Skeletal muscle myostatin mRNA expression is fiber-type specific and increases during hindlimb unloading

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Carlson, Christian J., Frank W. Booth, and Scott E. Gordon. Skeletal muscle myostatin mRNA expression is fiber-type specific and increases during hindlimb unloading. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R601–R606, 1999.—Transgenic mice lacking a functional myostatin (MSTN) gene demonstrate greater skeletal muscle mass resulting from muscle fiber hypertrophy and hyperplasia (McPhearron, A. C., A. M. Lawler, and S.-J. Lee. Nature 387: 83–90, 1997). Therefore, we hypothesized that, in normal mice, MSTN may act as a negative regulator of muscle mass. Specifically, we hypothesized that the predominately slow (type I) soleus muscle, which demonstrates greater atrophy than the fast (type II) gastrocnemius-plantaris complex (Gast/PLT), would show more elevation in MSTN mRNA abundance during hindlimb unloading (HU). Surprisingly, MSTN mRNA was not detectable in weight-bearing or HU soleus muscle, which atrophied 42% by the 7th day of HU in female ICR mice. In contrast, MSTN mRNA was present in weight-bearing Gast/PLT muscle and was significantly elevated (67%) at 1 day but not at 3 or 7 days of HU. However, the Gast/PLT muscle had only atrophied 17% by the 7th day of HU. Because the soleus is composed only of type I and IIa fibers, whereas the Gast/PLT expresses type II/d/x and IIb in addition to type I and IIa, it was necessary to perform a more careful analysis of the relationship between MSTN mRNA levels and myosin heavy-chain (MHC) isoform expression (as a marker of fiber type). A significant correlation (r = 0.725, P < 0.0005) was noted between the percentage of MHC isoform IIb expression and MSTN mRNA abundance in several muscles of the mouse hindlimb. These results indicate that MSTN expression is not strongly associated with muscle atrophy induced by HU; however, it is strongly associated with MHC isoform IIb expression in normal muscle.

mice; atrophy; myosin heavy-chain isoforms

MYOSTATIN (MSTN) is a recently identified member of the transforming growth factor (TGF)-β family of growth factors. The MSTN gene is highly conserved among many vertebrate species and is preferentially expressed in developing and adult skeletal muscle (18). Transgenic mice lacking the MSTN gene exhibit enlarged skeletal muscle mass, resulting from both hyperplasia and hypertrophy of muscle fibers (18). Additionally, the Belgian Blue and Piedmontese breeds of cattle, in which genetic mutations have presumably led to an inactive MSTN protein (11, 16, 19), are termed “double muscled” because of their skeletal muscle hypertrophy. Thus it has been suggested that the presence of MSTN in a wild-type animal may act as a negative regulator of muscle mass (18). We speculated that the upregulation of MSTN expression could cause muscle atrophy.

Skeletal muscle mass is sensitive to many factors, including load bearing and caloric intake. In rodents, removal of load bearing via hindlimb unloading results in a significant decrease in wet weight of the unloaded muscles within 1 wk (25). This muscle atrophy is accompanied by a decrease in protein synthesis rate and fiber cross-sectional area as well as a shift in expression of many muscle-specific proteins, such that the atrophying skeletal muscle becomes a faster, less metabolically active phenotype (25). The mechanisms by which skeletal muscle senses and responds to changes in load are not completely delineated.

An elevated MSTN gene expression is appealing as a possible factor contributing to skeletal muscle atrophy because of the hypertrophy observed in animals lacking the gene (18). Thus it was a hypothesis of this investigation that MSTN mRNA increases during the early stages of muscle atrophy induced by hindlimb unloading in adult mice. We expected to see the greatest increase in MSTN mRNA in the soleus muscle because it atrophies the most with hindlimb unloading (25). However, we were surprised that MSTN mRNA was in fact undetectable in either the weight-bearing or atrophying soleus muscle, whereas MSTN mRNA was detectable in the fast gastrocnemius-plantaris (Gast/PLT) muscle complex. Because the soleus is composed of type I and type IIa fibers, we wondered whether MSTN mRNA was expressed in type II/d/x or type IIb fibers present in the Gast/PLT muscle. This led us to a more careful analysis of the relationship between MSTN mRNA levels and percentage of various myosin heavy-chain (MHC) isoforms (as a marker of fiber type) in some of the mouse hindlimb muscles.

