Contractile properties of EDL and soleus muscles of myostatin-deficient mice

Christopher L. Mendias,1 James E. Marcin,2 Daniel R. Calerdon,3 and John A. Faulkner1,2

Departments of 1Molecular and Integrative Physiology, 2Biomedical Engineering, and 3Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, Michigan

Submitted 2 February 2006; accepted in final form 10 May 2006

Mendias, Christopher L., James E. Marcin, Daniel R. Calerdon, and John A. Faulkner. Contractile properties of EDL and soleus muscles of myostatin-deficient mice. J Appl Physiol 101: 898–905, 2006. — Myostatin is a negative regulator of muscle mass. The impact of myostatin deficiency on the contractile properties of healthy muscles has not been determined. We hypothesized that myostatin deficiency would increase the maximum tetanic force (P0), but decrease the specific P0 (sP0) of muscles and increase the susceptibility to contraction-induced injury. The in vitro contractile properties of extensor digitorum longus (EDL) and soleus muscles from wild-type (MSTN+/+) mice, heterozygous-null (MSTN+/-), and homozygous-null (MSTN-/-) adult male mice were determined. For EDL muscles, the P0 of both MSTN+/- and MSTN-/- mice were greater than the P0 of MSTN+/+ mice. For soleus muscles, the P0 of MSTN-/- mice was greater than that of MSTN+/- mice. The sP0 of EDL muscles of MSTN-/- mice was less than that of MSTN+/- mice. For soleus muscles, however, no difference in sP0 was observed. Following two lengthening contractions, EDL muscles from MSTN+/- mice had a greater force deficit than that of MSTN+/+ or MSTN-/- mice, whereas no differences were observed for the force deficits of soleus muscles. Myostatin-deficient EDL muscles had less hydroxyproline, and myostatin directly increased type I collagen mRNA expression and protein content. The difference in the response of EDL and soleus muscles to myostatin may arise from differences in the levels of a myostatin receptor, activin type IIB. Compared with the soleus, the amount of activin type IIB receptor was approximately twofold greater in EDL muscles. The results support a significant role for myostatin not only in the mass of muscles but also in the contractility and the composition of the extracellular matrix of muscles.

GDF-8; muscle morphology; muscle injury

MYOSTATIN (GDF-8) IS A MEMBER of the transforming growth factor-β (TGF-β) family of cytokines and functions as a negative regulator of skeletal muscle mass. Inactivation of the myostatin genes and postnatal inhibition of myostatin both result in significant increases in skeletal muscle mass (4, 5, 21, 42, 47, 65, 68, 69). Systemic and skeletal muscle-specific overexpression of myostatin induce skeletal muscle atrophy (52, 71). The Belgian Blue and Piedmontese breeds of cattle have a mutated form of the myostatin gene and are characterized by larger skeletal muscles than other breeds (20, 24). At the time of birth, a human child with apparent null mutations in his myostatin genes had a thigh muscle volume twofold greater in size than 10 age-matched controls (54). The child’s mother, who is heterozygous for the mutation, was also reported to be hypermuscular (54).

Myostatin circulates through the blood in a latent form bound to its propeptide and to follistatin (2). The myostatin gene (MSTN) encodes a precursor protein that undergoes proteolytic processing to generate a propeptide and a mature myostatin dimer (67). The propeptide binds myostatin noncovalently and inhibits the bioactivity of myostatin (42, 57, 67). Cleavage of the propeptide by the bone morphogenic protein-1/tolloid family of metalloproteinases results in the liberation and activation of myostatin (67). Activated myostatin binds to the activin type IIB (ActRIIB) and IB (ActRIB) receptors to initiate the Smad2/3 and p38 MAPK intercellular signal transduction cascades (29, 46, 50, 70). Myostatin appears to regulate skeletal muscle mass, at least in part, by inhibiting the proliferation, differentiation, and self-renewal of myoblasts (28, 39, 56, 58). Type II muscle fibers appear to be more responsive to the myostatin signaling pathway than type I muscles, but the mechanism responsible for this difference is unknown (10, 37, 41, 43).

