic responses to resistance training. In our recent work (3), we employed K-means cluster analysis as a novel means of statistically classifying 66 young and old human subjects as extreme responders (Xtr), modest responders (Mod), or nonresponders (Non), based on mean myofiber hypertrophy after 16 wk of resistance training. In this study, we compared across these three clusters myostatin protein and gene expression and four additional target mRNAs involved in cell cycle regulation. To our knowledge, this is the first work in humans to 1) assess protein levels of both MSTNCMPLX and MSTNPRO in muscle lysate, along with muscle myostatin mRNA expression and myostatin protein concentration in serum; 2) evaluate changes in all of these components across time in a resistance training model; and 3) investigate changes in myostatin between groups based on differential hypertrophy responsiveness.

METHODS

Subjects. Sixty-six healthy, sedentary adults were recruited from the Birmingham, Alabama metropolitan area. Inclusion criteria were based on age ranges of 60–75 yr for older adults (15 women, 14 men) and 20–35 yr for younger adults (16 women, 21 men), as described (3). Briefly, subjects were free of any musculoskeletal or other disorders that might have affected their ability to complete resistance training and testing for the study. Subjects were not obese (body mass index < 30), and none had undergone leg resistance training within the past 5 yr. None of the subjects was being treated with exogenous testosterone or other pharmacological interventions known to influence muscle mass. The study was approved by the Institutional Review Boards of both the University of Alabama at Birmingham (UAB) and the Birmingham Veterans Affairs Medical Center. Written, informed consent was obtained before participation in the research. Using K-means cluster analysis, subjects were classified post hoc as Xtr (n = 17), Mod (n = 32), or Non (n = 17), based on changes in vastus lateralis mean myofiber cross-sectional area (μm²) in response to 16 wk of resistance training (3). This form of analysis is an ideal model by which potential mechanisms underlying human intervention responsiveness may be revealed in an unbiased manner.

Progressive resistance training program. Subjects underwent 16 wk of resistance training (3 days/wk) focused primarily on the knee extensors. Resistance training consisted of three exercises, including knee extension, leg press, and squats. Each exercise was performed for three sets of 8–12 repetitions to volitional fatigue using resistance exercise stations or plate-loaded stations (barbell squats and linear 45° leg press). To accurately compare training and testing loads between weight stack and free weight stations, actual resistances were determined for each weight stack using a load cell and regression procedures, as our laboratory has described previously (33). Before each testing and training session, subjects warmed up on a cycle ergometer or treadmill for ~5 min. All sets were separated by 90-s rest intervals. Initially, training loads were based on 80% of baseline one-repetition maximum strength. As training progressed, resistance was increased when a subject completed 12 repetitions for at least two of the three total sets at a given resistance while maintaining proper form.

We previously reported no differences in program adherence, training intensity, or training volume among the three responder clusters (3).

Muscle biopsy and tissue preparation procedures. Muscle biopsies were performed in the Pittman General Clinical Research Center at UAB. Muscle specimens were collected under local anesthesia (1% lidocaine) from vastus lateralis muscle by percutaneous needle biopsy using a 5-mm Bergstrom biopsy needle under suction, as previously described (11). To avoid any residual effects of the initial biopsy taken from the left leg, the postexercise biopsy was taken from the right leg 24 h after the first full bilateral loading bout (four progressive bouts preceded this one), and the third, 16-wk postraining biopsy was obtained from the left leg 24 h after the final training session. Samples were quickly blotted with gauze and dissected free of visible connective and adipose tissues, and portions of each sample to be used for RNA and protein isolation were immediately weighed and snap-frozen in liquid nitrogen. A separate portion for immunohistochemistry was mounted cross sectionally on cork in optimum cutting temperature mounting medium mixed with tragacanth gum and frozen in liquid nitrogen-cooled isopentane. All samples were stored at −80°C until analysis.

