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Science 24 May 2002:  
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ABSTRACT

FULL TEXT

**Induction of Cachexia in Mice by Systemically Administered Myostatin**  
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 Kathy N. Tomkinson, Alexandra C. McPherron, Neil M. Wolfman, and Se-Jin Lee

### Supplementary Material

#### Materials and Methods

#### Analysis of serum myostatin protein

Serum was obtained from mice bearing a targeted deletion of the region of the myostatin gene encoding the biologically active COOH-terminus (1) or from wild type littermates. Western blotting was performed using antiserum raised against bacterially derived myostatin COOH-terminal mature peptide and chemiluminescent detection (Pierce SuperSignal, West Femto, Pierce Chemical Co., Rockford, IL). Myostatin biological activity was assayed using a pGL3-(CAGA)<sub>12</sub> reporter assay (2). In this assay, the Transforming Growth Factor- $\beta$  (TGF- $\beta$ )-responsive CAGA sequence derived from the promoter of the TGF- $\beta$ -induced gene PAI-1 was coupled to the luciferase gene and transfected into human A204 rhabdomyosarcoma cells, which were then stimulated with recombinant myostatin, serum, or myostatin diluted in null serum for generation of a standard curve.

#### Cell lines and analysis of mice

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The CHO cell line engineered to express myostatin from the metallothionein promoter (CHO-myostatin) is that described in Lee and McPherron (3). A clonal cell line transfected with the metallothionein expression vector and similarly selected but shown not to express recombinant protein was used as a control (CHO-control). Other cell lines used included similarly transfected and selected CHO lines expressing a Bone Morphogenetic Protein-2 (BMP-2)/Growth/Differentiation Factor-1 (GDF-1) fusion protein, BMP-9, GDF-5, GDF-12, cytokine responsive gene-2 (Crg-2), paired basic amino acid converting enzyme (PACE), or no recombinant protein. CHO cells engineered to express recombinant human myostatin pro-region (CHO-PRO), human follistatin (CHO-FSTN), or human interleukin-6 (IL-6) were generated using methods similar to those described above. Expression of recombinant protein was confirmed by Western blotting of conditioned media from these lines. Cells ( $5 \times 10^6$ ) were injected into the thighs of 5-6 week old athymic female nude mice (Harlan Sprague-Dawley, Indianapolis, IN, or National Cancer Institute, Bethesda, MD). Mice were housed four per cage in a microisolator facility with free access to mouse chow and sterile tap water or 25 mM ZnSO<sub>4</sub> in sterile tap water. For time course analysis, all mice were maintained on zinc water, and at least eight mice from each group were examined per timepoint. Food intake was measured as food consumed per mouse per cage for five cages over each interval indicated. Quantikine-M ELISA kits (R&D Systems, Minneapolis, MN) were used to measure serum TNF and IL-6 levels. Liver serum enzyme levels were determined by Antech Diagnostics, (Piscataway, NJ). Statistical analyses were performed using the Student's t-test.

#### Administration of recombinant myostatin

Five to six-week-old CD-1 female mice (Charles River Laboratories, Inc., Wilmington, MA) were given subcutaneous injections of 1  $\mu$ g myostatin mature region (3) or equivalent volume of carrier (0.1% TFA) in 0.2 ml 5% BSA in PBS at approximately 8AM and 8PM for seven days.

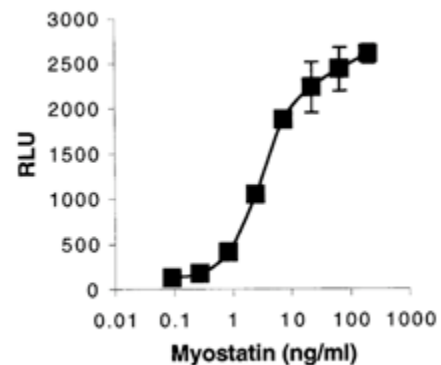
#### Supporting Figures

**Supplemental Figure 1.** Characterization of myostatin activity in the A204 reporter gene assay (2). Standard curve of recombinant myostatin COOH-terminal region activity in the A204 assay. RLU, relative luciferase units.