The inhibition of myostatin may be useful in the treatment of muscle injuries and muscle-wasting diseases by improving the contractile properties of muscle (17, 26, 45, 53, 55, 59, 61). Compared with wild-type mice, myostatin-deficient mice have increased bite force (9) and gross grip strength (65). Treating mdx mice with an antibody against myostatin increased the maximum tetanic force (P0) of extensor digitorum longus (EDL) muscles, but it did not change the specific P0 (sP0) (4). When mdx mice were treated with the propeptide of myostatin, both the P0 and sP0 of EDL muscles increased (5).

Myostatin may also be useful in treating muscle injuries and disease by regulating the collagen accumulation and scar tissue formation in the extracellular matrix (ECM) (17, 45). In addition to enhancing the contractile properties of dystrophic muscle, the deficiency of myostatin decreased the accumulation of scar tissue and ECM of mdx mice (4, 5, 63). Type I collagen is a major component of muscle ECM (31). The transcripts of two separate genes, col1a1 and col1a2, are used to synthesize the collagen I precursor molecule, procollagen I. Procollagen I is secreted into the ECM, where it undergoes cleavage and assembly to form mature collagen I (27). TGF-β has a well-established role as a positive regulator of type I collagen protein synthesis via the Smad2/3 and p38-MAPK signaling pathways (reviewed in Ref. 60). As myostatin is a member of the TGF-β family of cytokines and utilizes similar signal transduction pathways as TGF-β (29, 46, 50, 70), myostatin may have a direct role in the regulation of the type I collagen content of skeletal muscle ECM.

While a few studies have examined the effects of myostatin deficiency on the contractile properties and ECM of dystrophic muscles (4, 5, 63), how myostatin deficiency impacts on healthy, nondystrophic muscle is unknown. The overall aim of this study was to determine the effect of myostatin deficiency on the contractile properties, susceptibility to contraction-induced injury, and collagen composition of skeletal muscle tissue. As increases in P0 due to hypertrophy of muscle fibers...
often result in a corresponding decrease in sPo (15, 25, 30), we hypothesized that a deficiency of myostatin would increase the P Po, but decrease the sPo. Based on the observations that myostatin deficiency decreased the ECM accumulation in dystrophic muscle (4, 5, 63), and the similarities between the myostatin and TGF-β signal transduction pathways (29, 46, 50, 70), we formed the hypothesis that myostatin-deficient mice would have less muscle ECM and that myostatin would directly increase type I collagen expression in skeletal muscle tissue. If these assumptions proved to be correct, we hypothesized that the deficiency of myostatin would increase the force deficits of muscles following a protocol of damaging lengthening contractions.

MATERIALS AND METHODS

Animals

All experiments were conducted in accordance with the guidelines of the University of Michigan Committee on the Use and Care of Animals. Mice were housed in specific pathogen-free conditions and fed food and water ad libitum. MSTNlox/lox mice of a C57BL/6 background were a generous gift of Dr. Se-Jin Lee. The MSTN null allele was generated by replacing the portion of the third exon of the MSTN gene that encodes the COOH-terminal region of the mature myostatin protein with a neo cassette (42). Male MSTNlox/lox mice were crossed with MSTNlox/+ C57BL/6 female mice to generate an F1 MSTNlox/+ generation. The F1 generation was backcrossed to obtain an F2 generation containing all three genotypes. F2 male mice 10–12 mo of age were used in this study. The genotype of mice was determined by PCR-based analysis of genomic DNA samples obtained via tail biopsy. The MSTN wild-type allele was detected using a set of primers that generates a 247-bp amplicon from the third exon of the MSTN gene, and the MSTN null allele was detected using a set of primers that generate a 192-bp amplicon from the neo cassette that replaced the third exon of the MSTN gene. Amplicons from PCR reactions were separated on a 2% agarose gel.