Muscle myostatin immunoblotting. Immunoblotting was conducted to assess muscle protein lysate concentrations of MSTNCMPLX, MSTNPRO, and any detected amounts of bioactive myostatin. Frozen muscle samples (30–40 mg) were homogenized and supernatant assayed for total protein using the bicinchoninic acid technique with BSA as a standard as described (2). Twenty-five micrograms of total protein diluted in reducing Laemmli buffer were separated by SDS-PAGE (120 V constant) in 10% minigels (Bio-Rad MP3, Bio-Rad Laboratories, Hercules, CA). Proteins were transferred at constant current (1 mA/cm² × 30 min) to polyvinylidene difluoride membranes using a semidry transfer cell (Trans-Blot SD, Bio-Rad Laboratories). Within subjects, baseline, acute postexercise, and 16-wk postraining samples were loaded in adjacent lanes. To control for between-groups bias, each 12-lane gel contained samples for subjects in each group, with the different subject groups loaded in random order on each gel. Equal loading across lanes and equal transfer were verified by staining all gels (after transfer) with Coomassie blue.

We conducted a thorough series of antibody evaluations against various sequences along the full-length 375-AA myostatin monomer and utilized for this report an antibody (BL946, Bethyl Laboratories, Montgomery, TX) against a residue sequence that crosses the cleavage site (arginine residue 266), which we found to be immunoreactive against MSTNCMPLX (375-AA migrates to ~50 kDa) and MSTNPRO (243 AA, ~28 kDa) in human muscle, as well as recombinant bioactive 12-kDa peptide (US Biologicals, Swampscott, MA) under reducing conditions (Fig. 1). An additional band at 37 kDa was detected in several samples, which may be the glycosylated version of MSTNPRO (glycosylation site at Asp71). However, this was not confirmed, and, therefore, the band was not quantified. We verified the specificity of the 28-kDa band as MSTNPRO by testing additional primary antibodies raised against peptide sequences within the MSTNPRO fragment (Bethyl Laboratories, Montgomery, TX). All of these antibodies recognized the 28-kDa band but were not immunoreactive against recombinant bioactive myostatin (12 kDa), indicating the 28-kDa band was in fact MSTNPRO rather than a dimer or other modified form of the bioactive COOH-terminal peptide. As we have found repeatedly in muscle tissue lysates from humans and rats, there appears to be no measurable level of the bioactive myostatin monomer, suggesting cleavage from MSTNPRO occurs in close proximity to its receptor, followed by rapid degradation, entry into the vascular compartment, or binding to a binding protein, such as follistatin or MSTNPRO.

Membranes were blocked with 2% BSA, 2% milk in PBS-0.1% Tween-20 (PBST), while the rabbit polyclonal primary antibody (BL946, Bethyl Laboratories, 1:1,000) and horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (1:50,000) were diluted in 0.5% BSA, 0.5% milk in PBST. Membranes were
Table 1. Sequences of the specific sets of primers used

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<th>Target mRNA</th>
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<th>Product Size, bp</th>
<th>GenBank Accession No.</th>
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<td>Myostatin</td>
<td>5' sense: GTTTCAGAGCATGGGATCGATGATGAA&lt;br&gt;3' antisense: GGACGGGAAAGAGGCTCAGAC</td>
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<td>Cyclin D1</td>
<td>5' sense: GACCCGGCGCATGATCATATTG&lt;br&gt;3' antisense: GAAACGGAGTCGAGCTCAAC</td>
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<td>NM_001758</td>
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<tr>
<td>p21^{cip1}</td>
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<td>233</td>
<td>L25610</td>
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<tr>
<td>p27^{kip1}</td>
<td>5' sense: GCGGAGGGAGGCGAGGAGG&lt;br&gt;3' antisense: TTGGGGGACCTCGTGAAGAAAC</td>
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<td>NM_004064</td>
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<tr>
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<td>NM_001106</td>
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samples and standards were run in duplicate with 100 μl loaded into each well. Each 96-well plate assay contained high- and low-concentration quality controls, serum samples, and a 7-point standard curve (0.1, 0.2, 0.5, 1, 2, 5, and 10 ng/ml myostatin). Myostatin from serum samples and standards was captured in microtiter wells coated with monoclonal anti-human MSTNPRO antibody. Captured proteins were sandwiched by subsequent treatment with biotinylated polyclonal anti-human MSTNPRO antibody. Streptavidin-HRP conjugate was then bound during a 30-min incubation. Substrate solution (H₂O₂ and tetramethylbenzine) was added to each well and allowed to react with the bound HRP-conjugate for 10 min. The reaction was stopped by adding acidic solution, and absorbance was measured at 450 nm using a microplate reader (Bio-Rad Model 550, Bio-Rad Laboratories). Serum concentrations were determined in nanograms per milliliter from the log-linear standard curve. In our hands, the intra-assay CV averaged 4.98%, and the interassay CV averaged 8.18%. We noted a high degree of variability among individuals (range 0.1–328 ng/ml). Nearly 35% of the human serum samples contained myostatin concentrations above the detectable range of the assay (up to 10 ng/ml); thus we repeated those samples at 1:4 dilution and found that most, but not all, fell within the range of the assay. A small number of samples required further dilution to 1:20 to fall within range, and the samples for two of the subjects required 1:100 dilution. The number of high-concentration samples requiring further dilution was not biased by age or responder classification.

