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Myostatin Decreases with Aerobic Exercise and Associates with Insulin Resistance

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Abstract

Purpose—There is mounting evidence that skeletal muscle produces and secretes biologically active proteins or “myokines” that facilitate metabolic cross talk between organ systems. The increased expression of myostatin, a secreted anabolic inhibitor of muscle growth and development, has been associated with obesity and insulin resistance. Despite these intriguing findings, there have been few studies linking myostatin and insulin resistance.

Methods—To explore this relationship in more detail, we quantified myostatin protein in muscle and plasma from 10 insulin-resistant, middle aged (53.1 ± 5.5 years) men before and after 6 months of moderate aerobic exercise training (1200 kcal/wk at 40–55% peak $\dot{V}O_2$). To establish a cause-effect relationship we also injected C57/B16 male mice with high-physiologic levels of recombinant myostatin protein.

Results—Myostatin protein levels were shown to decrease in muscle (37%, $P=0.042$, $n=10$) and matching plasma samples (28.7 pre-training to 22.8 ng/ml post-training, $P=0.003$, $n=9$) with aerobic exercise. Furthermore, the strong correlation between plasma myostatin levels and insulin sensitivity ($R^2 = 0.82$, $P<0.001$, $n=9$) suggested a cause-effect relationship that was subsequently confirmed by inducing insulin resistance in myostatin-injected mice. A modest increase (44%) in plasma myostatin levels was also associated with significant reductions in the insulin-stimulated phosphorylation of AKT (Thr³⁰⁸) in both muscle and liver of myostatin treated animals.

Conclusions—These findings indicate that both muscle and plasma myostatin protein levels are regulated by aerobic exercise and furthermore, that myostatin is in the causal pathway of acquired insulin resistance with physical inactivity.

Keywords

Liver; Akt; phosphorylation; mouse

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INTRODUCTION

Myostatin (Mstn), a potent regulator of muscle development and size is a member of the transforming growth factor β (TGF β) superfamily of secreted proteins (7,24). As with all members of the TGF β family, it is translated as a precursor protein that is subsequently processed into a mature peptide dimer. Mature Mstn is then secreted from muscle so it can act locally or systemically via the activin type II A and B (ActRIIA/B) receptors (29,34). In postnatal skeletal muscle, Mstn inhibits the proliferation and differentiation of myoblasts and as well as the Akt/mTOR pathway which regulates protein synthesis (8,13). This is why the pharmacological or genetic inhibition of Mstn signaling produces a hyper-muscular phenotype in all vertebrate species (24,27).

The antagonism of Mstn and insulin signaling was first postulated when Mstn null mice exhibited immunity to dietary-induced obesity and insulin resistance (5). This finding is supported by observational studies of muscle from obese and insulin resistant human subjects that show increased expression of Mstn mRNA and protein (8,25,28). Furthermore, short-term administration of recombinant Mstn induces insulin resistance in Mstn null (36) animals.

Previously, we discovered that muscle and plasma Mstn protein levels increased with both body mass and the severity of insulin resistance in lean and extremely obese human subjects (8). However, a weakness of this study was that we had no way to determine if differences in lean or fat mass were contributing to elevated Mstn levels, as some have postulated (1,6). This “myostatin paradox” (14), a positive correlation between Mstn levels and muscle mass (one would expect the opposite), has been described by others and suggests there are other factors that govern Mstn expression levels in muscle. Also, resistance exercise (RE), aerobic exercise (AE) and weight loss reduce Mstn mRNA levels in muscle of rats and human subjects (10, 14,20–22,35,38). Because exercise and weight loss both improve whole body insulin sensitivity (9) we hypothesize that Mstn is an important component of signaling pathways that determine whole body insulin sensitivity and that insulin action should correlate with plasma Mstn protein levels in interventions that modulate insulin action (8). To test this hypothesis and expand upon previous studies that have studied Mstn mRNA levels alone, we quantified Mstn protein in paired muscle and plasma samples from insulin resistant men before and after 6 months of moderate AE training (9). Furthermore, to determine its effects on whole body insulin sensitivity we also injected mice with recombinant Mstn. To our knowledge this is the first study of Mstn protein levels with AE training in a pre-diabetic patient population.