Whole Muscle

Operative procedure. Mice were anesthetized with inrapertorinental injection of Avertin (400 mg/kg). Additional doses were provided as required to maintain a deep anesthesia throughout the experiment. The EDL and soleus muscles were removed from both the left and right legs of each mouse. Muscles used for fiber counts, hydroxyproline, histochemistry, or protein analysis were flash frozen in liquid nitrogen and stored at −80°C until use. A 5–0 silk suture was tied to the proximal and distal tendons of muscles used in the contractile properties experiments. These muscles were placed immediately in a bath that contained Krebs mammalian Ringer solution with 0.25 mM tubocurarine chloride. The bath was maintained at 25°C, and the solution was bubbled with 95% O2 and 5% CO2 to stabilize pH at 7.4. Following the removal of muscles, mice were euthanized with an overdose of anesthetic and induction of a pneumothorax.

Fiber counts of muscles. To determine the number of fibers present in muscles, the extracellular matrices of muscles were digested as described (38). Briefly, muscles were placed in a 5% HNO3 solution overnight at room temperature. Following digestion, the HNO3 solution was replaced with phosphate-buffered saline. Individual muscle fibers were teased apart from bundles and counted under a dissecting microscope. The lengths of 40 individual fibers per muscle were measured using digital calipers.

Measurement of maximum isometric tetanic force. Each muscle was immersed in the bath solution, and the distal tendon was attached to a servomotor (model 305B, Aurora Scientific, Aurora, ON). The proximal tendon was attached to a force transducer (model BG-50, Kulite Semiconductor Products, Leonia, NJ). The attachment of tendons to the servomotor and force transducer occurred just distal to the myotendinous junctions so that the impact of the tendon on the measurement of contractile properties was minimized. Muscles were stimulated by square pulses delivered by two platinum electrodes connected to a high-power biphasic current stimulator (model 701B, Aurora Scientific). An IBM-compatible personal computer and custom-designed software (LabVIEW 7.1, National Instruments, Austin, TX) controlled electrical pulse properties and servomotor activity and recorded data from the force transducer. The voltage of pulses was increased, and optimal muscle length (Lo) was subsequently adjusted to the length that resulted in maximum twitch force (6). The Lo was measured with digital calipers. Muscles were held at Lo and subjected to trains of pulses to generate an isometric contraction. Pulse trains were 300 ms for EDL muscles and 900 ms for soleus muscles. Stimulus frequency was increased until the P Po was achieved (6). The general shape of the force traces during twitch and isometric contractions was not different between the three genotypes of mice for EDL and soleus muscles, respectively. The sPo was determined by dividing Po relative to plantarflexes the muscle by 10.220.33.1 on July 11, 2017 http://jap.physiology.org/ Downloaded from
loaded into two 4% stacking, 7.5% separating polyacrylamide gels and subjected to electrophoresis. To verify equal protein loading, gels that were not used in immunoblotting were stained with Coomassie brilliant blue (Bio-Rad). Proteins were transferred to a 0.45-μm nitrocellulose membrane and stained with Ponceau S to verify equal protein transfer. Membranes were blocked using casein and an avidin-biotin blocking kit (Vector Laboratories, Burlingame, CA), rinsed, and incubated with a biotinylated monoclonal antibody against ActRIIB (R&D Systems, Minneapolis, MN) and an avidin-horseradish peroxidase conjugate (Vector Laboratories). Membranes were developed with SuperSignal West Dura enhanced chemiluminescent reagents (Pierce Biotechnology, Rockford, IL) and visualized using a chemiluminescent documentation system (Bio-Rad).

**Cultured Cells**

Satellite cell isolation and culture. Satellite cells were isolated from adult male MSTN+/− mice as described by Allen and colleagues (1). Mice were anesthetized with intraperitoneal injection of Avertin (400 mg/kg) and killed by cervical dislocation. The hindlimb muscles were quickly removed, minced, and digested in protease solution (1.25 mg/ml of Pronase E in PBS, Sigma) for 1 h at 37°C. Satellite cells were separated from muscle fiber fragments and from tissue debris by differential centrifugation and then plated on fibronectin-coated 60-mm tissue culture plates (BD Biosciences, San Jose, CA). Cultures were maintained in a humidified environment at 37°C and 5% CO2. Satellite cells were grown in DMEM + 20% FBS + 1% antibiotic-antimycotic (AbAm) until reaching 80% confluence, at which time the media was changed to DMEM + 2% horse serum + 1% AbAm to induce differentiation into myotubes.

