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Glucocorticoid-induced skeletal muscle atrophy is associated with upregulation of myostatin gene expression

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Ma, Kun, Con Mallidis, Shalender Bhasin, Vahid Mahabadi, Jorge Artaza, Nestor Gonzalez-Cadavid, Jose Arias, and Behrouz Salehian. Glucocorticoid-induced skeletal muscle atrophy is associated with upregulation of myostatin gene expression. *Am J Physiol Endocrinol Metab* 285: E363–E371, 2003. First published April 29, 2003; 10.1152/ajpendo.00487.2002.—The mechanisms by which excessive glucocorticoids cause muscular atrophy remain unclear. We previously demonstrated that dexamethasone increases the expression of myostatin, a negative regulator of skeletal muscle mass, in vitro. In the present study, we tested the hypothesis that dexamethasone-induced muscle loss is associated with increased myostatin expression in vivo. Daily administration (60, 600, 1,200 $\mu\text{g/kg}$ body wt) of dexamethasone for 5 days resulted in rapid, dose-dependent loss of body weight (-4.0 , -13.4 , -17.2% , respectively, $P < 0.05$ for each comparison), and muscle atrophy (6.3, 15.0, 16.6% below controls, respectively). These changes were associated with dose-dependent, marked induction of intramuscular myostatin mRNA (66.3, 450, 527.6% increase above controls, $P < 0.05$ for each comparison) and protein expression (0.0, 260.5, 318.4% increase above controls, $P < 0.05$). We found that the effect of dexamethasone on body weight and muscle loss and upregulation of intramuscular myostatin expression was time dependent. When dexamethasone treatment ($600 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) was extended from 5 to 10 days, the rate of body weight loss was markedly reduced to $\sim 2\%$ within this extended period. The concentrations of intramuscular myosin heavy chain type II in dexamethasone-treated rats were significantly lower (-43% after 5-day treatment, -14% after 10-day treatment) than their respective corresponding controls. The intramuscular myostatin concentration in rats treated with dexamethasone for 10 days returned to basal level. Concurrent treatment with RU-486 blocked dexamethasone-induced myostatin expression and significantly attenuated body loss and muscle atrophy. We propose that dexamethasone-induced muscle loss is mediated, at least in part, by the upregulation of myostatin expression through a glucocorticoid receptor-mediated pathway.

regulation; RU-486

GLUCOCORTICOIDS ARE COMMONLY USED in the treatment of a vast array of chronic inflammatory illnesses, such as systemic lupus erythematosus, sarcoidosis, rheuma-

toid arthritis, and bronchial asthma (3, 4, 7). However, administration of high doses of glucocorticoids causes muscular atrophy in humans and animals (8, 31). Similarly, hypercortisolism has a major role in muscular atrophy in Cushing's disease (2, 33). Many previous studies have suggested that glucocorticoids inhibit protein synthesis and stimulate protein degradation in skeletal muscle (2, 38, 39, 41, 42). Although a number of mechanisms have been put forward to explain the action of glucocorticoids on skeletal muscle (15, 16, 29, 49), the precise molecular mechanisms by which glucocorticoids induce muscle atrophy are still not well understood.

Myostatin, formerly known as growth and differentiation factor 8, a member of the transforming growth factor- β superfamily, is an important negative regulator of skeletal muscle mass. Disrupted myostatin gene expression, either by gene targeting in mice or as a consequence of naturally occurring mutations in cattle, is associated with increased skeletal muscle mass resulting from muscle fiber hyperplasia as well as hypertrophy (17, 24, 25, 30, 32). Conversely, increased serum myostatin concentration is associated with a loss of skeletal muscle mass in men with the AIDS wasting syndrome (11). Increases in myostatin expression have also been reported in experimental animals after exposure to microgravity during space flight (22) and after hindlimb suspension (6, 48). We (44) and other investigators (45) have demonstrated that recombinant myostatin protein inhibits C₂C₁₂ muscle cell proliferation and protein synthesis in vitro. The mechanisms that regulate myostatin gene expression remain poorly understood.

To investigate the regulation of myostatin gene expression, we recently cloned and characterized the 5'-upstream regulatory region of the human myostatin gene and found that the promoter contains a number of response elements important for muscle growth, including seven putative glucocorticoid response elements (GREs). We also demonstrated that dexamethasone dose-dependently increases endogenous myostatin transcription in C₂C₁₂ myocytes through a glucocorticoid receptor-mediated mechanism (1, 26).

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These findings led us to postulate that the increase in myostatin gene expression by glucocorticoids might contribute to the pathogenesis of glucocorticoid-induced skeletal muscle atrophy. In the present study, we tested this hypothesis *in vivo* in a rat model. First, we determined the effects of graded doses of dexamethasone on intramuscular myostatin gene expression. Second, we investigated the time course of dexamethasone effect on intramuscular myostatin gene expression. Finally, we examined whether the effects of dexamethasone on myostatin mRNA and protein can be reversed by concomitant administration of a glucocorticoid antagonist, RU-486.

MATERIALS AND METHODS

Animal care and experimental treatments. Male Sprague-Dawley rats, 10–12 wk of age, with initial body weights ranging from 250 to 420 g, were purchased from Harlan Laboratories. Each animal was housed individually in controlled environmental conditions (temperature 22°C, 12:12-h light-dark cycle period starting at 6:00 AM), and provided standard laboratory rodent chow and water. All experimental procedures on animals were approved by the Institutional Animal Care and Use Committee of the Charles R. Drew University of Medicine and were in accordance with National Institutes of Health guidelines for humane treatment of laboratory animals.

Study 1. We examined effects of different doses of dexamethasone administered intraperitoneally for 5 days on body weight, skeletal muscle weight, and intramuscular myostatin protein and mRNA expression. Sixty adult male rats were divided into six groups with even initial body weight distribution in each group. Three randomly selected groups, *I*, *II*, and *III*, received intraperitoneal injections of 1 ml of dexamethasone (Sigma) dissolved in saline (0.85% NaCl) at daily doses of 60, 600, and 1,200 µg/kg body wt, respectively. These doses were selected because administration of dexamethasone at 600 µg/kg body wt has been shown previously to cause muscle atrophy (12, 40). For each dose experiment, there was a control group also randomly chosen from the three groups. Thus each control animal, receiving a daily intraperitoneal injection of 1 ml of saline (vehicle, 0.85% NaCl) had a pair mate (matched by body weight) in the corresponding group receiving dexamethasone treatment. All dexamethasone-treated animals were allowed free access to food and water. Dexamethasone treatment has been reported to decrease food intake (40). It is not known whether food intake can affect myostatin expression independently in animals; therefore, each control animal was pair fed the same amount of food as was consumed by its dexamethasone-treated pair mate during the previous day.

Study 2. Forty rats were divided into four groups with equal total body weight distribution in each group. Two groups were randomly selected to receive dexamethasone treatment (600 µg/kg body wt) intraperitoneally daily for either 5 days (*group I*) or 10 days (*group II*), respectively. The 600 µg/kg body wt dose was selected because this dose resulted in significant loss of muscle mass in *study 1*. The remaining animals, used as controls, were randomly assigned to one of the two dexamethasone-treated groups. Each control animal, receiving daily intraperitoneal injection of vehicle (1 ml of 0.85% NaCl) had a pair mate (matched by body weight) in the corresponding dexamethasone-treated group. All dexamethasone-treated animals were allowed free access to food and water. Each control animal was allowed

free access to water but pair fed the same amount of food as was consumed by its dexamethasone-treated pair mate during the previous day.