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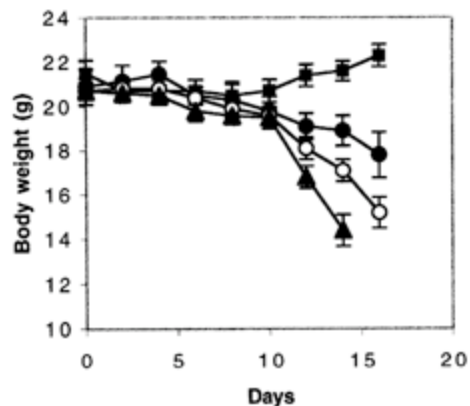


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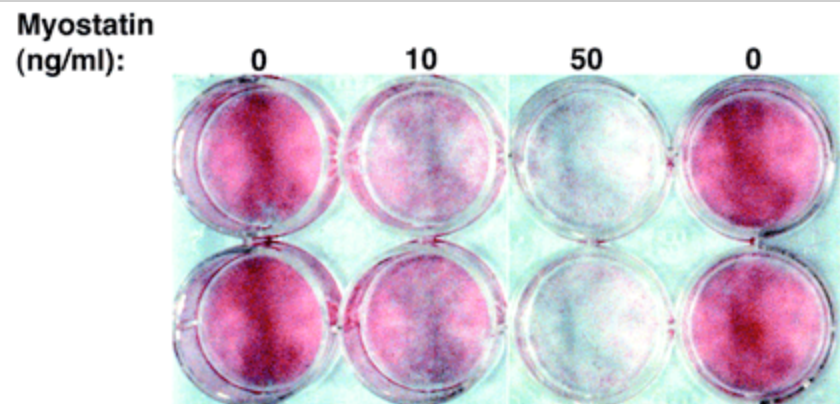
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**Supplemental Figure 2.** Enhancement of myostatin expression from the metallothionein promoter accelerates wasting. Northern blot analysis demonstrated increased *Mstn* expression in CHO-myostatin cells treated with media containing cadmium or zinc, although untreated cells demonstrated baseline expression of *Mstn* in the absence of heavy metals (4). Consistent with the enhancement of *Mstn* expression by zinc, CHO-myostatin tumor-bearing mice given tap water (closed circles) lost weight less rapidly than CHO-myostatin mice given water containing 25 mM ZnSO<sub>4</sub> (open circles). Interestingly, CHO-myostatin mice given tap water until day 10, then zinc water (closed triangles) lost weight even more rapidly, with no survivors past day 14. Tumor size was not significantly different in the three CHO-myostatin groups (4). Body weight of CHO-control tumor-bearing mice given water (closed squares) remained constant or increased over the course of the experiment, as did that of CHO-control mice on tap water (4). This analysis indicates that increased myostatin production in mice led to increased or accelerated weight loss. The less rapid weight loss in mice given zinc water than in mice switched to zinc water from tap water may suggest that mice exposed more chronically to high doses of myostatin may somehow compensate for increased myostatin signaling.



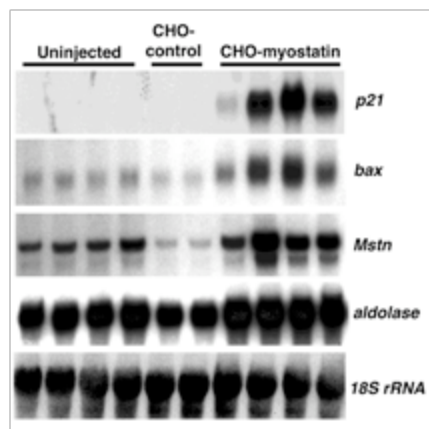
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**Supplemental Figure 3.** Effects of myostatin on adipose cells. 3T3 L1 fibroblasts were maintained and induced to differentiate into adipocytes as described (5). Recombinant myostatin added only to the differentiation-inducing media inhibited lipid accumulation in the post-induction period as assessed by staining with Oil Red O. Others have reported similar effects on 3T3-L1 adipocytes with myostatin administered chronically in the growth, differentiation and maintenance media (6). Moreover, in preliminary experiments we have observed increased Smad2 phosphorylation [a downstream effect of activin receptor activation (7)] in fat pads isolated from day12 CHO-myostatin mice versus same day CHO-controls. Taken together with the reduction in fat mass observed in CHO-myostatin nude mice and in normal mice treated with recombinant myostatin, these results suggest that excess myostatin may act directly on adipose tissue to negatively regulate fat mass. What role myostatin may play in regulating fat mass in normal animals is unclear, however. Clarification of that role is further complicated by the observation that older *Mstn*<sup>-/-</sup> mice are leaner than their wild-type counterparts, most likely due to an indirect, long-term effect of the increased muscle mass and its associated metabolic demand (8).