**RT-quantitative PCR.** Myotubes were treated with different concentrations of recombinant murine myostatin (R&D Systems) in DMEM + 1% AbAm for 8 h, rinsed with PBS and 0.5M EDTA, scraped, and homogenized in Laemmli’s sample buffer with 1.20 β-mercaptoethanol and 1.20 protease inhibitor cocktail (Sigma) and subsequently placed in boiling water for 5 min. Protein concentration, electrophoresis, and blotting occurred as described above. Membranes were blocked in 10% powered milk, rinsed, and incubated with a polyclonal antibody against procollagen I (Santa Cruz Biotechnology, Santa Cruz, CA) and a horseradish peroxidase-conjugated secondary antibody (Pierce Biotechnology), and developed as described above. Following detection of procollagen I, membranes were stripped and reprobed using a monoclonal antibody against β-tubulin (Developmental Studies Hybridoma Bank, Iowa City, IA).

**Statistical Analyses**

Results are presented as means ± SE. KaleidaGraph 4.02 software (Synergy Software, Reading, PA) was used to conduct statistical analyses. Differences between groups were tested using a one-way ANOVA with α = 0.05. Fisher’s least significant difference post hoc test was used to identify specific differences when significance was detected.

**RESULTS**

**Morphology**

The body mass, body length, tibial length, muscle mass, absolute numbers of fibers per muscle, fiber areas, Lf, and CSA values of EDL and soleus muscles from each of the three

<table>
<thead>
<tr>
<th>Table 1. Anatomical properties of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MSTN+/−</strong></td>
</tr>
<tr>
<td><strong>MSTN+-/−</strong></td>
</tr>
<tr>
<td><strong>MSTN−/−</strong></td>
</tr>
<tr>
<td>Body mass, g</td>
</tr>
<tr>
<td>Body length, mm</td>
</tr>
<tr>
<td>Tibia length, mm</td>
</tr>
<tr>
<td><strong>EDL muscles</strong></td>
</tr>
<tr>
<td>Wet mass, mg</td>
</tr>
<tr>
<td>Fibers per muscle</td>
</tr>
<tr>
<td>Fiber area, μm²</td>
</tr>
<tr>
<td>Lf, mm</td>
</tr>
<tr>
<td>Lo, mm</td>
</tr>
<tr>
<td>CSA, mm²</td>
</tr>
<tr>
<td>Hydroxyproline/dry muscle mass, μg/mg</td>
</tr>
<tr>
<td><strong>Soleus muscles</strong></td>
</tr>
<tr>
<td>Wet mass, mg</td>
</tr>
<tr>
<td>Fibers per muscle</td>
</tr>
<tr>
<td>Fiber area, μm²</td>
</tr>
<tr>
<td>Lf, mm</td>
</tr>
<tr>
<td>Lo, mm</td>
</tr>
<tr>
<td>CSA, mm²</td>
</tr>
<tr>
<td>Hydroxyproline/dry muscle mass, μg/mg</td>
</tr>
</tbody>
</table>

Values are means ± SE; N = 12 for tibia length and body length, n = 300 fibers from 3 muscles for each genotype, n = 5 muscles per genotype for fibers per muscle and hydroxyproline, and n = 6 muscles per genotype for all other values. MSTN, myostatin gene; EDL, extensor digitorum longus; Lf, optimal muscle length; Lo, fiber length; CSA, cross-sectional area. *Significantly different from MSTN+/− at P < 0.05. †Significantly different from MSTN−/− at P < 0.05.
groups of mice are shown in Table 1. Although no differences were observed for body masses or body lengths of the MSTN+/+, MSTN+-, or MSTN-- mice, the mean mass of the EDL muscles of MSTN-- mice was 66% greater than that of the MSTN+/+ mice and 51% greater than that of the MSTN+/-- mice. For MSTN-- mice, the mass of the soleus was 36% greater than that of the MSTN+/+ mice.