Immunofluorescence microscopy. Our laboratory routinely assesses myofiber-type distribution (I, IIA, IIX) and type-specific myofiber size via myosin heavy chain isoform immunofluorescence microscopy and has published these methods in detail elsewhere (21, 22). Images were analyzed by a single technician blinded to age, sex, and time point (pre- or posttraining) of each specimen. Mean myofiber size was determined as a weighted average based on distribution and size of type I, IIA, and IIX myofibers, before and after training. Myofiber-type distribution did not differ by cluster, as previously determined from 1,009 ± 39 myofibers at baseline and 851 ± 38 myofibers posttraining (3).

Statistical analysis. Data are reported as means ± SE. Between-cluster differences in preexercise descriptive variables were tested using one-way ANOVA. All variables measured across resistance training were analyzed using cluster × training repeated-measures ANOVA. Levels of muscle myostatin protein and all target transcripts were assessed across all three biopsy time points (baseline, 24 h acute, 16 wk) and were thus analyzed via 3 (cluster) × 3 (time) repeated-measures ANOVA. Serum myostatin levels were only assessed at baseline and 16 wk and were thus tested via 3 (cluster) × 2 (time) repeated-measures ANOVA. Of the 66 subjects originally clustered (17 Non, 32 Mod, 17 Xtr) (3), transcript results were obtained for 59 subjects (15 Non, 30 Mod, 14 Xtr), and muscle and serum protein results were obtained for n = 49 (12 Non, 29 Mod, 8 Xtr). For each ANOVA model with a significant main or interaction effect, Tukey honestly significant difference tests were performed post hoc to localize the effect(s). Statistical significance was accepted at P < 0.05 for all tests.

RESULTS

For a detailed description of the ranges of myofiber hypertrophy and the age-sex composition within each of the three responder clusters, the reader is referred to Bamman et al. (3). An advantage of cluster analysis is the ability to identify factors associated with the hypertrophy adaptation without forced groupings based on independent variables such as age and/or sex. It is noteworthy that all four age-sex groups were represented in each of the three clusters, although Non contained only one young male, while Mod contained ~50% of each age-sex cohort. Briefly, the change in mean fiber area averaged +2,475 ± 140 μm² in Xtr, +1,111 ± 46 μm² in Mod, and −16 ± 99 μm² in Non. By design, K-means cluster analysis prevents any overlap among clusters. Notably, in this prior report, we ruled out the possibility that responsiveness (mean myofiber hypertrophy) was influenced by program adherence or training intensity (3). We also reported essentially identical IIX-to-IIa myofiber type shifts among all three clusters, indicating the resistance training intensity and frequency were at least sufficient to induce this training adaptation in all clusters, despite the failed hypertrophy in Non (3).

Muscle gene expression. A main time effect was found for total RNA concentration (P < 0.001), with significant stepwise increases from 297 ± 8 ng/mg muscle at baseline to 346 ± 10 ng/mg after the first full resistance loading bout (P < 0.001), and a further rise to 413 ± 10 mg/mg by week 16 (P < 0.001). As determined by subsequent post hoc testing, Xtr drove the early response as the only cluster with a significant acute increase in total RNA concentration (26%, P < 0.05). Significant increases (P < 0.001) were found by week 16 in each of the three clusters (Non 37%, Mod 40%, Xtr 40%).