METHODS

Human Subjects

The skeletal muscle biopsies and plasma used in this study were obtained from 10 men participating in the Studies of Targeted Risk Reduction Interventions Through Defined Exercise (STRRIDE) study (9,33). Briefly, subjects ranged from 40 to 64 years of age, were sedentary (exercise < once weekly; peak $\dot{V}O_2$ 25.8 to 34.6 ml·kg⁻¹·min⁻¹), overweight (BMI 27.9 to 36.1) and exhibited signs of fasting hyper-insulinemia (fasting insulin 12.3 to 18.8 IU/ml) with mild to moderate lipid abnormalities (LDL 132 to 163 mg·dl⁻¹ and HDL 27 to 40 mg·dl⁻¹). Exclusion criteria included no medications that could alter carbohydrate metabolism and evidence for diabetes, orthopedic conditions prohibiting exercise, hypertension, and heart disease. All subjects provided written, informed consent. This study was performed in accordance with the Declaration of Helsinki and received ethics approval from Duke University Medical Center.

Human Exercise training

Subjects were prescribed a moderate-intensity aerobic exercise regimen (1,200 kcal/wk kJ/wk at 40–55% peak VO_2 , 14 kcal·kg⁻¹·wk⁻¹). The lower exercise training intensity (40–55% VO_2 peak) was chosen because it approximates brisk walking or moderate-intensity exercise levels advised in current health guidelines. The exercise modes included cycle ergometer, treadmill, and elliptical trainers to enhance variety and adherence. Once the exercise volume (kcal·kg⁻¹·wk⁻¹) was calculated, subjects selected, with the assistance of an exercise physiologist, an appropriate exercise frequency and duration to achieve their weekly exercise dose. All exercise sessions were verified by direct supervision and/or use of a heart rate monitor that provided recorded data (Polar Electro, Woodbury, NY). To minimize musculoskeletal injury there was an initial ramp period of 2 to 3 months followed by 6 months at the appropriate exercise prescription. Subjects were counseled to maintain baseline body weight so as to establish the body mass independent value of increased fitness. Body mass and diet were closely monitored to determine if subjects were purposely changing diet to lose weight. We observed no systematic changes in diet among the groups. Body composition was determined using the sum of 4 skinfolds measured with Lange calipers (Beta Technology Inc, Cambridge, MD) and the sex-specific formulas of Jackson and Pollock as described previously (32,33).

Human Insulin Sensitivity

Insulin sensitivity was determined with a 3-h intravenous glucose tolerance test (IVGTT) as described previously (9). Briefly, the IVGTT was performed ~24 h after the final exercise bout. After fasting samples were obtained, glucose (50%) was injected into a catheter placed in an antecubital vein at a dose of 0.3 g/kg body mass. Insulin, at a dose of 0.025 U/kg body mass was injected at minute 20. Blood samples were obtained at minutes 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 and the samples were centrifuged, and plasma frozen at -80°C for the subsequent determination of insulin and glucose. Insulin was determined with immunoassay (Access Immunoassay System, Beckman Coulter, Fullerton, CA) and glucose was determined with an oxidation reaction (YSI model 2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, OH). An insulin sensitivity index (SI) was calculated by using the minimal model where a higher SI indicates enhanced insulin sensitivity.

Human Muscle Biopsies

Skeletal muscle biopsies were obtained with the percutaneous needle biopsy technique using 1% lidocaine without epinephrine. Two serial *vastus lateralis* muscle biopsies (100 to 200 mg) were obtained with a triple pass of the bioprobe needle: one upon entry into the study and one after 9 months (3 months ramp up, 6 months training) of exercise training (within 24 hours after the last bout of exercise). Biopsy samples were flash frozen for histology and other analyses. Sample of approximately 25 mg/biopsy were used for the analyses described here.

Mouse Maintenance and Experimentation

Procedures were approved by the University of Calgary Animal Care and Use Committee and abide by the Canadian Association for Laboratory Animal Science guidelines for animal experimentation. Animals were maintained in a humidity controlled room with a 12-h light:dark cycle. Following weaning (3 wk of age), male C57BL/6J littermates were randomly segregated into two groups (n=16 each) and maintained in microisolator cages for one week. Following this acclimation period, treated animals received 15 µg/kg/day of recombinant myostatin (MSTN) (R&D Systems, Minneapolis, MN) whilst the control group received 100 µL of phosphate buffered saline (PBS) as described by Wilkes *et al.* (9). The recombinant myostatin was diluted in 100 µL of PBS and administered daily by subcutaneous injection. Food and

water were provided *ad libitum* throughout the experiment. Insulin tolerance tests were determined in 6 hour fasted mice.