Conflicting reports have been published regarding the role of myostatin in determining the number of fibers per muscle (21, 36, 42, 47, 51, 68, 69). Each of these reports counted the number of muscle fibers present in a cross section of muscle. The counting of the number of fibers present in a cross section of a muscle does not necessarily provide an accurate indication of the total number of fibers present in that muscle (38). To address this issue, the absolute number of fibers in muscles from MSTN+/+, MSTN+-, and MSTN-- mice was counted. The EDL muscles of MSTN-- mice had 60% more muscle fibers than MSTN+/+ mice and 39% more fibers than MSTN+/-- mice, and the MSTN+/+ mice had 16% more fibers than MSTN+/+ mice (Table 1). For soleus muscles, MSTN-- mice had 31% more fibers than MSTN+/+ mice and 9% more than MSTN+/-- mice, and the MSTN+/+ mice had 20% more fibers than MSTN+/+ mice (Table 1).

The mean fiber areas and CSA of EDL muscles of MSTN+/+ and MSTN-- mice were greater than that of MSTN+/-- mice. For soleus muscles, the mean fiber areas and CSA of MSTN+/+ mice were greater than those of MSTN+/-- mice (Table 1).

The EDL and soleus muscles of mice both originate on the proximal tibia and run the entire length of the tibia before inserting on the phalanges or calcaneus, respectively. No differences in the lengths of tibias of MSTN+/+, MSTN+/-- mice, and MSTN-- mice were observed (Table 1). Furthermore, the Lo and Le values of EDL and soleus muscles, respectively, were not different among the three genotypes.

Collagen Content of Muscles

The amino acid hydroxyproline makes up ~14% of the dry mass of fibrillar collagens (44) and is commonly used as an indicator of collagen content. The relative hydroxyproline content of EDL muscles of MSTN-- was 75% less than that of MSTN+/+ mice (Table 1 and Fig. 1A). MSTN+/-- mice had 59% less hydroxyproline than MSTN+/+ mice. For soleus muscles, no difference was observed for the relative amounts of hydroxyproline among the three genotypes (Table 1 and Fig. 1B).

Myostatin-Mediated Type I Collagen Synthesis

The marked decrease in the collagen content of the EDL muscles from myostatin-deficient mice lead us to hypothesize that myostatin induces the expression of type I collagen in muscle tissue. We found a dose-dependent increase in col1a2 expression (Fig. 2A) and procollagen I protein content (Fig. 2B) of primary myotubes treated with myostatin. Similar results were observed in C2C12 myotubes and primary fibroblasts isolated from mouse tendon (data not shown).

Contractile Properties

Myostatin deficiency had a more profound impact on the contractile properties of EDL muscles than soleus muscles (Table 2 and Fig. 3). The P0 of MSTN-- mice was 34% greater than the P0 of MSTN+/+ mice and 19% greater than the P0 of MSTN+/-- mice. MSTN+/+ mice had a 13% greater P0.
Table 2. Contractile properties of EDL and soleus muscles

