

# Myostatin Gene Expression Is Reduced in Humans with Heavy-Resistance Strength Training: A Brief Communication

STEPHEN M. ROTH,<sup>\*,†,1</sup> Gregory F. Martel,<sup>†,‡</sup> Robert E. Ferrell,<sup>\*</sup> E. Jeffrey Metter,<sup>§</sup> Ben F. Hurley,<sup>†</sup> and Marc A. Rogers<sup>†</sup>

<sup>\*</sup>Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA 15261; <sup>†</sup>Department of Kinesiology, University of Maryland, College Park, Maryland 20742; <sup>‡</sup>Department of Physical Therapy, University of Maryland Eastern Shore, Princess Anne, Maryland 21853; <sup>§</sup>Laboratory of Clinical Investigation, Intramural Research Program, National Institute on Aging, Baltimore, Maryland 21224

This study examined changes in myostatin gene expression in response to strength training (ST). Fifteen young and older men ( $n = 7$ ) and women ( $n = 8$ ) completed a 9-week heavy-resistance unilateral knee extension ST program. Muscle biopsies were obtained from the dominant vastus lateralis before and after ST. In addition to myostatin mRNA levels, muscle volume and strength were measured. Total RNA was reverse transcribed into cDNA, and myostatin mRNA was quantified using quantitative PCR by standard fluorescent chemistries and was normalized to 18S rRNA levels. A 37% decrease in myostatin expression was observed in response to ST in all subjects combined ( $2.70 \pm 0.36$  vs  $1.69 \pm 0.18$  U, arbitrary units;  $P < 0.05$ ). Though the decline in myostatin expression was similar regardless of age or gender, the small number of subjects in these subgroups suggests that this observation needs to be confirmed. No significant correlations were observed between myostatin expression and any muscle strength or volume measure. Although further work is necessary to clarify the findings, these data demonstrate that myostatin mRNA levels are reduced in response to heavy-resistance ST in humans. *Exp Biol Med* 228:706–709, 2003

**Key words:** GDF8; skeletal muscle; transcription

**M**yostatin (growth and differentiation factor 8 [GDF8]) is a transforming growth factor- $\beta$  superfamily member with importance as a negative growth regulator for skeletal muscle, such that mutations in the myostatin gene result in a hypermuscular phenotype in

mice and cattle (1–4). How myostatin influences muscle phenotypes is unclear. Higher levels of myostatin immunoreactivity have been reported in the muscle and serum of HIV-infected men with muscle wasting (5) and in the plasma of young men exposed to prolonged bed rest (6). Reardon *et al.* (7) reported higher levels of myostatin mRNA in muscle samples from patients with chronic disuse atrophy associated with hip osteoarthritis compared with healthy control subjects. In rats, two reports have demonstrated that myostatin expression is reduced from elevated levels in response to reloading after atrophy-inducing conditions (8, 9). Thus, increases in myostatin expression have been observed in conditions associated with muscle atrophy, and myostatin expression appears to be responsive to muscle loading after atrophy.

In the present study, we sought to determine whether myostatin expression was altered by increased muscle loading in healthy individuals. Presumably, for muscle hypertrophy to occur in response to a stimulus, intrinsic regulators of muscle cell size must be altered in some way. To address this issue, we studied the response of myostatin expression in humans to heavy-resistance strength training (ST), which is associated with increases in muscle strength and mass (10, 11), and we hypothesized that ST would reduce myostatin expression.

The subjects studied consisted of properly consented young (20–30 years old; 4 men, 4 women) and older (65–75 years old; 3 men, 4 women) healthy, sedentary men and women. All subjects performed a 9-week unilateral heavy-resistance ST program as previously described in detail (12). Briefly, the training protocol consisted of unilateral ST of the dominant leg performed by the knee extensors using a Keiser K-300 pneumatic resistance machine 3 days per week. Each supervised ST session resulted in 50 completed repetitions performed at near maximal resistance, thus resulting in a highly strenuous muscle loading stimulus. Specified rest periods (90–180 sec) were allowed between

This work was supported by the Competitive Medical Research Fund of the UPMC Health System, Grants DK-46204 and AG-42148. S.R. was supported by Grant AG-05893.

<sup>1</sup> To whom requests for reprints should be addressed at Department of Kinesiology, University of Maryland, College Park, MD 20742. E-mail: sroth1@umd.edu

Received July 1, 2002.

Accepted January 7, 2003.

1535-3702/03/2286-0706\$15.00

Copyright © 2003 by the Society for Experimental Biology and Medicine

sets, and progressive increases in resistance occurred throughout the 9-week training program. All subjects were reminded throughout the study not to alter their regular activity levels or dietary habits for the duration of the investigation.