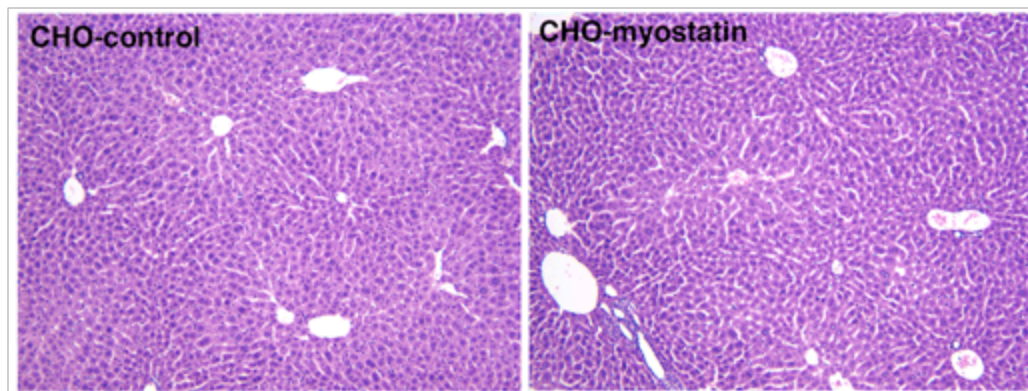
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**Supplemental Figure 4.** Effects of excess myostatin on skeletal muscle. Northern blot analysis of 20  $\mu$ g total RNA isolated from pooled triceps, pectoralis, quadriceps, gastrocnemius, plantaris, soleus and tibialis anterior muscles taken from uninjected nude mice or from the non-tumor bearing sides of day 16 CHO-control or CHO-myostatin mice was performed as described (1). Note increased expression of *p21* and *bax* in CHO-myostatin muscles, consistent with a previous report documenting induction of p21 protein and inhibition of cell proliferation by myostatin on C2C12 myoblasts (9). Wasted muscle from CHO-myostatin mice also exhibited increased expression of the endogenous myostatin gene, which suggests the possibility that myostatin may regulate its own expression. Aldolase and 18S rRNA probes were used to assess uniformity of loading.



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**Supplemental Figure 5.** Overexpression of myostatin is not hepatotoxic. Liver to body mass ratios were slightly reduced compared to uninjected nude mice in CHO–myostatin mice on days 4 and 8, but not later. Despite this transient decline in liver to body mass ratio, liver histology from CHO–myostatin mice was normal on days 4, 8, and 12. For example, hematoxylin and eosin staining of formalin–fixed, paraffin embedded liver sections (5  $\mu$ m) from CHO–control (left) and CHO–myostatin (right) mice killed on day 12 after injection demonstrated normal liver histology in both samples. By day 16, however, CHO–myostatin livers exhibited evidence of apoptotic hepatocytes scattered throughout the otherwise normal liver parenchyma, perhaps reflecting compensatory apoptosis to normalize the liver to body mass ratio during this period of dramatic weight loss. Furthermore, liver enzyme levels (AST, ALT, alkaline phosphatase, and GGTP) in serum from day 4, 12, and 16 CHO–myostatin mice were not different from those measured in uninjected nude mice (day 8 CHO–myostatin sera were not analyzed) (4). These observations contrast sharply with the massive centrilobular hepatocyte necrosis associated with cachexia induced by excess activin activity (10, 11). Our results indicate that myostatin–induced wasting is not due to liver failure, and further suggest that in vivo, myostatin signaling is distinguishable from activin signaling, despite the ability of both to bind the Activin receptors type IIA and IIB in vitro (3, 12). One possible explanation for the distinct effects of myostatin and activin in vivo may be that the two ligands utilize different receptor subtypes; in this regard, activin–induced wasting was shown to be completely blocked by loss of ActRIIA (11). However, other possible mechanisms such as differences in protein distribution or availability may also account for the different effects in myostatin versus activin overexpressing mice.



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## Supporting References and Notes

1. A. C. McPherron, A. M. Lawler, S. J. Lee, *Nature* **387**, 83–90 (1997).
2. R. S. Thies *et al.*, *Growth Factors* **18**, 251 (2001).
3. S. J. Lee, A. C. McPherron, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 9306 (2001).
4. Data not shown.
5. R. A. Ignatz, J. Massague, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 8530 (1985).
6. H. S. Kim, L. Liang, R. G. Dean, D. B. Hausman, D. L. Hatzell, C. A. Baile, *Biochem. Biophys. Res. Commun.* **281**, 902 (2001).
7. K. Miyazono, K. Kusanagi, H. Inoue, *J. Cell. Physiol.* **187**, 265 (2001).
8. A. C. McPherron, S.-J. Lee, *J. Clin. Invest.* **109**, 55 (2002).
9. M. Thomas *et al.*, *J Biol Chem* **275**, 40235–43. (2000).
10. M. M. Matzuk *et al.*, *Proc. Natl. Acad. Sci. .U.S.A.* **91**, 8817 (1994).
11. K. A. Coerver *et al.*, *Mol. Endocrinol.* **10**, 534 (1996).
12. M. M. Matzuk, *Trends Endocrinol. Metab.* **6**, 120 (1995).

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