Study 3. We examined whether the glucocorticoid receptor antagonist RU-486 could inhibit the effect of dexamethasone on intramuscular myostatin protein and mRNA expression. In this experiment, 50 rats were divided into five groups with an even distribution of initial body weight in each group. The animals were randomly selected for receiving the following treatments for five consecutive days. Group Dex received dexamethasone (600 µg/kg body wt) daily; group Dex/RU-486 received dexamethasone (600 µg/kg body wt) and RU-486 (1,313 µg/kg body wt or 2× molarity of dexamethasone) daily; group RU-486 received RU-486 (1,313 µg/kg body wt) daily; control group PC was given daily saline injections and pair fed; and control group FC was given daily saline injections and free access to food. Animals in groups Dex and FC were allowed free access to the food and water, and their food intakes were recorded daily. Individuals in groups Dex/RU-486, RU-486, and PC were allowed free access to water but were pair fed with the same amount of food as was consumed by their dexamethasone-treated pair mates. In all studies, each individual's body weight was recorded daily.

Muscle collection and calculation of change of muscle mass. After each experimental protocol, animals were killed by CO₂ asphyxiation. The muscles of the gastrocnemius and flexor digitorum superficialis (G/FDS) complex from both legs were excised together, weighed on an analytical balance, and quickly frozen on dry ice and stored at –80°C for subsequent RNA and protein extractions. Although we tried to group animals with an even distribution of initial body weight, there were still minor differences in the averages of initial total body weights among experimental groups. Because the wet muscle weight can be measured only at the end of each experiment, we adjusted the average muscle weight of each experimental group by multiplying its measured average muscle weight (MW) by the ratio of its initial average body weight (BW) vs. that of the control group, i.e.

$$\text{adjusted MW}_{\text{Group A}} = \text{measured MW}_{\text{Group A}} \times (\text{BW}_{\text{control}} \div \text{BW}_{\text{Group A}})$$

Northern blot analysis. Total RNA was extracted from the G/FDS muscle complex with TRIzol (GIBCO-BRL), following the instructions provided by the manufacturer. The extracted RNA pellets were dissolved in diethyl pyrocarbonate-treated water. After the concentrations were determined by a spectrometer at 260 nm, all RNA samples were kept at –80°C until use. To determine the relative abundance of myostatin and the reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNAs, 25 µg of heat-denatured total RNA for each muscle were electrophoresed (4 V/cm for 4 h) on a 0.8% agarose denaturing gel prepared in MOPS [40 mM 3-(N-morpholino)-2-hydroxypropanesulfonic acid], 10 mM sodium acetate, 1 mM EDTA, and 6.6% formaldehyde (all from Sigma). The RNA was transferred to a Hybond-XL nylon membrane (Amersham) by overnight capillary action in 20× SSC (3 M NaCl + 0.3 M Na₃citrate, pH 7.0). Membranes were treated with ultraviolet cross-link (UV Stratelinker 1800; Strategene) at 70,000 µJ, as recommended by the manufacturer. Membranes that carried the RNA samples were first prehybridized in hybridization buffer (5× Denhardt's, 6× SSC, 2.5% SDS, and 100 µg/ml denatured, sonicated salmon sperm DNA, 5 ml/100 cm² filters) in hybridization bottles at 60°C for ≥2 h. DNA probes were radiolabeled by random priming with [α-³²P]dCTP (ICN) with a High Prime kit (Roche), following the manufacturer's instructions.

Table 1. Dexamethasone dose effects on body weight and wet muscle mass

Group (n)	Dex Treatment, $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1a}$	Body Weight			G/FDS Weight, g	Dex-Induced G/FDS Weight Changes, %
		Initial, g	Final, g	Changes, %		
<i>I</i>	C	367.7 \pm 5.0	369.7 \pm 3.7	0.54 \pm 0.44	5.10 \pm 0.05	
(2 \times 10)	60	369.4 \pm 4.9	354.5 \pm 5.2	-4.02 \pm 0.44*	4.80 \pm 0.05 ^b	-6.3 \pm 0.44*
<i>II</i>	C	379.9 \pm 4.4	375.3 \pm 3.5	-1.21 \pm 0.28	5.12 \pm 0.03	
(2 \times 10)	600	375.4 \pm 4.9	325.0 \pm 4.1	-13.42 \pm 0.28*†	4.30 \pm 0.03 ^b	-15.0 \pm 0.28*†
<i>III</i>	C	378.3 \pm 5.1	361.2 \pm 5.1	-4.53 \pm 0.44	4.96 \pm 0.05	
(2 \times 10)	1,200	381.7 \pm 5.2	314.9 \pm 5.6	-17.2 \pm 0.44*†	4.17 \pm 0.05 ^b	-16.6 \pm 0.44*†

Values are expressed as means \pm SE. C, control; G/FDS, gastrocnemius and flexor digitorum superficialis muscle complex. ^aDose of dexamethasone (Dex) per kg body wt daily; ^badjusted value (see MATERIALS AND METHODS). Statistical analysis was carried out by 2-way ANOVA and Tukey's test. *Significantly different from corresponding pair-fed group ($P < 0.05$); †significantly different from Dex (60 μg) dose group ($P < 0.05$).

For myostatin mRNA detection, a rat myostatin cDNA fragment of 748 bp (corresponding to nt 178–925) was used as the probe. For GAPDH mRNA detection, a rat GAPDH cDNA fragment of 1.2 kb was used as the probe.

A radiolabeled probe was boiled for 5 min before being added to the prehybridized membranes at 60°C. After being incubated with the probe overnight at 60°C, the membranes were washed (in SSC + 0.1% SDS at 60°C) to the desired stringency by reducing the SSC concentration. The membranes were then exposed to Kodak Biomax film for 2–5 days at -80°C to visualize bands corresponding to myostatin and GAPDH transcripts. Concentrations of myostatin and GAPDH mRNA were quantified by densitometry scanning (Fluor-s Multiimager; Bio-Rad, Hercules, CA). The uniformity of loadings was adjusted by the optical density (OD) of the GAPDH band.

Western blot analysis. Protein was extracted from G/FDS complex by means of a denaturing-reducing lysis buffer containing 1% SDS, Tris-HCl, and a 1:20 dilution of β -mercaptoethanol. Samples (40 μg each) were heat denatured (95°C for 5 min), electrophoretically separated using 12% Tris-glycine polyacrylamide gels (ReadyGel; Bio-Rad), and the proteins were visualized using Coomassie brilliant blue staining. The electrophoretically separated samples were then transferred to a nitrocellulose membrane (Hybond-ECL; Amersham) and immunodetected using the previously described procedure with the myostatin polyclonal antibody "B" (11). An anti-rabbit-IgG secondary antibody linked to horseradish peroxidase (HRP) was used. Blots were developed with an enhanced chemiluminescent substrate for HRP and exposed to film (ECL Hyperfilm; Amersham). To adjust the protein sample loading, the membranes were stripped by incubating them in stripping buffer (100 mM β -mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl, pH 6.7) at 50°C for 30 min and then reprobing them with a rat monoclonal antibody for GAPDH. The concentrations of myostatin and GAPDH proteins were quantified by densitometry scanning (Fluor-s Multiimager). The uniformity of loadings was adjusted by the OD of the GAPDH band.