Both before and after ST, subjects completed tests aimed at characterizing muscle phenotypes. One repetition maximum (1RM) strength was measured as described in detail elsewhere (12) with standard techniques using the same Keiser equipment used for the strength training. Percentage of body fat was determined using dual-energy X-ray absorptiometry using standard techniques, and muscle volume of the quadriceps was measured using magnetic resonance imaging (MRI) as previously described (13). Briefly, the dominant thigh of each subject was scanned before and at least 48 hr after the last ST session to obtain axial slices of the quadriceps muscle group extending from the superior border of the patella to the anterior superior iliac spine. The cross-sectional area (CSA) of each quadriceps slice was determined from the images using NIH Image version 1.61 software and the quadriceps muscle volume was calculated. The details of the study design, as well as complete analyses of the response of muscle phenotypes to ST, have been previously published (11–14).

Biopsies were obtained using standard needle biopsy techniques from the vastus lateralis muscle of the trained leg 2 weeks before ST and again 48 to 72 hr after the last ST session. Muscle samples were not available from the untrained leg. Total RNA was extracted from the samples using a standard phenol-based extraction method (Ambion, Austin, TX), quantified by determining absorbance at 260 nm in triplicate, treated with DNase I (Ambion), and reverse transcribed using the Reverse Transcription Reagents kit (Applied Biosystems, Foster City, CA) with random hexamers.

Myostatin expression was determined by quantitative, or real time, PCR (qPCR) using the ABI 7700 DNA Sequence Detection System (TaqMan; Applied Biosystems) using standard fluorescent chemistries and thermal cycling conditions as directed by the manufacturer. Primer and probe sequences were designed for myostatin mRNA (NM\_005259) using Primer Express software (Applied Biosystems) as follows:

Forward primer: 5'-AGGTATACTGGAATCC GATCTCTGA-3'

Reverse primer: 5'-CACTGTCTTCACATCAAT GCTCTG-3'

Probe: 5'-CTTGACATGAACCCAGGCACTGG TATTTG-3'

Sequences were chosen to exclude known sites of sequence variability (15). The probe was labeled in the standard manner with FAM reporter (5') and TAMRA quencher (3') dyes. 18S rRNA was used as an internal expression control and was amplified using the Ribosomal RNA Control Reagents kit (Applied Biosystems). For each myostatin qPCR reaction, 14 ng of cDNA was added to optimized

primer and probe concentrations, with 25  $\mu$ l of PCR Master Mix. A corresponding well contained 7 ng of cDNA with reaction reagents for the qPCR of 18S rRNA. All reactions were performed in duplicate. Thermal cycling conditions were as specified by the manufacturer: 50°C for 2 min, 95°C for 10 min, and 40 cycles as follows: 95°C for 15 sec and ramp to 60°C for 1 min. Known concentration standards were developed using cDNA (obtained as outlined above) from a commercially available skeletal muscle total RNA source (Ambion), and optimization reactions were performed to ensure that all experimental samples fell within the range of the resulting standard curves. Standard curves were generated for both myostatin and 18S rRNA, and the  $C_T$  (Cycle Threshold) value for each sample was then used to calculate relative expression based on the respective standard curve equation, according to the manufacturer's instructions. Myostatin expression level was normalized to the 18S rRNA control gene quantity after determining no change in 18S rRNA expression in response to ST.

One-way analysis of variance (ANOVA) with repeated measures was used to test for changes in myostatin expression in response to ST, with LSD *post hoc* analysis. Independent sample *t* tests were used to confirm no age and sex differences in myostatin expression, thus allowing the subjects to be pooled for statistical analysis. For the pooled samples, a paired-sample *t* test was used to test for ST-related (before versus after ST) changes in normalized myostatin expression. Analysis of non-normalized myostatin expression (e.g., without normalizing to 18S rRNA) revealed similar results (data not shown). Linear regression analysis was performed to determine potential associations of myostatin expression with muscle strength and mass phenotypes.