<table>
<thead>
<tr>
<th>Muscle</th>
<th>MSTN&lt;sup&gt;++/+&lt;/sup&gt;</th>
<th>MSTN&lt;sup&gt;+/−/−&lt;/sup&gt;</th>
<th>MSTN&lt;sup&gt;−/−/−&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>P&lt;sub&gt;t&lt;/sub&gt;, mN</td>
<td>110.78±4.84</td>
<td>136.76±12.33</td>
<td>160.87±8.07*</td>
</tr>
<tr>
<td>sP&lt;sub&gt;t&lt;/sub&gt;, mN/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>58.20±1.75</td>
<td>63.27±5.10</td>
<td>53.25±3.38</td>
</tr>
<tr>
<td>TTP&lt;sub&gt;r&lt;/sub&gt;, ms</td>
<td>23.68±2.80</td>
<td>20.91±1.32</td>
<td>23.86±2.46</td>
</tr>
<tr>
<td>l/2RT&lt;sub&gt;i&lt;/sub&gt;, ms</td>
<td>22.56±1.48</td>
<td>28.80±1.02*</td>
<td>15.51±0.25*†</td>
</tr>
<tr>
<td>dP/dt, mN/ms</td>
<td>13.07±0.05</td>
<td>15.64±1.29</td>
<td>19.00±1.24*†</td>
</tr>
<tr>
<td>P&lt;sub&gt;n&lt;/sub&gt;, mN</td>
<td>461.19±14.69</td>
<td>520.53±18.48*</td>
<td>616.93±17.10*†</td>
</tr>
<tr>
<td>sP&lt;sub&gt;n&lt;/sub&gt;, mN/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>243.32±10.04</td>
<td>242.12±8.22</td>
<td>199.86±7.37*‡</td>
</tr>
</tbody>
</table>

Soleus muscles

<table>
<thead>
<tr>
<th>Muscle</th>
<th>MSTN&lt;sup&gt;++/+&lt;/sup&gt;</th>
<th>MSTN&lt;sup&gt;+/−/−&lt;/sup&gt;</th>
<th>MSTN&lt;sup&gt;−/−/−&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>P&lt;sub&gt;t&lt;/sub&gt;, mN</td>
<td>47.12±2.78</td>
<td>60.43±5.00</td>
<td>65.52±5.85*</td>
</tr>
<tr>
<td>sP&lt;sub&gt;t&lt;/sub&gt;, mN/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>42.08±1.90</td>
<td>47.51±2.30</td>
<td>45.06±3.81</td>
</tr>
<tr>
<td>TTP&lt;sub&gt;r&lt;/sub&gt;, ms</td>
<td>40.61±4.68</td>
<td>32.41±0.99</td>
<td>27.38±3.75</td>
</tr>
<tr>
<td>l/2RT&lt;sub&gt;i&lt;/sub&gt;, ms</td>
<td>50.10±5.42</td>
<td>47.55±2.13</td>
<td>37.57±1.92</td>
</tr>
<tr>
<td>dP/dt, mN/ms</td>
<td>4.20±0.21</td>
<td>4.91±0.33</td>
<td>6.08±0.80</td>
</tr>
<tr>
<td>P&lt;sub&gt;n&lt;/sub&gt;, mN</td>
<td>295.84±7.49</td>
<td>324.94±18.52</td>
<td>385.06±17.57*‡</td>
</tr>
<tr>
<td>sP&lt;sub&gt;n&lt;/sub&gt;, mN/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>265.81±10.10</td>
<td>257.15±6.34</td>
<td>264.84±8.50</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 muscles per genotype. P<sub>t</sub>, peak twitch force; sP<sub>t</sub>, specific P<sub>t</sub>; TTP<sub>r</sub>, time to peak twitch tension; l/2RT<sub>i</sub>, half-relaxation time; dP/dt, maximum rise in tension. *Significantly different from MSTN<sup>++/+</sup> at P < 0.05. †Significantly different from MSTN<sup>−/−/−</sup> at P < 0.05.

than MSTN<sup>++/+</sup> mice. When P<sub>n</sub> was normalized by the CSA, MSTN<sup>++/+</sup> mice had an 18% lower value for sP<sub>n</sub> than either MSTN<sup>+/−/−</sup> or MSTN<sup>−/−/−</sup> mice. For soleus muscles, the P<sub>n</sub> of MSTN<sup>−/−/−</sup> mice was 30% greater than that of MSTN<sup>++/+</sup> mice, but the values for sP<sub>n</sub> were not different.