Mouse Insulin Tolerance Test

Insulin (Novolin R; Novo Nordisk, Copenhagen, Denmark) was administered to conscious mice by intraperitoneal injection (0.75 U/kg). Tail vein blood glucose was measured in duplicate using a One-Touch Ultra glucometer (One Touch, Lifescan, Burnaby, BC) at 0, 20, 40, 60 and 80 minutes. Animals were allowed to rest overnight, then weighed and anesthetized (Pentobarbital) with the exception of six animals from each treatment group that were first injected with 10 U/kg of insulin for assessment of maximum insulin-stimulated Akt phosphorylation. Whole blood (~1 mL) was obtained by a cardiac puncture and placed on ice and allowed to clot for 30 min. Samples were then centrifuged for 10 min (3000 rpm) and sera collected prior to storage at -80 C. Tissues were harvested and flash frozen at -80 C.

Mouse Serum Hormone levels

Serum insulin, leptin, adiponectin, resistin and TNF α were measured in duplicate by the LINCoplex assay (Linco Research, Inc. St Charles Missouri, USA). This assay relied on the use of polystyrene beads, each with a unique signature mix of fluorescent dyes that can be discriminated by a laser-based detection instrument, the Luminex 100 (Luminex Corp., Austin, TX). Each bead type was coated with a specific antibody to the cytokine of interest. The cytokine antibody pairs in this multiplex assay do not cross react with other analytes in the panel. The intra-assay coefficient of variation (CV) was below 10%.

Myostatin ELISA

Mature Mstn protein levels in human plasma and mouse serum were determined in duplicate using an ELISA assay according to the manufacturers instructions (Immundiagnostik, Bensheim, Germany). Samples were read using a microtiter plate reader at 450 nm against 620 nm as a reference. A 4-parameter-algorithm was used to calculate the standard curve against which the concentration of Mstn in human and mouse samples were determined. The coefficient of variance between replicates was consistently below 10%.

Western Blotting

Nitrogen pulverized muscle and liver were extracted using GSK-3 buffer (10% Glycerol, 150 mM NaCl, 50 mM HEPES (pH 7.5), 1% NP-40, 2mM EDTA (pH 8.0), 1mM CaCl₂, 1mM Mg Cl₂, 1X Halt™ Protease and Phosphatase Inhibitor Cocktail (Pierce, Rockford, IL), 3mM Benzamide) with gentle inversion at 4°C for 1 hour. Samples were then centrifuged at 4°C for 1 hour at 17500g and protein concentration determined using the Bio-Rad protein assay dye reagent, following the manufacturer's instructions (Bio-Rad, Hercules, CA). 20 μ g of cellular protein were then separated on precast 4–12% gradient SDS-PAGE gels (Invitrogen, Carlsbad, CA) and transferred to Immobilon-P PVDF Membrane (Millipore, Billerica, MA). The primary anti-Mstn antibody used in this study (AB3239, Chemicon/Millipore) was raised in rabbits against the active C-terminal domain of Mstn as previously described (6). Anti-AKT and anti-phospho-AKT(Thr³⁰⁸) antibodies were purchased from Cell Signal (Beverly, MA). An alpha-actin mouse monoclonal antibody (Sigma St. Louis, MO) and anti-AKT antibodies were used on stripped membranes as a loading control. Membranes were incubated in 1/1000 in primary antibody in TBST overnight (~14 hours) at 4°C. After washing, primary antibodies were detected using HRP-conjugated secondary antibodies (Santa-Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1/5000 and a SuperSignal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL). Images were acquired and quantified using a ChemiGenius Bioimaging System (Syngene, Frederick, MD).

Statistics

Relative and absolute changes (after training/before training) in Mstn protein levels were compared with paired Student's t-test. Insulin tolerance tests were analyzed by ANOVA and t-tests as appropriate. Correlations (Pearson product) were performed between circulating Mstn levels (ng/ml) and selected variables (SI, Lean and Fat Mass). Statistical significance was indicated at or below the $P = 0.05$ level. Data are presented as means $\pm$ SEM.

RESULTS

Exercise Training

The baseline and post-exercise training characteristics of STRRIDE study participants have been described previously (2,9). The characteristics of the 10 men used for our study are described in Table 1. There were no significant changes in total weight, lean or fat mass and BMI whereas Si fasting insulin and peak VO_2 changed significantly with aerobic exercise training.

Myostatin Western Blots

When compared with pre-trained skeletal muscle (Figure 1) Mstn levels decreased significantly with exercise training relative to alpha-actin (37% decrease, $P=0.042$, $n=10$). These results are consistent with time-frame for the depression of Mstn mRNA in muscle with both AE and RE training in human subjects (11,14,20). Using the same antibody, a recent study found decrease in 28 and 10 kDa immunoreactive bands with 90 days of resistance training followed by a significant increase after 30 days of detraining (12). Presumably, these proteins represent the dimeric and monomeric forms of the mature myostatin protein. While there is some controversy regarding the specificity of this antibody, in our hands the titration of pure myostatin protein eliminated the 26 kDa immunoreactive band from our Western blots. Furthermore, we used less stringent reducing conditions during sample preparation because of significant variability in the reduction of the dimeric form of Mstn to the monomeric form, which makes quantification by Western blot difficult.