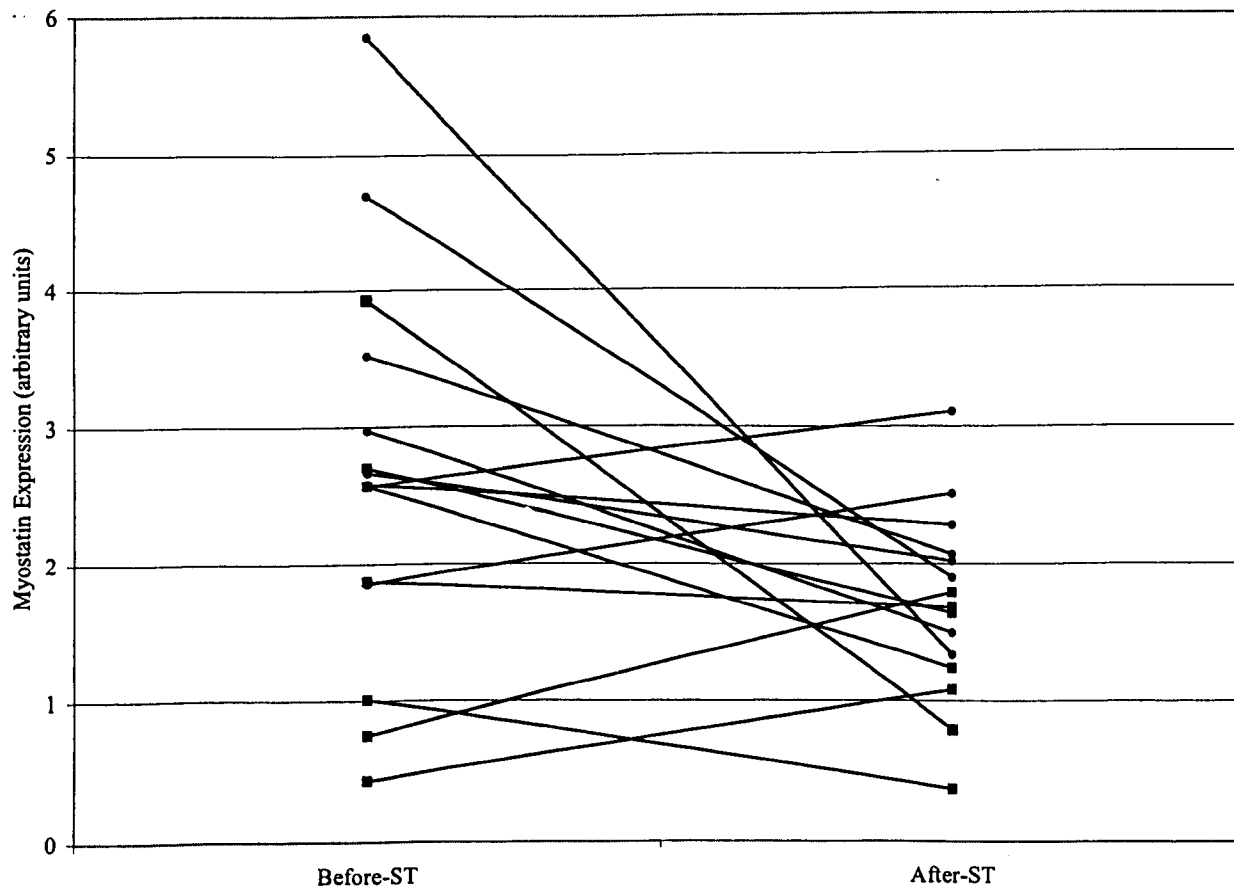
Subject characteristics are shown in Table I. ST significantly reduced normalized myostatin expression by 37% ( $2.70 \pm 0.36$  vs  $1.69 \pm 0.18$  U, arbitrary units;  $P < 0.05$ ; Fig. 1), but had no effect on 18S rRNA expression. ANOVA analysis revealed a significant change in myostatin expres-

**Table I. Subject Characteristics**

	Men	Women
<i>n</i>	7	8
Height (cm)	173 $\pm$ 2	162 $\pm$ 2
Before ST weight (kg)	71.3 $\pm$ 2.9	62.4 $\pm$ 4.2
After ST weight (kg)	71.3 $\pm$ 2.8	63.0 $\pm$ 4.3
Before ST body fat (%)	22.4 $\pm$ 2.1	34.6 $\pm$ 3.1
After ST body fat (%)	21.9 $\pm$ 2.2	34.7 $\pm$ 2.7
Before ST muscle volume (cm <sup>3</sup> )	1891 $\pm$ 196	1227 $\pm$ 188
After ST muscle volume (cm <sup>3</sup> )	2017 $\pm$ 177 <sup>a</sup>	1329 $\pm$ 180 <sup>a</sup>
Before ST 1RM (kg)	86.7 $\pm$ 6.3	47.1 $\pm$ 4.9
After ST 1RM (kg)	106.5 $\pm$ 8.2 <sup>a</sup>	64.6 $\pm$ 5.8 <sup>a</sup>

Note. Data are means  $\pm$  SE.