Main time effects were noted for two of five transcripts (myostatin and cyclin D1). Changes in myostatin mRNA expression are shown in Fig. 2A. Myostatin transcript expression decreased throughout the resistance training program in all three responder clusters (P < 0.001). Myostatin mRNA decreased acutely (~44%, P < 0.001) and was also suppressed at week 16 (~52%, P < 0.001), but not differentially across clusters. Cyclin D1 transcript results are shown in Fig. 2B. Cyclin D1 mRNA increased robustly following the first full exercise bout (38%, P < 0.001) and rose significantly further by week 16 (74%, P < 0.001). Post hoc testing revealed that the early increase was driven by Xtr (62%, P < 0.005), the only cluster with a within-groups increase at the acute response time point. Additionally, Xtr had the largest elevation at week 16 (82%, P < 0.001) and a main cluster effect resulted from significantly higher average levels of cyclin D1 mRNA in Xtr compared with the other two clusters. No effects of cluster or training were found for transcript levels of ActRIIB, p21^(CPI), or p21^(CPI) (data not shown).

Muscle protein lysate levels of myostatin. Levels of full-length MSTNCMPLX and its propeptide MSTNPRO are shown in Fig. 3A and B. A main training effect (P < 0.001) was noted for levels of MSTNCMPLX, with an overall 44% increase from baseline to week 16. There were no differences among clusters (P = 0.15) and no significant cluster × training interaction (P = 0.82), although post hoc testing showed that Mod was the only cluster with a statistically significant increase from baseline to week 16 (P < 0.01). For MSTNPRO (Fig. 3B), there was no effect of cluster (P = 0.82), no cluster × training interaction (P = 0.38), and resistance training did not alter MSTNPRO concentration in any cluster (P = 0.72–0.99).

Serum myostatin concentration. Circulating myostatin concentrations by cluster are shown in Fig. 4. A remarkable degree of variability was noted between subjects, and the data were not normally distributed. Circulating myostatin did not change with resistance training in any cluster; thus the distribution of concentrations shown in Fig. 4 are based on within-subject mean levels between baseline and week 16. Serum myostatin concentration was <1 ng/ml for approximately one-third of the subjects in each cluster, while ~15% of Non and Mod subjects had myostatin concentrations in excess of 100 ng/ml.
DISCUSSION

We have taken a novel approach to studying the potential inhibitory role of myostatin during skeletal muscle hypertrophy in humans. Making use of K-means cluster analysis enabled us to directly ask whether key components of the myostatin pathway were differentially expressed during resistance training in subjects who responded robustly with myofiber hypertrophy vs. those who showed little to no growth adaptation. In prior work comparing young and old women and men, we found resistance training-induced hypertrophy to be most robust (22) in subjects who were adept at increasing muscle stem cell number and incorporating new myonuclei, which likely promoted the superior growth (34). The present study was undertaken to explore myostatin as a likely barrier to muscle stem cell proliferation that we predicted would be differentially expressed across training in Non, Mod, and Xtr responder clusters.

Clearly there is a large degree of interindividual variability in the ability to hypertrophy myofibers as an adaptive response to resistance training. Based on the recent flurry of myostatin-related findings as a negative regulator of muscle mass, myostatin seemed a likely target of differential regulation in humans showing disparate myofiber hypertrophy across 16 wk of resistance training. The bulk of our findings, however, do not support the concept that myostatin plays a major role as an inhibitor of resistance training-induced hypertrophy, at least not in normal healthy adults expressing a fair amount of muscle myostatin protein and mRNA. While MSTN\textsubscript{Cmplx} expression increased significantly and muscle myostatin mRNA was reduced across 16 wk of resistance training, none of the myostatin measurements were differentially affected by resistance training among the three responder clusters. Additionally, levels of mRNA expression among the myostatin receptor (ActRIIB) and cell cycle inhibitors associated with myostatin signaling (p27\textsuperscript{kip1}, p21\textsuperscript{cip1}) were not different between clusters and unaffected by resistance training.