METHODS

Animals. All procedures were approved by the Animal Welfare Committee at the University of Texas Health Science Center at Houston. All animals were housed in an American Association for Accreditation of Laboratory Animal Care-accredited facility with a 12:12-h light-dark cycle and were maintained on a diet of standard rodent chow (Harlan). Female ICR mice (21–24 g, Harlan) were used for all experiments in this investigation. In the first experiment, 44 mice were divided into six groups, corresponding to 1, 3, and 7 days of either hindlimb unloading (HU) or weight-bearing (WB) control. A subsequent pair-feeding experiment used 10 mice that were body-weight matched to 1-day HU animals and assigned to one of two groups: WB animals pair fed (PF) to 1-day HU animals and WB animals fed ad libitum.

HU. HU was performed by using a modification of the protocol used for rats by Babij and Booth (3), as described by Criswell et al. (7). For the unloading procedure, the applica-
tion of adhesive tape to the tails of the HU animals was accomplished after the animals were lightly anesthetized with an intraperitoneal injection of ketamine (100 mg/kg body wt). All WB and PF animals were also anesthetized in this manner at the "preunloading" time point. With the exception of the PF group in the pair-feeding experiment, all mice had ad libitum access to chow and water throughout the experimental protocol.

Pair-feeding experiment. It has been observed that food intake decreases briefly after the onset of HU (17). Because the physiological factors involved with regulating MSTN gene expression were unknown, it was necessary to determine whether HU-induced changes in MSTN mRNA were due to a decreased caloric intake. Food intake (by weight) was assessed in a subset of five of the 1-day HU animals in the initial experiment. In a follow-up experiment, an identical amount of food as consumed by 1-day HU animals was given to and consumed by five appropriate body weight-matched WB animals during a 24-h period (PF). During the same 24-h period, five additional WB animals of the same body weight were fed ad libitum and served as a comparison group for the PF animals.

Tissue sampling. Immediately after each experimental protocol, mice were anesthetized with an intraperitoneal injection of a cocktail containing ketamine (73.9 mg/kg), xylazine (3.7 mg/kg), and acepromazine (0.7 mg/kg) at a dose of 1.8 ml/kg body mass. All HU animals were anesthetized before removal from the unloading device so that no WB occurred before extraction of muscles. Post-HU body mass was then measured. To examine differences in MSTN expression and fiber type, two synergistic muscles of the mouse hindlimb with differing fiber types were analyzed. The Gast/PLT and soleus muscles were removed from WB and HU animals and processed for RNA extraction. To obtain a greater mass of tissue for RNA extraction, the Gast/PLT muscles were not separated and were considered as one muscle. Soleus muscles were analyzed as pooled samples consisting of muscle tissue from three to four animals. To further characterize the fiber type-specific expression of MSTN mRNA, the following muscles were also removed from some of the WB animals and processed for RNA extraction and MHC analysis: tibialis anterior, extensor digitorum longus, visibly white portion of the quadriceps, and visibly red portion of the quadriceps. The mixed portion of the quadriceps was discarded. All muscles were weighed on an analytical balance (Mettler) and quick-frozen in liquid nitrogen. After this procedure, the anesthetized mice were killed by cervical dislocation. All muscles were powdered under a mortar and pestle cooled in liquid nitrogen and aliquoted for RNA isolation and protein analysis, where appropriate. Because of the small size of muscle masses, analysis of fiber-type expression of MSTN mRNA and MHC isoforms was performed on pooled samples, with each sample consisting of muscles from three to four WB animals that were pooled before powdering.

RNA isolation. Total RNA was extracted by using the guanidinium thiocyanate method of Chomczynski and Sacchi (5) with Trizol (GIBCO BRL, Gaithersburg, MD). The extracted RNA was dissolved in diethyl pyrocarbonate-treated water, and the RNA concentration was determined spectrophotometrically at 260 nm.