Contraction-Induced Injury

During lengthening contractions, the average force developed by EDL muscles was not different, but, compared with MSTN<sup>++/+</sup> mice, the work done to lengthen the muscles was 12 and 37% less for MSTN<sup>+/−/−</sup> and MSTN<sup>−/−/−</sup> mice, respectively (Table 3), indicating a decrease in the stiffness of these muscles. After the lengthening contraction protocol, muscles from MSTN<sup>−/−/−</sup> mice had a force deficit that was 15% greater than that of MSTN<sup>++/+</sup> mice (Table 3 and Fig. 4). During stretches of soleus muscles, the average force developed by MSTN<sup>−/−/−</sup> mice was ~18% greater than that of MSTN<sup>++/+</sup> mice. During lengthening contractions of soleus muscles, no differences in the work done to stretch the muscles were observed. Following the lengthening contraction protocol, the force deficits of soleus muscles were not different.
**ActRIIB Content of EDL and Soleus Muscles**

The deficiency of myostatin had a more profound impact on the morphological and contractile properties of EDL muscles than soleus muscles. Since myostatin appears to act systemically (71), the difference in the amount of ActRIIB present in EDL and soleus muscles was determined. Compared with soleus muscles, the amount of ActRIIB was greater in EDL muscles (Fig. 5).

**DISCUSSION**

The $P_o$ of skeletal muscle can be increased by either hypertrophy of existing fibers or by hyperplasia. In either case, as whole muscle CSA increases, the angle of pennation of muscle fibers ($\theta$) also increases (38). The transmission of the force developed by single muscle fibers from tendon to tendon along the line of tension development of the muscle is proportional to the cosine of $\theta$. As $\theta$ increases from 0 to 90°, the cosine of $\theta$ decreases from 1 to 0. Therefore, as a muscle undergoes hypertrophy or hyperplasia, the net force per CSA is expected to decrease. Our hypothesis that myostatin deficiency would increase the $P_o$ but decrease the $sP_o$ of muscles is supported by the observations of the contractile properties of the EDL muscles of $MSTN^{-/-}$ mice. In contrast, for soleus muscles, the complete deficiency of myostatin increased the $P_o$ less than that of the EDL muscle and had no effect on the $sP_o$. Furthermore, EDL muscles of $MSTN^{+/+}$ mice displayed a greater $P_o$ than those of $MSTN^{+/+}$ mice, but showed no change in $sP_o$. While $\theta$ was not measured directly, using a model of muscle architecture that estimates $\theta$ based upon the number of fibers in a muscle, $L_e$, $L_o$, and fiber areas (38), compared with $MSTN^{+/+}$ mice, we estimate that $MSTN^{-/-}$ mice had a 38% greater $\theta$ for EDL muscles, and a 17% greater $\theta$ for soleus muscles, respectively. The greater increase in $\theta$ for EDL than soleus muscles may explain the observed decrease in $sP_o$ for EDL muscles and the lack of change in $sP_o$ for soleus muscles. In terms of the magnitude of the increases in muscle CSA, mass, and $P_o$, a threshold appears to exist for initiating a decrease in $sP_o$, wherein large increases in muscle CSA, mass, and $P_o$ initiate decreases in $sP_o$, whereas, with small increases, $sP_o$ does not change.

Following contraction-induced injury to muscles, the immediate force deficit results from the mechanical disruption of the ultrastructure of sarcomeres (8, 18, 33, 34). The magnitude of this force deficit is a function of strain and the work done to stretch the contractile component (CC) of muscle (8). The aponeurosis (intramuscular tendon) and the tendon, composed chiefly of type I and III collagen (12, 22, 27), form the series elastic component (SEC) of muscle (23). A positive correlation exists between the collagen content and stiffness of a muscle (16, 19, 48). During a lengthening contraction, the total displacement of the muscle is the sum of the displacement of the CC and the SEC; therefore, displacement of the SEC protects the CC from contraction-induced injury. During a lengthening contraction, the protection afforded to the CC by the SEC increases as the displacement of the SEC relative to the CC increases. Despite this potential for protection, if the strain and work done during a lengthening contraction are great enough, the SEC can become damaged and no longer provide protection for the CC. Consequently, an advantageous arrangement for a muscle is to have an SEC with a stiffness that allows for a moderate amount of displacement during a lengthening contraction, but not too compliant as to become damaged during a lengthening contraction. Our results suggest that, for EDL muscles, the complete deficiency of myostatin decreases the stiffness of the SEC in such a way that the susceptibility to contraction-induced injury is increased.