Separation and quantitation of MHC isoforms. The MHC isoforms of myofibrils were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method developed by Talmadge and Roy (43). Each gel was composed of two continuous parts, stacking gel (4% acrylamide, 30% glycerol) and separating gel (8% acrylamide, 30% glycerol). SDS-PAGE was run at a constant voltage of 70 V at 4°C for 24 h. The gel was then stained with Coomassie blue. The concentrations of the MHC isoforms were quantified by densitometry scanning (Fluor-s Multiimager).

Statistical analysis. All data are reported as means \pm SE. All statistical tests were performed using the Jandel Sigma-

Stat statistical software package. $P < 0.05$ is taken as the level of statistical significance. For *study 1*, the key outcome variables were body weight, weights of G/FDS complex, and myostatin protein and mRNA concentrations, corrected by GAPDH protein and mRNA. Treatment effects were analyzed by use of a two-way ANOVA model with factors for dose (60, 600, or 1,200 $\mu\text{g}/\text{kg}$ body wt) and treatment (saline or dexamethasone). Comparisons between groups were performed with Tukey's test. For *study 2*, the data were analyzed by use of a two-way ANOVA model with factors for treatment (saline or dexamethasone) and treatment duration (5 or 10 days). Comparisons between groups were performed with the Tukey test. For *study 3*, the treatment effects in the five groups were analyzed with a one-way ANOVA model. Comparisons between groups were performed with Tukey's test.

RESULTS

Study 1: effects of 5-day dexamethasone treatment on body and muscle weight. Food intake decreased markedly in animals, particularly in the first 3 days after dexamethasone-treatment was initiated. The average food intakes of rats from each dexamethasone-treated group (60, 600, and 1,200 $\mu\text{g}/\text{kg}$ body wt) on the day after the third dexamethasone injection were 19, 43, and 54% less than what they had eaten on the day before the dexamethasone treatment (data not shown).

Administration of dexamethasone caused a progressive, dose-dependent loss of body weight in rats (Table 1). The mean body weight reductions from baseline in dexamethasone-treated rats in *groups I, II, and III* (-4.0 \pm 0.4, -13.4 \pm 0.3, and -17.2 \pm 0.4%, respectively) were significantly greater than those in the corresponding pair-fed controls (0.5 \pm 0.4, -1.2 \pm 0.3, and -4.5 \pm 0.4%, respectively, $P < 0.05$; Table 1). The loss of body weight in pair-fed controls was apparently due to the restriction of their food intake.

The mean mass of the G/FDS complex was significantly lower in dexamethasone-treated rats than that in pair-fed controls within each dose group (Table 1). The rats treated with 600 and 1,200 $\mu\text{g}/\text{kg}$ body wt daily had significantly lower muscle mass than those treated with the 60 $\mu\text{g}/\text{kg}$ body wt ($P < 0.05$ for each comparison). As shown in Table 1, the losses of wet weight of G/FDS complex in dexamethasone-treated rats from *groups I, II, and III* were significantly (6.3 \pm 0.4, 15.0 \pm 0.3, and 16.6 \pm 0.4%, respectively, all $P <$

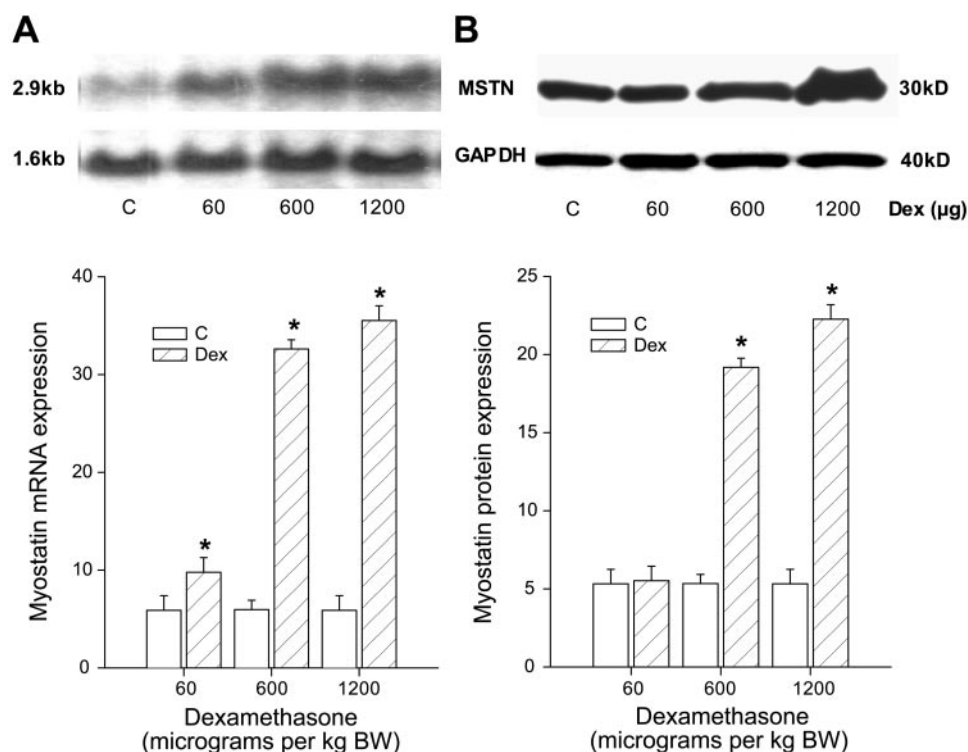


Fig. 1. Dexamethasone (Dex) dose effects on myostatin (MSTN) expression in rats after 5-day treatment. *Top*: myostatin mRNA and protein expression in gastrocnemius and flexor digitorum superficialis (G/FDS) complex analyzed by Northern (A) and Western blot (B). *Bottom*: myostatin mRNA and protein quantified as optical density (OD). Sample loadings were adjusted by the OD of GAPDH. C, control; BW, body weight. * $P < 0.05$, ** $P < 0.01$, significantly different from the corresponding pair-fed control. * $P < 0.05$, significantly different from 60-µg dose.

0.05) higher than those of their corresponding pair-fed controls. Because the 600 µg/kg body wt dose produced near-maximal loss of muscle mass, we selected this dose in the subsequent time course experiments.

Dexamethasone treatment increases intramuscular myostatin gene expression. To determine whether dexamethasone administration affects myostatin expression in skeletal muscle from rats, we measured myostatin protein and mRNA concentrations in the G/FDS complex. The intramuscular myostatin mRNA concentrations, indicated by the abundance of a 2.9-kb transcript by Northern blot analysis, were significantly higher in dexamethasone-treated animals than in the corresponding pair-fed controls ($P < 0.01$). The dexamethasone-induced increase in myostatin mRNA expression in rats was dose dependent. In rats that received five consecutive daily injections of dexamethasone at 60, 600, or 1,200 µg/kg body wt, the myostatin mRNA expression in the G/FDS complexes was 66.3 ± 0.60 , 450 ± 72.4 ($P < 0.01$), and $527.6 \pm 79.8\%$ ($P < 0.01$) higher, respectively, compared with their corresponding pair-fed controls (Fig. 1). Similarly, dexamethasone treatment also dose-dependently increased intramuscular myostatin protein expression. The myostatin protein expression, indicated by the abundance of a 30-kDa immunoreactive band by Western blot analysis in dexamethasone-treated rats at 600 or 1,200 µg/kg body wt, was $260.5 \pm 22.3\%$ ($P < 0.01$) or $318.4 \pm 44.8\%$ ($P < 0.01$) higher than that in their corresponding controls (Fig. 1). In contrast, rats treated with 60 µg/kg body wt dexamethasone showed no significant difference in myostatin protein expression compared with controls (Fig. 1). The sizes of the myostatin mRNA

transcript and myostatin immunoreactive band detected in this study are consistent with the previously reported findings (6, 11, 48).