Muscle myostatin protein concentration. Although there is evidence of decreased muscle myostatin protein levels in response to loading in rodents (19), a recent study in humans (50) reported upregulation of myostatin protein in young men after both 6 and 12 wk of relatively intense knee extensor exercise training using a regimen much like ours (3 days/wk, 3 sets at 85–90% one-repetition maximum of knee extension, leg press, and leg curl). While assessments of muscle myostatin at

![Figure 2](fig2.jpg)

**Fig. 2.** Transcript levels of myostatin (**A**) and a primary myostatin target, cyclin D1 (**B**), among non- (Non), modest (Mod), and extreme (Xtr) responders across three biopsy time points (baseline, 24-h acute response to the first loading bout, and after 16 wk of resistance training). Resistance training led to coordinate downregulation of myostatin mRNA (–44% after one loading bout and –52% by week 16) and increased expression of cyclin D1 (–38% after one loading bout and +74% by week 16) (P < 0.001). The myostatin responses were similar across responder clusters, while extreme responders drove the acute increase in cyclin D1. Values are means ± SE. †Main training effect, P < 0.001. §Main cluster effect, P < 0.001. *Different from previous time point within group, P < 0.05. #Different from baseline within group, P < 0.001.

![Figure 3](fig3.jpg)

**Fig. 3.** Muscle protein lysate levels of latent full-length myostatin complex (**A**) and its propeptide (**B**) among Non, Mod, and Xtr responders across three biopsy time points (baseline, 24-h acute response to the first loading bout, and after 16 wk of resistance training). Values are means ± SE. †Main training effect, P < 0.001. #Different from baseline within group, P < 0.005.
ELISA after 12 wk of resistance training in 12 subjects. The we report as MSTNPRO but did not recognize the bioactive sequences within the propeptide recognized the 28-kDa band following cleavage from MSTNPRO and, presum-

The fate of the bioactive 12-kDa COOH-terminal myostatin is remarkable, based on the extreme variability we found in these subjects. Recent work on 17 humans reported a 20% decline in serum myostatin concentration after 10 wk of heavy resistance training, with the bulk of this drop occurring 24 h after the first bout. Alterations in gene expression after a perturbation such as resistance loading are not doubt transient (and will return to baseline given sufficient time with no additional stimulus). The duration of the myostatin mRNA transient is not known, but suppression as many as 48 h postresistance exercise has been reported (17). In the present study, the magnitudes of myostatin mRNA downregulation did not differ between the two biopsies collected 24 h after the first and last loading bouts of the 16-wk training program, suggesting expression may have remained suppressed during the 48 h between loading bouts.

Myostatin or growth and differentiation factor-8 inhibits cell proliferation in part by upregulating cell cycle inhibitors such as p21cip1 (24, 44). Studies have shown that C2C12 myoblasts in growth media are arrested in the G1 phase of the cell cycle by the addition of myostatin, which concomitantly leads to marked induction of p21cip1 (24, 44). Growth and differentiation factor-11, a myostatin homolog, has been shown to inhibit neurogenesis by inducing p27kip1 and cell cycle arrest (51). Furthermore, myostatin expression is reduced in p27kip1 knockout mice, which experience robust muscle hypertrophy (26), and we have previously found a correlation between muscle transcript levels of myostatin and p27kip1 in humans (20). These combined data indicate p21cip1 is a myostatin target, while myostatin may itself be a target of p27kip1 in proliferation suppression. In the present study, myostatin mRNA and protein levels were not different across clusters; thus it is not surprising that these markers of cell cycle arrest were similar among clusters. However, because myostatin mRNA was uniformly suppressed with training, one might expect to find overall training effects for p21cip1 and p27kip1 transcripts. Of course, the interpretation of these data is tempered by the limitations of only assessing transcript levels.