Protein isolation. Powdered muscle was homogenized in 0.1% Triton X-100, 50 mM HEPES (pH = 7.4), 4 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), 10 mM EDTA, 15 mM Na3P2O7·10H2O, 100 mM β-glycerophosphate, 25 mM NaF, 5 mM NaVO4, and 50 µg/µl leupeptin, pepstatin, and aprotinin. The resulting homogenate was diluted 1:10 in 50% glycerol, 50 mM sodium pyrophosphate, 2.5 mM EGTA, and 1 mM β-mercaptoethanol and stored at −20°C until analysis.

MHC gel electrophoresis and analysis. MHC isoforms were separated on SDS-polyacrylamide gel electrophoresis gels by using a modification of the protocol developed by Talmadge and Roy (24). Briefly, protein samples were diluted 1:8 in Laemmli sample buffer and boiled for 2 min before separation on an 8% acrylamide-30% glycerol gel. Gel electrophoresis was performed at 8°C for 30 h. Proteins were visualized with Coomassie brilliant blue. After being destained and dried, the gels were scanned with the use of a flatbed scanner, and the area under the curve for each MHC peak was determined by using the gel plotting program associated with NIH Image (version 1.61; National Institutes of Health, Bethesda, MD).

Northern blot analyses. Northern blot analysis was used to assess the relative abundance of MSTN mRNA, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA, and 18S RNA. Extracted total RNA for each muscle was loaded onto denaturing 1% agarose-formaldehyde gel [0.1 M 3-(N-morpholino)propanesulfonic acid and 6.7% formaldehyde] and electrophoresed at 4 V/cm for 3 h. The RNA was then transferred to a nylon membrane (Hybond-N; Amersham, Arlington Heights, IL) by capillary action and ultraviolet cross-linked to the membrane. Radiolabeled cDNA probes were prepared by random priming with [32P]deoxyctydine triphosphate (Amersham). A 749-base pair fragment of the MSTN cDNA (generously provided by Dr. S. Lee) corresponding to bases 1928–2676 of the mouse cDNA was used for the probe. Small cDNAs corresponding to portions of the mouse GAPDH mRNA and 18S RNA were purchased from Ambion (Austin, TX). Membranes containing the RNA were hybridized in hybridization buffer (Quick-Hyb; Stratagene, La Jolla, CA). The bands corresponding to MSTN, GAPDH, or 18S RNA were visualized by autoradiography and quantified by scanning densitometry (Bioimage; Millipore, Ann Arbor, MI). The integrated optical densities (IODs) of MSTN bands were divided by the IODs for GAPDH or 18S RNA bands from the same lane to correct for variations in loading and/or transfer. Normalization to 18S RNA was performed in the fiber-type experiment because GAPDH mRNA is expressed in a fiber type-specific manner (6).

Statistical analyses. Statistical differences were determined via the use of multivariate ANOVAs in this investigation (Statistica; StatSoft, Tulsa, OK). Repeated measures were employed for multivariate ANOVAs requiring a within-subjects analysis. Where appropriate, subsequent post hoc comparisons were accomplished with a Fisher's least significant difference test. Pearson product-moment analyses were used to calculate correlation coefficients. Statistical significance in this study was chosen as P < 0.05.

RESULTS

Effects of HU on body and muscle weights. Table 1 displays the body and muscle weights of WB and HU mice. HU resulted in a decrease in body weight (P = 0.055 at day 1, P < 0.05 by days 3 and 7). Significant decreases of 11 and 17% in Gast/PLT wet weights occurred after 3 and 7 days of unloading, respectively. Soleus muscle wet weight was also decreased significantly by 16 and 42% after 3 and 7 days of unloading, respectively. Previous HU experiments in mice (7, 14) have reported significant muscle atrophy within 2 wk of HU. The results of the current study further demon-
Effects HU on MSTN gene expression. Figure 1 illustrates the effect of 1, 3, and 7 days of HU on MSTN mRNA abundance in Gast/PLT muscles. MSTN mRNA was significantly elevated (67%) over WB in Gast/PLT muscles after 1 day of HU. However, MSTN mRNA was not different from WB after 3 or 7 days of HU. These results were the same regardless of whether MSTN mRNA was corrected for GAPDH mRNA or 18S RNA. Despite the substantial atrophy, MSTN mRNA was not detected by Northern blot analysis in the soleus muscles of control or HU mice at any time point examined.