Study 2: time course of dexamethasone's effects on body and muscle weight and intramuscular myostatin gene expression. To investigate whether extension of dexamethasone treatment would affect muscle weights and intramuscular myostatin protein and mRNA expression, we treated the rats with a daily injection of 600 µg/kg body wt dexamethasone for either 5 days (group I) or 10 days (group II). Again, dexamethasone administration induced a greater loss of body weight in both groups compared with their corresponding pair-fed controls. The average body weight changes in dexamethasone-treated rats from groups I and II, compared with their initial body weights, were -13.4 ± 0.6 ($P < 0.05$) and $-15.3 \pm 0.9\%$ ($P < 0.05$), respectively. In contrast, the body weight changes of their corresponding pair-fed controls were -1.21 ± 1.0 and $0.42 \pm 0.8\%$, respectively. However, the dexamethasone-induced changes in body weights between animals treated for 5 days and for 10 days were not significantly different from each other.

Overall, the average wet weights of G/FDS complex in dexamethasone-treated rats were 13.9 ± 0.71 and $16.5 \pm 1.2\%$ lower after 5 and 10 days of treatment compared with their pair-fed controls ($P < 0.05$ for each of the two comparisons). However, the wet weights of the G/FDS complex in rats treated with dexamethasone for 5 days (4.0 ± 0.2 g) were not significantly different from those in rats treated for 10 days (3.9 ± 0.3 g; Table 2). Interestingly, rats treated with dexamethasone for 5 days showed a marked de-

Table 2. Dexamethasone time course effects on body weight and weight and wet muscle mass

Group (n)	Treatment, days	Body Weight			G/FDS Weight, g	Dex-Induced G/FDS Weight Changes, %
		Initial, g	Final, g	Changes, %		
I (2 × 10)	C(5)	335.8 ± 14.0	331.7 ± 12.3	-1.21 ± 0.6	4.61 ± 0.2	0
	Dex ^a (5)	339.4 ± 14.9	293.9 ± 12.8	-13.4 ± 0.7*	3.91 ± 0.3 ^b	-15.1 ± 0.7*
II (2 × 10)	C(10)	336.2 ± 14.2	337.6 ± 13.1	0.42 ± 0.8	4.67 ± 0.3	0
	Dex ^a (10)	338.7 ± 14.7	287.3 ± 12.3	-15.2 ± 0.9*	3.90 ± 0.3 ^b	-16.5 ± 1.2*

Values are expressed as means ± SE. ^aDex at 600 µg per kg body wt daily; ^badjusted value (see MATERIALS AND METHODS). Statistical analysis was carried out by 2-way ANOVA and Tukey's test. *Significantly different from corresponding pair-fed group ($P < 0.05$).

crease of 42.6% ($P < 0.05$) in the concentration of MHC II(A, X, B) isoforms in the G/FDS complex compared with the pair-fed controls. However, the concentration of MHC II(A, X, B) isoforms in animals treated with dexamethasone for 10 days was only 14.0% ($P < 0.05$) lower than that in the pair-fed controls (Fig. 2). No significant change was detected for MHC I in either dexamethasone-treated group.

As expected, the intramuscular myostatin mRNA expression in rats treated with dexamethasone for 5 days was (densitometry intensity reading 32.3 ± 1.5) significantly (4.50-fold, $P < 0.01$) higher than that (densitometry intensity reading 5.9 ± 1.4) in their pair-fed controls. The myostatin protein expression in these rats was (densitometry intensity reading 19.2 ± 1.4) also significantly higher (2.6-fold, $P < 0.01$) than that in their pair-fed controls (densitometry intensity reading 5.3 ± 1.0 ; Fig. 3). However, after 10 days of treatment, intramuscular myostatin protein concentrations were not significantly different between the control and dexamethasone-treated rats (6.3 ± 1.5 vs. 5.8 ± 1.5 , $P = 0.82$ for mRNA and 6.0 ± 1.1 vs. 5.7 ± 1.0 , $P = 0.86$ for protein; Fig. 3).

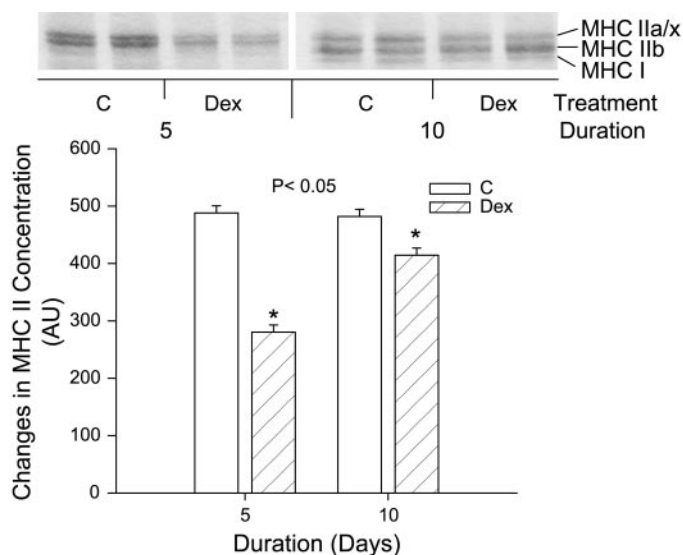


Fig. 2. Dexamethasone time effect on myosin heavy chain (MHC) in rats treated for 5 and 10 days. Top: MHC expression in G/FDS complex. Bottom: MHC II (quantified as OD). Sample loadings were adjusted by the OD of GAPDH. AU, arbitrary units. * $P < 0.05$, significantly different from the corresponding pair-fed control ($600 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$).

Study 3: RU-486 inhibits dexamethasone's effect on body and muscle weights and myostatin gene expression. RU-486 is known to antagonize the effects of glucocorticoids by binding to the glucocorticoid receptors (20, 21, 28). To determine whether RU-486 antagonizes the effects of dexamethasone on myostatin mRNA and protein expression in vivo, we divided rats into five groups of equal body weight distribution. Three randomly selected groups were treated with dexamethasone ($600 \mu\text{g}/\text{kg}$ body wt), dexamethasone ($600 \mu\text{g}/\text{kg}$ body wt) plus RU-486 ($1,313 \mu\text{g}/\text{kg}$ body wt), or RU-486 ($1,313 \mu\text{g}/\text{kg}$ body wt) alone, respectively, for 5 days. To determine whether restriction of food intake could influence myostatin expression, we set up two groups of animals as controls, the pair-fed control (PC) and the free-access-to-food control (FC). Animals from both groups were given daily intraperitoneal injections of the vehicle (0.85% NaCl). The animals were killed after five consecutive daily treatments. Changes in their body weight, G/FDS mass, and myostatin gene expression were analyzed.