<sup>a</sup> Significant increase compared with Before ST ( $P < 0.05$ ). Both groups included four young and three to four older subjects, with young individuals aged 20 to 30 years, and older individuals age 65 to 75 years.



**Figure 1.** Myostatin expression levels (arbitrary units) for before ST ( $n = 15$ ) versus after ST ( $n = 15$ ) for each individual. ●, males; ■, females. A significant decrease in myostatin expression was observed in response to ST,  $P < 0.05$ , with no group differences.

sion in response to ST ( $P < 0.05$ ), with no significant age or gender differences. Figure 1 shows the individual responses for each subject, demonstrating that the majority of individuals showed a reduction in myostatin expression, with no gender differences in the response. Subjects with lower baseline levels of myostatin were more likely to show little or no change in expression in response to ST. No significant relationships were observed between myostatin expression and muscle volume, strength, or body mass for either baseline expression or change in expression in response to ST.

The present study is the first to examine the influence of muscle loading on myostatin expression in normally ambulatory, healthy human muscle. We observed a significant decrease in myostatin expression in response to 9 weeks of heavy-resistance ST in previously sedentary, healthy men and women. The ST-mediated decline in myostatin expression appeared to be independent of age or gender, but the small number of subjects in these subgroups will require additional studies to confirm that finding.

Myostatin is well established as a negative regulator of muscle growth in animal models (16). The mechanism by which myostatin inhibits muscle growth is uncertain, although Carlson *et al.* (17) and others (18, 19) have suggested an inhibitory effect on skeletal muscle satellite cells. We pursued the present investigation with the hypothesis

that myostatin mRNA levels would be reduced in response to a program of heavy-resistance ST designed to increase muscle mass and strength (i.e., inhibition of the growth inhibitor). Wehling *et al.* (8) demonstrated that periodic reloading of otherwise unloaded rat plantaris muscle resulted in less elevated myostatin levels (55% increase versus ambulatory controls) compared with a continuously unloaded condition (110% increase versus ambulatory controls). Moreover, Lalani *et al.* (9) reported increased myostatin mRNA and protein in several rat muscles in response to microgravity, and a normalization of those levels after the return to normal gravitation. Recent work by Kawada *et al.* (20) demonstrated that myostatin expression was significantly reduced upon reloading of hindlimb suspended muscle in mice, with no change in expression in response to either aging-related or unloading-induced atrophy. These data indicate that myostatin is responsive to muscle loading in some contexts, and thus might represent an important cell size regulator for skeletal muscle.

In the present study, the issue was whether myostatin expression was responsive to additional muscle loading in normal, ambulatory (although sedentary) individuals. As Lalani *et al.* (9) postulate, a homeostatic balance likely exists between positive (e.g., insulin-like growth factors) and negative (e.g., myostatin) growth regulators in skeletal

muscle, thus providing for maintenance of muscle fiber size over moderate amounts of time. This balance would be expected to shift during loading or atrophy conditions, thus resulting in changes in muscle cell size. Our results indicate that heavy-resistance ST leads to a significant reduction in skeletal muscle myostatin expression in young and older men and women, with no age or gender differences. No significant correlations were observed with changes in muscle strength or volume, which is consistent with previous observations in animal models (8, 17). Future investigations will be required to verify and extend the findings of the present work.

In conclusion, ST significantly reduces skeletal muscle myostatin (GDF8) expression in sedentary, healthy men and women. These data provide evidence that myostatin is responsive to muscle loading in healthy adult humans, which may have relevance in future work where myostatin is studied as a therapeutic target for muscle wasting disorders.

We thank the volunteers who participated in this study, as well as Drs. Fred Ivey, Jeff Lemmer, Brian Tracy, Diane Hurlbut, and Mary Lott for help with strength training, biopsies, and study coordination.