Effects of acute caloric restriction on MSTN gene expression. Although not statistically significant, food intake was reduced by 21% in the selected subset of 1-day HU animals in the first experiment (n = 5, data not shown). Because others have also observed that caloric intake briefly decreases after the onset of HU in mice (17), the subsequent experiment tested whether our observed significant increase in skeletal muscle MSTN mRNA abundance during 1 day of HU was the result of reduced caloric intake. During a 24-h period, an identical amount of food (by weight) to that consumed by 1-day HU animals was subsequently given to five appropriate body weight-matched animals not undergoing HU (PF). After 1 day, the body weights of HU animals were 6% lower than the body weights of the PF animals, which were unchanged over the 24-h period. Caloric restriction alone (PF) did not result in an elevated MSTN mRNA abundance over ad libitum-fed WB animals (Fig. 2).

MSTN mRNA abundance correlates with the percentage of MHC isoform IIb expression. To further examine the potential fiber type-specific expression of MSTN mRNA, MHC isoforms were examined from several hindlimb muscles of normal WB young adult mice (Table 2). Figure 3 displays the significant positive correlation found between the percentage of MHC isoform IIb (MHC IIb) in a particular muscle and the abundance of MSTN mRNA in that muscle. The greatest amount of MSTN mRNA was found in the white portion of the quadriceps muscle, which was composed of 100% MHC IIb. In contrast, the soleus muscle did not contain a detectable amount of MHC IIb or MSTN mRNA. Moreover, the relationship between MHC IIb content and MSTN mRNA content also apparently exists regionally within a single muscle group. The red portion of the quadriceps, composed of ~50% MHC IIb, had ~17% of the MSTN mRNA found in the white portion of the quadriceps, which was composed of 100% MHC IIb. MSTN mRNA concentration was not significantly correlated with percent content of any other MHC protein.

DISCUSSION

The results of this experiment provide evidence that MSTN gene expression is not strongly involved in the

Table 1. Body and muscle weights of experimental groups

<table>
<thead>
<tr>
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<th>Body Wt, g</th>
<th>Muscle Wt, mg/pair</th>
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<tr>
<td></td>
<td>n Pre Post</td>
<td>Gast/PLT Soleus</td>
</tr>
<tr>
<td>1 day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WB 7</td>
<td>24.9 ± 0.5</td>
<td>25.1 ± 0.6</td>
</tr>
<tr>
<td>HU 7</td>
<td>24.9 ± 0.5</td>
<td>23.8 ± 0.5</td>
</tr>
<tr>
<td>3 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WB 8</td>
<td>24.9 ± 0.4</td>
<td>24.4 ± 0.3</td>
</tr>
<tr>
<td>HU 8</td>
<td>25.0 ± 0.5</td>
<td>23.6 ± 0.7</td>
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<tr>
<td>7 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WB 7</td>
<td>25.4 ± 1.0</td>
<td>25.4 ± 0.6</td>
</tr>
<tr>
<td>HU 7</td>
<td>25.0 ± 0.1</td>
<td>23.5 ± 1.0</td>
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</table>

Body and muscle weights of experimental groups at unloading durations of 1, 3, and 7 days. Values are means ± SE; n = no. of animals. WB, weight-bearing control; HU, hindlimb unloaded; pre, preunloading; post, postunloading; Gast/PLT, gastrocnemius-plantaris muscle complex. *Significantly different (P ≤ 0.05) from WB within specified unloading duration. †Significantly different (P ≤ 0.05) from preunloading body wt for specified group.