As shown in Table 3, animals in the FC group gained on average $7.8 \pm 0.2\%$ in body weight ($P < 0.05$) compared with their initial body weight, whereas the PC group lost $1.2 \pm 0.3\%$ ($P < 0.05$) in body weight. Dexamethasone-treated rats showed a much greater loss in body weight ($-13.4 \pm 0.3\%$, $P < 0.05$) compared with the PC group. Interestingly, Dex/RU-486 animals had significantly fewer losses in body weight ($-6.4 \pm 0.25\%$, $P < 0.05$) than those treated with dexamethasone alone, even though both groups of rats were fed the same amount of food. The change of body weight in the RU-486 group showed no significant difference from that in the PC group (Table 3).

The changes of body weights in the animals were mirrored by the wet muscle weights of each corresponding group. The average wet weight of G/FDS complex of the dexamethasone-treated rats (3.8 ± 0.1 g) was significantly lower than those of groups FC (4.9 ± 0.1 g, $P < 0.05$), PC (4.5 ± 0.1 g, $P < 0.05$), Dex/RU-486 (4.1 ± 0.1 g, $P < 0.05$), and RU-486 (4.5 ± 0.2 , $P < 0.05$), respectively. There was no significant difference between the weights of wet G/FDS complex in the PC rats and in those treated with RU-486 alone (Table 3).

To determine whether RU-486 could alter the dexamethasone-induced increase in intramuscular myostatin expression in animals, we examined the mRNA and protein expression in the G/FDS complex from these

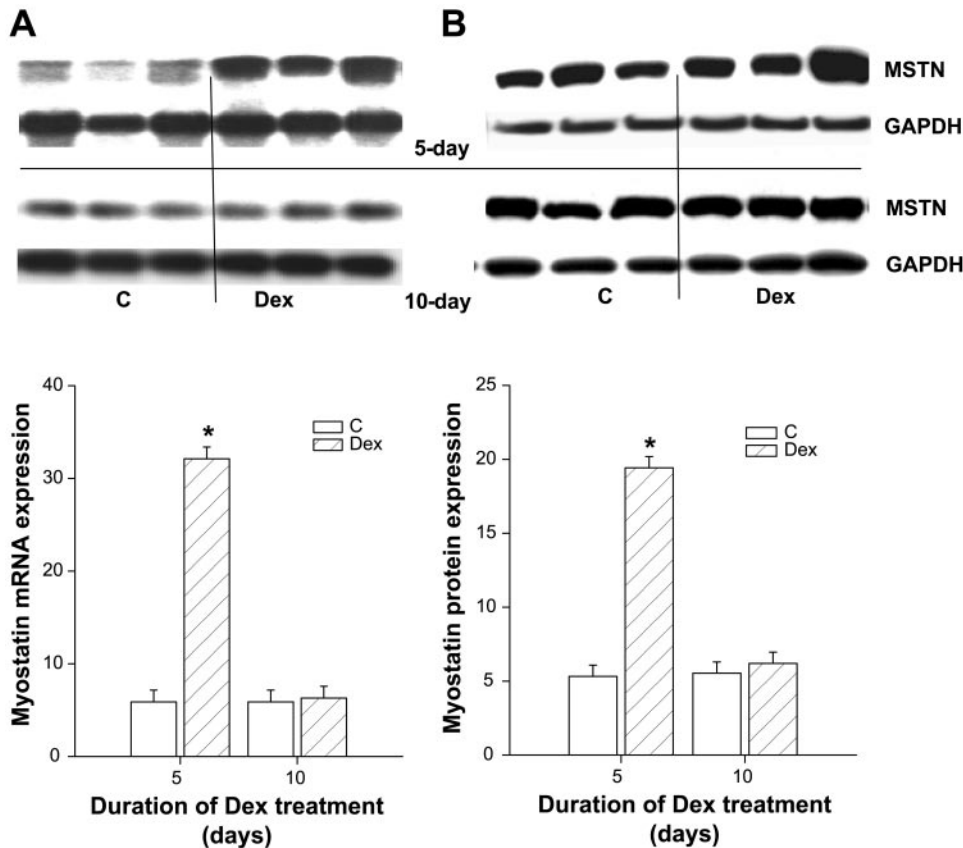


Fig. 3. Dexamethasone time effect on myostatin expression in rats treated for 5 and 10 days. *Top*: myostatin mRNA and protein expression in G/FDS muscle complex analyzed by Northern (A) and Western blot (B). *Bottom*: myostatin mRNA and protein quantified as OD. Sample loadings were adjusted by OD of GAPDH. * $P < 0.01$, significantly different from the corresponding pair-fed controls.

animals by using Northern and Western blot analyses. As displayed in Fig. 4, we found no significant difference in the intramuscular myostatin mRNA expression among animals from Groups FC, PC, and RU-486 (densitometry intensity readings 5.9 ± 0.9 , 5.8 ± 0.9 , and 5.6 ± 1.0 , respectively, $P < 0.05$). The intramuscular myostatin mRNA expressions were ~4.5-fold higher in dexamethasone-treated rats compared with their pair-fed controls (densitometry intensity readings 32.3 ± 1.6 vs. 5.8 ± 0.2 , $P < 0.01$). The action of dexamethasone treatment on myostatin mRNA expression was effectively nullified by RU-486 administration, as the intramuscular myostatin mRNA expression in Dex/RU-486 rats (densitometry intensity reading 6.4 ± 1.0 , $P < 0.05$) showed no significant difference compared with that in groups PC or FC (Fig. 4A).

Similarly, there was no significant difference in the intramuscular myostatin protein expression among animals from the FC, PC, and RU-486 groups (densitometry intensity readings 5.3 ± 0.3 , 5.3 ± 0.2 , and 5.4 ± 0.3 , respectively, $P < 0.05$). The intramuscular myostatin protein expression in the dexamethasone-treated rats (densitometry intensity reading 19.2 ± 1.4 , $P < 0.01$) was significantly higher than those in groups FC (5.3 ± 0.2 , $P < 0.05$), PC (5.3 ± 0.2 , $P < 0.05$), and RU-486 (5.2 ± 0.2 , $P < 0.05$). Again, the dexamethasone-induced increase in myostatin protein expression was completely blocked by RU-486, because the myostatin protein expression (densitometry intensity reading 5.4 ± 0.3 , $P < 0.05$) in animals concurrently administered dexamethasone plus RU-486 showed no significant difference from that in the PC group (Fig. 4B).