The progression of cells through the G1 phase of the cell cycle requires D- and E-type cyclins. Recent evidence indicates cyclin D1 is another downstream target of myostatin, as treatment of C2C12 cells with myostatin arrests the cell cycle in G1 by inducing proteasome-dependent degradation of cyclin D1 (52). Furthermore, overexpression of cyclin D1 overrides the inhibitory influence of myostatin on proliferation (52). In the present study, the transcript for cyclin D1 was the only target found to be differentially expressed by cluster, as only Xtr subjects realized an acute increase after the first resistance loading bout. Additionally, a main cluster effect was driven by...
higher average levels of cyclin D1 in Xtr across the 16-wk training program. Based on these data, combined with the finding of Yang et al. that cyclin D1 overexpression negates the influence of myostatin (52), we suggest cyclin D1 induction may be a more meaningful indicator of proliferative potential during resistance training than myostatin suppression. Enhanced cyclin D1 expression has been found in a number of acute resistance loading models (1, 6, 20), but to our knowledge the current findings are the first to demonstrate an up-regulation of cyclin D1 mRNA during long-term training, which differed concomitant with the hypertrophic adaptation among clusters. While cyclin D1 is an integral component of the general cell cycle machinery (i.e., not muscle stem cell specific), we have previously shown strong relationships between changes in its expression and changes in the expression of the muscle-specific mitogen, mechanogrowth factor (or IGF-I isoform Ec) (20). We, therefore, suggest the early and robust induction of cyclin D1 in Xtr may have led to superior mitogenic activity among the resident myogenic stem (satellite) cells in Xtr compared with Non. Although this is limited to speculation, since we did not assess cyclin D1 protein levels, the concept is supported by our prior work demonstrating robust increases in satellite cell number and myonuclei per myofiber in subjects experiencing the most myofiber growth (34).

Recent findings from Coffey et al. (8, 9) demonstrate the influence of training history and type of exercise training on acute gene expression responses in skeletal muscle. These investigators found that several mRNA level responses to acute resistance exercise thought to promote myogenesis, including myostatin downregulation, were observed in subjects unaccustomed to resistance loading (i.e., endurance trained) but were not seen in experienced strength-trained subjects, suggesting a blunted transcriptional response to each loading bout after some period of training (8). Our previously untrained subjects, who continued to show load-mediated mRNA responses after 16 wk of training in this and other studies (3, 22, 34), would not be considered “highly resistance trained” after such a 16-wk training program. Reaching the training state at which responsiveness to loading is blunted may require several months or years. We (20, 21) and others (5, 6, 35, 36, 49, 53) have studied gene expression following acute resistance loading in humans with the general goal of determining which factors involved in myogenesis are responsive to mechanical load. While these data are certainly valuable and progress our understanding of the powerful influence mechanical load exerts on local cellular and molecular processes, our present findings suggest, at least when analyzed 24 h after resistance exercise in either untrained (acute response) or trained (16 wk) muscle, that down-regulation of myostatin is not predictive of one’s potential for long-term resistance training-mediated myofiber hypertrophy.

In addition, training-induced changes in full-length MSTN\textsuperscript{CMPLX} protein appeared to be opposite from changes in transcript levels in the present study (Fig. 3A). Our findings fit well with the model of “myostatin auto-regulation” proposed by Forbes and colleagues (12). They suggest that myostatin protein mediates a decrease in myostatin mRNA expression via a negative feedback loop through a Smad7-dependent mechanism. Although further studies are necessary, in the present study, we observed a modest inverse relationship ($r = -0.36, P < 0.05$) between levels of MSTN\textsuperscript{CMPLX} protein and myostatin mRNA expression in posttraining muscles at week 16.

In summary, it is abundantly clear from the literature that myostatin is perhaps the single most powerful negative regulator of muscle mass during mammalian development. Homozygous mutant animals (31) and humans (41) realize marked increases in both myofiber number and size early in the postnatal period, resulting in adult muscle masses considered extreme. Based on the early seminal findings in myostatin discovery and characterization, investigators have feverishly pursued myostatin blockade or inhibition as a potential therapeutic in adult conditions of muscle wasting or atrophy. We report herein that myostatin protein levels quantified in serum and muscle, along with muscle myostatin transcript levels, do little to determine individual responsiveness to a resistance training stimulus for muscle growth in adult humans, including a relatively large cohort of sarcopenic elderly.

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MYOSTATIN AND MYOFIBER HYPERTROPHY