Fig. 1. Data from Northern blot analyses of myostatin (MSTN) mRNA in gastrocnemius-plantaris (Gast/PLT) muscles of weight-bearing (WB) or hindlimb-unloaded (HU) mice for 1, 3, or 7 days (n = 7 animals/group for 1 and 7 days; n = 8 animals/group for 3 days). A: integrated optical density (IOD) of MSTN mRNA normalized to IOD of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA (arbitrary units). *P < 0.05, significantly different from WB control within specified unloading duration. B: representative autoradiograph showing detection of a 2.9-kilobase (kb) band corresponding to MSTN mRNA and a 1.6-kb band corresponding to GAPDH mRNA. Hybridization with a GAPDH probe was used to ensure that equivalent amounts of RNA (15 µg) were loaded onto gel and transferred to nylon membrane.
regulation of muscle mass in wild-type mice. We found upregulation of MSTN mRNA at day 1 in fast fibers but not on days 3 or 7 of unloading, when significant muscle atrophy was observed. Thus the upregulation of MSTN mRNA preceded a measurable loss in Gast/PLT muscle wet weight. Furthermore, no MSTN mRNA expression was found in the soleus muscles, which experienced the greatest magnitude of muscle atrophy. A decreased caloric intake during the early period of suspension in mice has been reported (present study and Ref. 17). Thus it was necessary to examine the effects of short-term caloric restriction on skeletal muscle MSTN mRNA expression to determine whether the unloading-induced increase in MSTN mRNA was due to a decreased caloric intake. We found that MSTN mRNA was not increased in the Gast/PLT muscles of WB animals for which food intake was restricted to a level equal to that of weight-matched animals undergoing 1 day of HU. Ji et al. (15) have similarly reported that MSTN mRNA was not elevated by 3 days of starvation in young pigs. Collectively, these observations lead to the conclusion that MSTN mRNA expression may be transiently elevated during the early period of unloading in predomi-
nately fast muscle and that this elevation is probably not due to reduced energy availability.

Although an elevated MSTN mRNA was observed in the Gast/PLT complex with HU in this investigation, our Northern blot analysis was unable to detect MSTN mRNA in the soleus in either the WB or HU groups. However, as Criswell et al. (7) and others (17) have previously observed in mice, HU elicited a much greater atrophy in the soleus than in the Gast/PLT muscles. It still remains possible that MSTN is expressed at a level below the detection limits of Northern blot analysis. Nevertheless, because the soleus muscle displayed the greatest percentage of muscle atrophy during HU without detectable MSTN expression, it must be concluded that high amounts of MSTN mRNA are not necessary for atrophy of the soleus muscle.

Current and recent observations suggest that there may be fundamental differences in MSTN expression in various muscle-wasting conditions. Whereas a recent paper by Gonzalez-Cadavid et al. (10) reported an increased MSTN expression associated with muscle wasting in human immunodeficiency virus (HIV)-infected men, we only observed a transient increase in MSTN mRNA during unloading-induced atrophy of muscle predominately expressing fast MHC. HIV is a chronic disease, with muscle mass loss appearing over months; in contrast, muscle atrophy from unloading is acute with muscle mass, reaching its new lower steady-state level within 3 wk (25). Thus the rapid and transient change in MSTN mRNA expression in the unloaded Gast/PLT muscle makes sense because the stimulus (i.e., unloading) is drastic and occurs very rapidly.

Further evidence that MSTN gene expression is not involved in the regulation of muscle fiber size was the inverse relationship between MSTN mRNA abundance and muscles with large fiber sizes. Typically, muscles containing predominately MHC IIb have much larger fiber cross-sectional areas than those containing predominately MHC isoform I (20). Remarkably, a significant positive correlation between MHC IIb content and MSTN mRNA abundance was detected across a range of several muscles (Fig. 3). This is in apparent contrast to the hypothesis that MSTN is a negative determinant of muscle fiber size. In the present study, muscles expressing greater MHC IIb protein content had the highest MSTN mRNA levels and presumably greater fiber cross-sectional areas. Conversely, transgenic animals lacking a functional MSTN protein demonstrate an increased muscle fiber cross-sectional area (18).

Thus a paradox exists in which normal mice exhibit the highest MSTN mRNA expression associated with muscle fiber hypertrophy (18). This implies that, under normal physiological conditions, MSTN gene expression does not regulate muscle fiber size directly; rather, MSTN may act on other pathways that may influence muscle fiber size or the expression of the fast phenotype.