Table 3. Effects of dexamethasone plus RU-486 on body weight and wet muscle mass

Treatment Group (n)	Body Weight			G/FDS Weight, g	Dex-Induced G/FDS Weight Changes, %
	Initial, g	Final, g	Changes, %		
FC(10)	329.7 ± 16.3	355.5 ± 16.6	7.84 ± 0.18	4.91 ± 0.12	0
PC(10)	332.1 ± 17.7	328.2 ± 16.1	-1.2 ± 0.29	4.53 ± 0.10	0
Dex ^a (10)	325.2 ± 18.5	281.6 ± 17.2	-13.4 ± 0.29*	3.8 ± 0.08 ^b	-16.7 ± 0.14*
Dex/RU-486(10)	326.2 ± 15.3	305.3 ± 17.1	-6.4 ± 0.25*	4.2 ± 0.13 ^b	-7.3 ± 0.13*
RU-486 ^c (10)	328.0 ± 14.7	324.4 ± 14.4	-1.1 ± 0.21	4.52 ± 0.16 ^b	0

Values are expressed as means ± SE. FC, controls with free access to food; PC, pair-fed control; Dex/RU-486, Dex plus RU-486. ^aDex at 600 µg per kg body weight daily; ^badjusted value (see MATERIALS AND METHODS). ^cRU-486 at 1,313 µg per kg body weight daily. Statistical analysis was carried out by 1-way ANOVA and Tukey's test. *Significantly different from corresponding pair-fed group ($P < 0.05$).

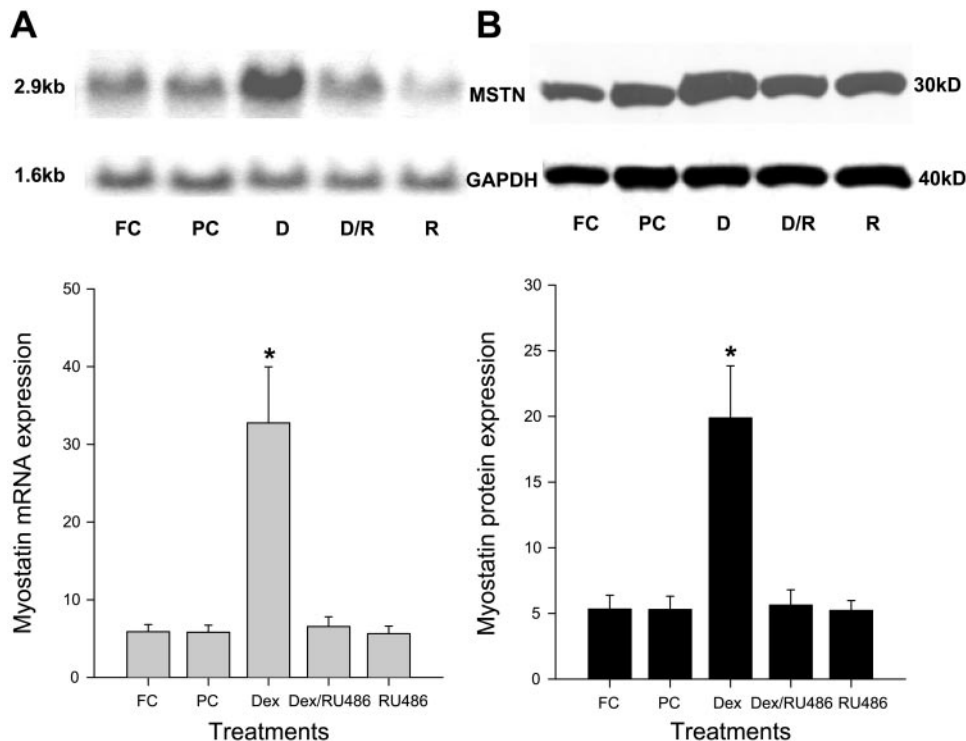


Fig. 4. Effects of dexamethasone and RU-486 on myostatin expression in rats after 5-day treatment. *Top*: myostatin mRNA and protein expression in G/FDS muscle complex analyzed by Northern (A) and Western blot (B). *Bottom*: myostatin mRNA and protein quantified as OD. Sample loadings were adjusted by the OD of GAPDH. * $P < 0.01$, significantly different from control with free access to food (FC), pair-fed control (PC), dexamethasone + RU-486 (Dex/RU-486, D/R), and RU-486-alone (R) treatments.

DISCUSSION

Our study, although confirming previous observations that dexamethasone treatment leads to a dose-dependent loss of total body weight and skeletal muscle mass (2, 38, 39, 41, 42), is the first to demonstrate that this loss is associated with a dose-dependent upregulation of intramuscular myostatin mRNA and protein expression in vivo. These increases in intramuscular myostatin protein and mRNA concentrations were pronounced after 5 days of the treatment; however, the hyperexpression was not sustained, in either myostatin mRNA or protein expression, when treatment was extended to 10 days. Coinciding with the changes in myostatin expression, a marked decrease was found in MHC II protein expression. This decline of MHC expression was particularly obvious on *day 5* of treatment, when myostatin expression was at its peak. Unlike myostatin, the decline in MHC II expression was maintained after 10 days of treatment, albeit at a decreased rate (14% compared with the 42.6% after 5 days). The addition of the potent glucocorticoid receptor antagonist RU-486 reversed the dexamethasone-induced hyperexpression of intramuscular myostatin, suggesting that the glucocorticoid's influence on myostatin expression may be mediated through glucocorticoid receptor-mediated mechanisms.

Because loss of body weight during dexamethasone administration is largely attributed to skeletal muscle atrophy (10, 12, 39, 41), it was not surprising that the reduction in total body weight found in our dexamethasone-treated rats was proportionately less than the associated loss of G/FDS mass. The mechanism(s) by which glucocorticoids induce such catabolic and anti-

anabolic effects on muscle remains unclear. Previous studies have shown that glucocorticoids promote protein degradation and impair protein synthesis, which result in muscle atrophy (2, 10, 12, 39, 41); however, the precise molecular mechanisms of these actions are yet to be elucidated.

A large body of emerging data suggests that myostatin is an important negative regulator of skeletal muscle mass (11, 17, 30, 32, 36, 37, 44, 45). Disruption of myostatin gene expression is associated with dramatic increases in skeletal muscle mass due to muscle fiber hyperplasia and/or hypertrophy (13, 17, 30, 32). Conversely, increased myostatin levels are associated with loss of skeletal muscle mass in conditions as disparate as AIDS wasting syndrome (11), exposure to microgravity during space flight (22), and hindlimb suspension (6, 48). Glucocorticoid-induced protein degradation resulting in muscle atrophy is known to occur predominantly in fast-twitch muscle fibers (MHC IIB), (9, 39, 47). Interestingly, myostatin gene expression appears to be much higher in muscles (e.g., G/FDS complex and extensor digitorum longus), which are mainly composed of fast-twitch fibers, than in those composed of slow-twitch fibers (e.g., soleus) (6, 18, 48). Our previous *in vitro* observations that recombinant myostatin protein inhibits muscle cell proliferation and protein synthesis (36, 37, 44, 45) suggest that it may have an antianabolic effect.

Previously, we identified multiple putative GREs in the human myostatin gene (26) and demonstrated that, *in vitro*, dexamethasone dose-dependently up-regulated endogenous myostatin mRNA and protein expression by increasing myostatin gene transcription

(1, 26). Our present in vivo observation emulates the previous finding (i.e., dexamethasone treatment resulted in significant dose-dependent increase in myostatin expression and a rapid decline of G/FDS mass), suggesting that dexamethasone-induced muscle atrophy is also associated with the upregulation of myostatin expression.