1. Grobet L, Martin LJR, Poncelet D, Pirottin D, Brouwers B, Riquet J, Schoeberlein A, Dunner S, Menissier F, Massabanda J, Fries R, Hanset R, Georges M. A deletion in the bovine myostatin gene causes the double-muscling phenotype in cattle. *Nat Genet* **17**:71–74, 1997.
2. Kambadur R, Sharma M, Smith TPL, Bass JJ. Mutations in myostatin (GDF8) in double-muscling Belgian Blue and Piedmontese cattle. *Genome Res* **7**:910–916, 1997.
3. McPherron AC, Lawler AM, Lee S-J. Regulation of skeletal muscle mass in mice by a new TGF- $\beta$  superfamily member. *Nature* **387**:83–90, 1997.
4. McPherron AC, Lee S-J. Double muscling in cattle due to mutations in the myostatin gene. *Proc Natl Acad Sci U S A* **94**:12457–12461, 1997.
5. Gonzalez-Cadavid NF, Taylor WE, Yarasheski KE, Sinha-Hikim I, Ma K, Ezzat S, Shen R, Lalani R, Asa S, Mamita M, Nair G, Arver S, Bhasin S. Organization of the human myostatin gene and expression in healthy men and HIV-infected men with muscle wasting. *Proc Natl Acad Sci U S A* **95**:14938–14943, 1998.
6. Zachwieja JJ, Smith SR, Sinha-Hikim I, Gonzalez-Cadavid NF, Bhasin S. Plasma myostatin-immunoreactive protein is increased after prolonged bed rest with low-dose T3 administration. *J Gravit Physiol* **6**:11–15, 1999.
7. Reardon KA, Davis J, Kapsa RMI, Choong P, Byrne E. Myostatin, insulin-like growth factor-1, and leukemia inhibitory factor mRNAs are upregulated in chronic human disuse muscle atrophy. *Muscle Nerve* **24**:893–899, 2001.
8. Wehling M, Cai B, Tidball JG. Modulation of myostatin expression during modified muscle use. *FASEB J* **14**:103–110, 2000.
9. Lalani R, Bhasin S, Byhower F, Tarnuzzer R, Grant M, Shen R, Asa S, Ezzat S, Gonzalez-Cadavid NF. Myostatin and insulin-like growth factor-I and -II expression in the muscle of rats exposed to the microgravity environment of the Neurolab space shuttle flight. *J Endocrinol* **167**:417–428, 2000.
10. Roth SM, Ivey FM, Martel GF, Lemmer JT, Hurlbut DE, Siegel EL, Metter EJ, Fleg JL, Fozard JL, Kostek MC, Wernick DM, Hurley BF. Muscle size responses to strength training in young and older men and women. *J Am Geriatr Soc* **49**:1428–1433, 2001.
11. Lemmer JT, Hurlbut DE, Martel GF, Tracy BL, Ivey FM, Metter EJ, Fozard JL, Fleg JL, Hurley BF. Age and gender responses to strength training and detraining. *Med Sci Sports Exerc* **32**:1505–1512, 2000.
12. Roth SM, Martel GF, Ivey FM, Lemmer JT, Tracy BL, Hurlbut DE, Metter EJ, Hurley BF, Rogers MA. Ultrastructural muscle damage in young vs. older men after high-volume, heavy-resistance strength training. *J Appl Physiol* **86**:1833–1840, 1999.
13. Ivey FM, Roth SM, Ferrell RE, Tracy BL, Lemmer JT, Hurlbut DE, Martel GF, Siegel EL, Fozard JL, Metter EJ, Fleg JL, Hurley BF. Effects of age, gender and myostatin genotype on the hypertrophic response to heavy resistance strength training. *J Gerontol Med Sci* **55A**:M641–M648, 2000.
14. Roth SM, Martel GF, Ivey FM, Lemmer JT, Metter EJ, Hurley BF, Rogers MA. High volume, heavy resistance strength training and muscle damage in young and older women. *J Appl Physiol* **88**:1112–1118, 2000.
15. Ferrell RE, Conte V, Lawrence EC, Roth SM, Hagberg JM, Hurley BF. Frequent sequence variation in the human myostatin (GDF8) gene as a marker for analysis of muscle-related phenotypes. *Genomics* **62**:203–207, 1999.
16. Oldham JM, Martyn JAK, Sharma M, Jeanplong F, Kambadur R, Bass JJ. Molecular expression of myostatin and MyoD is greater in double-muscling than normal-muscling cattle fetuses. *Am J Physiol Regulatory Integrative Comp Physiol* **280**:R1488–R1493, 2001.
17. Carlson CJ, Booth FW, Gordon SE. Skeletal muscle myostatin mRNA expression is fiber-type specific and increases during hindlimb unloading. *Am J Physiol* **277**:R601–R606, 1999.
18. Sharma M, Langley B, Bass J, Kambadur R. Myostatin in muscle growth and repair. *Exerc Sport Sci Rev* **29**:155–158, 2001.
19. Yamanouchi K, Soeta C, Naito K, Tojo H. Expression of myostatin gene in regenerating skeletal muscle of the rat and its localization. *Biochem Biophys Res Commun* **270**:510–516, 2000.
20. Kawada S, Tachi C, Ishii N. Content and localization of myostatin in mouse skeletal muscles during aging, mechanical unloading and reloading. *J Musc Res Cell Motil* **22**:627–633, 2001.