It has also been previously demonstrated that muscles that predominately express fast MHC have lower satel-

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Table 2. MHC isoform distributions of selected muscles

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Sample Size</th>
<th>Percent Distribution</th>
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<tr>
<td></td>
<td></td>
<td>MHC I</td>
</tr>
<tr>
<td>Soleus</td>
<td>4 (10)</td>
<td>70.4±6.3</td>
</tr>
<tr>
<td>Red quad</td>
<td>4 (10)</td>
<td>5.4±0.6</td>
</tr>
<tr>
<td>TA</td>
<td>4 (10)</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>EDL</td>
<td>3 (7)</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>White quad</td>
<td>4 (10)</td>
<td>0.0±0.0</td>
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</table>

Values are means ± SE. Sample size is no. of pooled homogenates, with total no. of animals listed in parentheses (2 or 3 animals/homogenate; see METHODS for further description of pooling). MHC, myosin heavy chain; quad, quadriceps; TA, tibialis anterior; EDL, extensor digitorum longus.
lite cell densities compared with muscles expressing slow MHC (9, 23). Interestingly, TGF-β has an inhibitory activity on satellite cell proliferation and differentiation in culture (1, 2). Thus it is possible that MSTN, as a member of the TGF-β family, may also affect satellite cell activity. Under normal physiological conditions, it is possible that higher MSTN mRNA expression in MHC IIb fibers is actually a negative feedback mechanism to inhibit satellite cell proliferation and thus inhibit any further increase in fiber size. This paradigm is consistent with the two phenomena seen in the MSTN knockout, skeletal muscle hypertrophy and hyperplasia, neither of which occur if satellite cell proliferation is blocked in normal animals (13, 21).

The peak MSTN mRNA expression in fast muscle after 1 day of HU found in this study occurred at the same time at which satellite cell proliferation has been shown to be decreased because of HU (8, 22). Thus it is possible that an elevated MSTN level contributes to this decrease in satellite cell proliferation when skeletal muscle is unloaded. Still, Schultz et al. (22) report that the decrease in satellite cell proliferation because of HU was greater in the soleus muscle than in the fast extensor digitorum longus muscle. Because we found that MSTN mRNA was not detectable with Northern blot analysis in either control or 1-day HU soleus muscle, it appears that MSTN may not be necessary for a decrease in satellite cell proliferation in this muscle during unloading-induced atrophy. Although it is appealing to speculate that MSTN may play a role in satellite cell proliferation in fast skeletal muscle, the experimental evidence is incomplete and unclear.

It is possible that muscle fiber type-specific gene expression could be modulated by MSTN. Recently Chin et al. (4) showed that cyclosporin A administration increased the percentage of fibers expressing fast MHC. Cyclosporin A inhibits calcineurin, preventing the dephosphorylation of the nuclear factor-activated T cell (NFAT) transcription factor and its subsequent translocation to the nucleus, where it may interact with myocyte-enhancer factor (MEF)-2 to activate transcription (4). Chin et al. proposed that when NFAT proteins are unavailable for DNA binding and for MEF-2 protein interactions at target promoters, the slow fiber-specific program is downregulated, and genes encoding fast fiber-specific proteins are transcribed. Interestingly, MEF-2 is not expressed in cultured muscle cells when treated with TGF-β (11). Because MSTN is a member of the TGF-β family, it may also downregulate the expression of MEF-2, leading to a response similar to the absence of NFAT. Future experiments should test the possibility that MSTN may act to positively reinforce the fast phenotype via fast fiber-specific gene activation.

**Perspectives**

Inactivation of the MSTN gene in transgenic animals has been reported to produce muscle hypertrophy and hyperplasia and thus is a potential target in the development of therapies for musculodegenerative diseases (18). However, an understanding of the physiological expression of MSTN in normal skeletal muscle would be required for the development of MSTN-based therapies. This paper attempted to examine the possibility that MSTN may act as a negative regulator of skeletal muscle fiber size in normal adult mice. With the use of Northern blot analysis, a transient upregula-
tion of expression of the MSTN gene has been found to occur during atrophy of fast muscles induced by HU but was not associated with the substantial atrophy of the slower soleus muscle. Furthermore, we have also shown that in the early stages of muscle unloading, transient upregulation of MSTN expression is probably not due to a decreased caloric intake. Additional evidence that MSTN does not regulate muscle fiber size comes from the fact that the highest MSTN mRNA expression was associated with those muscle fiber types of largest diameter. Thus it was concluded that MSTN likely does not regulate fiber size directly but could act on pathways that may influence muscle fiber size or the fast muscle phenotype. Future experiments should consider potential associations between MSTN expression and satellite cells as well as muscle fiber type-specific gene expression.

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REFERENCES