RU-486 inhibits glucocorticoid receptor binding and thus prevents dexamethasone-induced muscle atrophy (14, 20, 46). In the present study, dexamethasone's effects on skeletal muscle mass and myostatin protein and mRNA concentrations were antagonized by concurrent treatment with RU-486. This suggests that dexamethasone affects myostatin expression predominantly through a glucocorticoid receptor pathway. Although dexamethasone's effects on myostatin protein and mRNA were almost completely blocked by RU-486 (Fig. 4), its effects on body weight and skeletal muscle mass, albeit significantly reduced, were not completely prevented (Table 3). Similar partial attenuation of glucocorticoid action has been reported previously (14, 20, 46).

In a previous study, rats given a single injection of dexamethasone showed increases in myostatin mRNA expression of 60 and 270% 4 and 24 h, respectively, after treatment (23). Our study expands this finding, showing that dexamethasone-induced upregulation of myostatin is not only dose dependent but also time dependent. We found that, although the marked upregulation of myostatin mRNA and protein expression induced by dexamethasone could be seen for 5 days after treatment, this hyperexpression was not sustained by extending the treatment to 10 days (Fig. 2). The changes in myostatin expression coincided with those in muscle weight and MHC II. The majority of muscle loss occurred in the first 5 days of treatment, whereas animals treated for 10 days did not experience significantly greater muscle loss than those treated for 5 days (Table 2). Carlson et al. (6) also reported a significant upregulation of intramuscular myostatin after the first day of hindlimb unloading but a return to basal levels after 3–7 days of hindlimb suspension. Rats given high doses of corticosterone have significantly increased muscle tyrosine levels, an important indicator of net protein degradation (5, 15, 19, 35), after 2–5 days of treatment; however, after 5 days, the changes in tyrosine release are not sustained (5). Glucocorticoids are known to upregulate proteolysis and stimulate the expression of components of the ubiquitin-proteolytic pathways in both skeletal muscle and myocytes (8, 15, 27, 31, 34, 46); however, significant changes in ubiquitin are not observed after long-term glucocorticoid excess (36). The molecular adaptations that prevent continued loss of muscle mass and attenuate increases in myostatin expression during long-term exposure to catabolic stimuli remain unknown.

In addition to multiple GREs, the myostatin promoter contains other elements important for muscle growth. The presence of myocyte enhancer factor-2 and nuclear factor- κ B, among others (26), indicates that myostatin is probably regulated not only by glucocorticoids but also by other factors. The role of these

putative factors in acute and chronic regulation of myostatin gene expression during illness and other catabolic states remains to be elucidated.

In summary, we have demonstrated that dexamethasone dose- and time-dependently induces loss of body and muscle weights, upregulates intramuscular myostatin protein and mRNA concentrations, and is associated with a decline in MHC II expression. These effects can be blocked by the addition of RU-486, suggesting that myostatin expression is mediated via glucocorticoid receptors. We propose that muscle loss associated with dexamethasone administration is mediated, in part, by the upregulation of myostatin expression through a glucocorticoid receptor-mediated pathway. The speculation that dexamethasone induces muscle loss by upregulating myostatin transcription should now be further tested.

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DISCLOSURE

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REFERENCES

1. Artaza JN, Bhasin S, Mallidis C, Taylor W, Ma K, and Gonzalez-Cadavid NF. Endogenous expression and localization of myostatin and its relation to myosin heavy chain distribution in C2C12 skeletal muscle cells. *J Cell Physiol* 190: 170–179, 2002.
2. Auclair D, Garrel DR, Chaouki Zerouala A, and Ferland LH. Activation of the ubiquitin pathway in rat skeletal muscle by catabolic doses of glucocorticoids. *Am J Physiol Cell Physiol* 272: C1007–C1016, 1997.
3. Barnes PJ. Scientific rationale for inhaled combination therapy with long-acting beta2-agonists and corticosteroids. *Eur Respir J* 19: 182–191, 2002.
4. Barnes PJ and Adcock IM. Transcription factors and asthma. *Eur Respir J* 12: 221–234, 1998.
5. Bowes SB, Jackson NC, Papachristodoulou D, Umpleby AM, and Sonksen PH. Effect of corticosterone on protein degradation in isolated rat soleus and extensor digitorum longus muscles. *J Endocrinol* 148: 501–507, 1996.
6. Carlson CJ, Booth FW, and Gordon SE. Skeletal muscle myostatin mRNA expression is fiber-type specific and increases during hindlimb unloading. *Am J Physiol Regul Integr Comp Physiol* 277: R601–R606, 1999.
7. Cole CH. Postnatal glucocorticosteroid therapy for treatment and prevention of neonatal chronic lung disease. *Expert Opin Investig Drugs* 9: 53–67, 2000.
8. Dardevet D, Sornet C, Taillandier D, Savary I, Attaix D, and Grizard J. Sensitivity and protein turnover response to glucocorticoids are different in skeletal muscle from adult and old rats. Lack of regulation of the ubiquitin-proteasome proteolytic pathway in aging. *J Clin Invest* 96: 2113–2119, 1995.
9. Fang CH, Li BG, Tiao G, Wang JJ, Fischer JE, and Haselgren PO. The molecular regulation of protein breakdown following burn injury is different in fast- and slow-twitch skeletal muscle. *Int J Mol Med* 1: 163–169, 1998.
10. Gardiner PF, Montanaro G, Simpson DR, and Edgerton VR. Effects of glucocorticoid treatment and food restriction on