The effect of myostatin deficiency on the structure and function of EDL and soleus muscles was quite different. Compared with the content of the primary myostatin receptor, ActRIIB, in the soleus muscles of $MSTN^{+/+}$ mice, the content in the EDL muscles was approximately twofold greater. The greater quantity of ActRIIB in the EDL muscles of $MSTN^{+/+}$ mice appeared to make the EDL muscles more responsive than soleus muscles to the presence of myostatin. Consequently, with the absence of myostatin in the $MSTN^{-/-}$ mice, the EDL muscles experience a much greater relative increase in mass and number of fibers than experienced by the soleus muscles.

---

**Fig. 5.** Activin type IIB receptor (ActRIIB) protein content of EDL and soleus muscles from $MSTN^{+/+}$ mice. Compared with soleus muscles, the amount of ActRIIB protein is greater in EDL muscles (immunoblot). Sarcomeric myosin proteins are shown as loading controls (Coomassie brilliant blue staining).
The treatment of muscle injuries and disease often involves a two-pronged therapeutic regimen of improving the strength of muscle and decreasing the formation of collagenous scar tissue (13, 49). Much of the interest behind the potential use of myostatin inhibitors is the ability of these inhibitors to enhance the regeneration of skeletal muscle and also decrease the accumulation of scar tissue in murine models of muscle injuries and disease (17, 40, 45, 61–63). Myostatin inhibitors may therefore be a useful therapeutic adjunct to traditional athletic training and physical therapy by directly improving contractility and decreasing fibrosis. The enhanced regenerative capacity of myostatin-deficient muscle is likely due to an increase in satellite cell activity, as myostatin is a negative regulator of satellite cell proliferation and migration (28, 39, 40, 56, 58, 62).

The increased satellite cell activity produced by the deficiency of myostatin does not explain how myostatin deficiency decreases the fibrosis normally present in dystrophic muscle (4, 5, 63) and following snake venom-induced muscle injury (40, 62). The decreased content of collagen in otherwise healthy myostatin-deficient muscles, along with the observation that myostatin increases directly the expression of type I collagen in cultured muscle tissue, suggest a direct role for myostatin in the signal transduction pathways that regulate the collagen content of the ECM of skeletal muscle tissue.

Pharmaceutical inhibition of myostatin likely suppresses, but does not eliminate, myostatin signaling completely. The haploinsufficient MSTN+/− mouse provides a useful model for the investigation of the effects of partial suppression of myostatin signaling. In the present study, compared with their MSTN+/+ littermates, the EDL muscles of MSTN+/− mice developed a greater P0 but, unlike the MSTN−/− mice, had no difference in sPo or force deficit after injury. The collagen content and stiffness of the EDL muscles of MSTN+/− mice was less than that of MSTN+/+ mice, but this did not increase the susceptibility of these muscles to contraction-induced injury. Such a decrease in the stiffness of muscle might be beneficial in the treatment of diseases that involve severe muscle fibrosis, such as Duchenne muscular dystrophy. Patients with Duchenne muscular dystrophy suffer from respiratory insufficiency due to impaired contractility of the diaphragm muscle and to the increased stiffness of the diaphragm muscle (3, 64). Therefore, partial inhibition of myostatin may provide a useful treatment for fibrotic muscle diseases through an increase in the contractile forces and a decrease in the stiffness of the muscles.

ACKNOWLEDGMENTS

We acknowledge the generosity of Dr. Se-Jin Lee for providing transgenic mice for this study, Cheryl Hassett for providing assistance with animal surgeries, and Kimberly Gates for assistance with animal breeding.

GRANTS

This work was supported by National Institute on Aging Grants AG-13283 and AG-020591.

REFERENCES


62. Zhu X, Topouzis S, Liang LF, and Stotish RL. Myostatin signaling through Smad2, Smad3 and Smad4 is regulated by the inhibitory Smad7 through a negative feedback mechanism. *Cytokine* 26: 262–272, 2004.