- rat hindlimb muscles. *Am J Physiol Endocrinol Metab* 238: E124–E130, 1980.
11. **Gonzalez-Cadavid NF, Taylor WE, Yarasheski K, Sinha-Hikim I, Ma K, Ezzat S, Shen R, Lalani R, Asa S, Mamita M, Nair G, Arver S, and Bhasin S.** Organization of the human myostatin gene and expression in healthy men and HIV-infected men with muscle wasting. *Proc Natl Acad Sci USA* 95: 14938–14943, 1998.
 12. **Goodlad GA and Clark CM.** Glucocorticoid-mediated muscle atrophy: alterations in transcriptional activity of skeletal muscle nuclei. *Biochim Biophys Acta* 1097: 166–170, 1991.
 13. **Grobet L, Martin LJ, Poncelet D, Pirottin D, Brouwers B, Riquet J, Schoeberlein A, Dunner S, Menissier F, Massabanda J, Fries R, Hanset R, and Georges M.** A deletion in the bovine myostatin gene causes the double-muscling phenotype in cattle. *Nat Genet* 17: 71–74, 1997.
 14. **Hall-Angeras M, Angeras U, Zamir O, Hasselgren PO, and Fischer JE.** Effect of the glucocorticoid receptor antagonist RU 38486 on muscle protein breakdown in sepsis. *Surgery* 109: 468–473, 1991.
 15. **Hasselgren PO.** Glucocorticoids and muscle catabolism. *Curr Opin Clin Nutr Metab Care* 2: 201–205, 1999.
 16. **Hasselgren PO, Wray C, and Mammen J.** Molecular regulation of muscle cachexia: it may be more than the proteasome. *Biochem Biophys Res Commun* 290: 1–10, 2002.
 17. **Kambadur R, Sharma M, Smith TP, and Bass JJ.** Mutations in myostatin (GDF8) in double-muscling Belgian Blue and Piedmontese cattle. *Genome Res* 7: 910–916, 1997.
 18. **Kawada S, Tachi C, and Ishii N.** Content and localization of myostatin in mouse skeletal muscles during aging, mechanical unloading and reloading. *J Muscle Res Cell Motil* 22: 627–633, 2001.
 19. **Kettelhut IC, Wing SS, and Goldberg AL.** Endocrine regulation of protein breakdown in skeletal muscle. *Diabetes Metab Rev* 4: 751–772, 1988.
 20. **Konagaya M, Bernard PA, and Max SR.** Blockade of glucocorticoid receptor binding and inhibition of dexamethasone-induced muscle atrophy in the rat by RU38486, a potent glucocorticoid antagonist. *Endocrinology* 119: 375–380, 1986.
 21. **Konagaya M and Max SR.** A possible role for endogenous glucocorticoids in orchietomy-induced atrophy of the rat levator ani muscle: studies with RU 38486, a potent and selective anti-glucocorticoid. *J Steroid Biochem* 25: 305–308, 1986.
 22. **Lalani R, Bhasin S, Byhower F, Tarnuzzer R, Grant M, Shen R, Asa S, Ezzat S, and 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.
 23. **Lang CH, Silvis C, Nystrom G, and Frost RA.** Regulation of myostatin by glucocorticoids after thermal injury. *FASEB J* 15: 1807–1809, 2001.
 24. **Lee SJ and McPherron AC.** Myostatin and the control of skeletal muscle mass. *Curr Opin Genet Dev* 9: 604–607, 1999.
 25. **Lee SJ and McPherron AC.** Regulation of myostatin activity and muscle growth. *Proc Natl Acad Sci USA* 98: 9306–9311, 2001.
 26. **Ma K, Mallidis C, Artaza J, Taylor W, Gonzalez-Cadavid N, and Bhasin S.** Characterization of 5'-regulatory region of human myostatin gene: regulation by dexamethasone in vitro. *Am J Physiol Endocrinol Metab* 281: E1128–E1136, 2001.
 27. **Marinovic AC, Zheng B, Mitch WE, and Price SR.** Ubiquitin (UbC) expression in muscle cells is increased by glucocorticoids through a mechanism involving Sp1 and MEK1. *J Biol Chem* 277: 16673–16681, 2002.
 28. **Max SR, Thomas JW, Banner C, Vitkovic L, Konagaya M, and Konagaya Y.** Glucocorticoid receptor-mediated induction of glutamine synthetase in skeletal muscle cells in vitro. *Endocrinology* 120: 1179–1183, 1987.
 29. **McKinsey TA, Zhang CL, and Olson EN.** Control of muscle development by dueling HATs and HDACs. *Curr Opin Genet Dev* 11: 497–504, 2001.
 30. **McPherron AC and Lee SJ.** Double muscling in cattle due to mutations in the myostatin gene. *Proc Natl Acad Sci USA* 94: 12457–12461, 1997.
 31. **Mitch WE and Goldberg AL.** Mechanisms of muscle wasting. The role of the ubiquitin-proteasome pathway. *N Engl J Med* 335: 1897–1905, 1996.
 32. **Nishi M, Yasue A, Nishimatsu S, Nohno T, Yamaoka T, Itakura M, Moriyama K, Ohuchi H, and Noji S.** A missense mutant myostatin causes hyperplasia without hypertrophy in the mouse muscle. *Biochem Biophys Res Commun* 293: 247–251, 2002.
 33. **Odedra BR, Bates PC, and Millward DJ.** Time course of the effect of catabolic doses of corticosterone on protein turnover in rat skeletal muscle and liver. *Biochem J* 214: 617–627, 1983.
 34. **Penner G, Gang G, Sun X, Wray C, and Hasselgren PO.** C/EBP DNA-binding activity is upregulated by a glucocorticoid-dependent mechanism in septic muscle. *Am J Physiol Regul Integr Comp Physiol* 282: R439–R444, 2002.
 35. **Quan ZY and Walser M.** Effect of corticosterone administration at varying levels on leucine oxidation and whole body protein synthesis and breakdown in adrenalectomized rats. *Metabolism* 40: 1263–1267, 1991.
 36. **Rios R, Carneiro I, Arce VM, and Devesa J.** Myostatin regulates cell survival during C2C12 myogenesis. *Biochem Biophys Res Commun* 280: 561–566, 2001.
 37. **Rios R, Carneiro I, Arce VM, and Devesa J.** Myostatin is an inhibitor of myogenic differentiation. *Am J Physiol Cell Physiol* 282: C993–C999, 2002.
 38. **Rouleau G, Karpati G, Carpenter S, Soza M, Prescott S, and Holland P.** Glucocorticoid excess induces preferential depletion of myosin in denervated skeletal muscle fibers. *Muscle Nerve* 10: 428–438, 1987.
 39. **Roy RR, Gardiner PF, Simpson DR, and Edgerton VR.** Glucocorticoid-induced atrophy in different fibre types of selected rat jaw and hind-limb muscles. *Arch Oral Biol* 28: 639–643, 1983.
 40. **Savary I, Debras E, Dardevet D, Sornet C, Capitan P, Prugnaud J, Mirand PP, and Grizard J.** Effect of glucocorticoid excess on skeletal muscle and heart protein synthesis in adult and old rats. *Br J Nutr* 79: 297–304, 1998.
 41. **Seene T.** Turnover of skeletal muscle contractile proteins in glucocorticoid myopathy. *J Steroid Biochem Mol Biol* 50: 1–4, 1994.
 42. **Shah OJ, Anthony JC, Kimball SR, and Jefferson LS.** Glucocorticoids oppose translational control by leucine in skeletal muscle. *Am J Physiol Endocrinol Metab* 279: E1185–E1190, 2000.
 43. **Talmadge RJ and Roy RR.** Electrophoretic separation of rat skeletal muscle myosin heavy-chain isoforms. *J Appl Physiol* 75: 2337–2340, 1993.
 44. **Taylor WE, Bhasin S, Artaza J, Byhower F, Azam M, Willard DH Jr, Kull FC Jr, and Gonzalez-Cadavid N.** Myostatin inhibits cell proliferation and protein synthesis in C₂C₁₂ muscle cells. *Am J Physiol Endocrinol Metab* 280: E221–E228, 2001.
 45. **Thomas M, Langley B, Berry C, Sharma M, Kirk S, Bass J, and Kambadur R.** Myostatin, a negative regulator of muscle growth, functions by inhibiting myoblast proliferation. *J Biol Chem* 275: 40235–40243, 2000.
 46. **Tiao G, Fagan J, Roegner V, Lieberman M, Wang JJ, Fischer JE, and Hasselgren PO.** Energy-ubiquitin-dependent muscle proteolysis during sepsis in rats is regulated by glucocorticoids. *J Clin Invest* 97: 339–348, 1996.
 47. **Tiao G, Lieberman M, Fischer JE, and Hasselgren PO.** Intracellular regulation of protein degradation during sepsis is different in fast- and slow-twitch muscle. *Am J Physiol Regul Integr Comp Physiol* 272: R849–R856, 1997.
 48. **Wehling M, Cai B, and Tidball JG.** Modulation of myostatin expression during modified muscle use. *FASEB J* 14: 103–110, 2000.
 49. **Wolfe RR.** Control of muscle protein breakdown: effects of activity and nutritional states. *Int J Sport Nutr Exerc Metab* 11: S164–S169, 2001.