Myostatin ELISA

When compared against pre-training levels, circulating Mstn protein decreased from 28.7 ± 3.1 to 22.8 ± 2.0 ng/ml with AE training (Table 1 $P=0.003$, $n=9$). Of note, the significant intra-individual variability in pre and post training levels of plasma Mstn suggests that genetics may play an important role regulation of expression levels (17). Supporting this notion is the high degree of correlation ($R^2 = 0.9$, $P<0.001$, $n=9$) between pre and post-AE training Mstn levels. Not surprisingly, plasma myostatin levels were elevated in Mstn-treated vs PBS-treated mice (Table 2) though only modestly (44% increase, $P = 0.06$). Compared to previous assessments of circulating Mstn in human subjects our values were an order of magnitude higher (18) or lower (6) than those previously described. We assume that this discrepancy relates to differences in the sensitivity and specificity of different antigen/antibody combinations used by distinct ELISA assays.

Human Myostatin Associations

We found an inverse relationship between plasma Mstn levels and Si, which supports a direct correlation between Mstn and insulin sensitivity ($R^2 = 0.82$, $P<0.001$, $n=9$) (Figure 2). Furthermore, this association was less pronounced, but still significant after exercise training ($R^2 = 0.49$, $P=0.024$, $n=10$). We also found that pre-training plasma Mstn levels correlated better with insulin sensitivity than lean ($R^2 = 0.07$, NS, $n=9$) or fat mass ($R^2 = 0.14$, NS, $n=9$).

Mouse Insulin Tolerance and Serum Hormones

To determine if Mstn could induce insulin resistance in mice we randomly segregated healthy male littermates of similar body mass into two treatment groups. Significant insulin resistance was induced in Mstn-treated mice relative to PBS-treated control animals (Figure 3). Fasting glucose and insulin were also elevated (though not significantly) in Mstn-treated animals (Table 2). The concentration and duration of myostatin treatment were not sufficient to induce significant changes on body, gastrocnemius or soleus muscle mass. Unlike previous studies we did not find that Mstn treatment produced an increase in circulating TNF α , which is known to induce insulin resistance a number of tissues (9) (Table 2). Furthermore, we found no significant differences in circulating levels of adipocyte-derived metabolic hormones leptin, resistin or adiponectin.

Mouse Akt Western Blots

Measurements of insulin-stimulated AKT phosphorylation at Thr³⁰⁸ in muscle and liver extracts showed that insulin signaling was reduced by 68% in gastrocnemius muscle and 45% in vastus lateralis of Mstn-treated animals (Figure 4). Even more impressive was the 87% reduction in insulin-stimulated AKT phosphorylation in livers of Mstn-Treated animals. All results were significant with $P < 0.01$. These results confirm and expand upon the findings of Wilkes *et al* (36) who were the first to suggest the antagonism of myostatin and insulin signaling in the liver. Although both Thr³⁰⁸ and Ser⁴⁷³ are required for the complete activation of AKT, the Thr³⁰⁸ antibody was chosen because of studies that show a complete absence of GLUT4 translocation when blocking the Thr³⁰⁸ but not the Ser⁴⁷³ phosphorylation of AKT in response to insulin (15).

DISCUSSION

Aerobic and resistance exercise reduces muscle and circulating Mstn levels in human subjects whether acute or chronic (11,20,30). While there have been prior studies of circulating Mstn protein in relation to body mass, age, gender and exercise (6,8,18,35,37) ours is the first to describe a significant decrease in circulating Mstn protein with aerobic exercise training. This is also the first study to show a significant correlation between circulating Mstn and insulin sensitivity. While this did not prove a cause-effect relationship *per se*, we were able to induce whole-body insulin resistance in mice with the short-term administration of recombinant Mstn (36). This finding suggests that small changes in circulating Mstn can produce significant changes in insulin sensitivity in both liver and muscle. However, since myostatin levels were assayed > 12 hours after their last injection it is possible that serum levels were much higher at an earlier time point. These findings also support previously published studies in our laboratory in which we hypothesized a relationship between circulating Mstn and insulin resistance (8).

Interestingly, the post-natal suppression of circulating Mstn protein using anti-Mstn antibodies, soluble ActRIIB receptor and Mstn propeptide prevents dietary induced insulin resistance in mice (19,39). In fact, recent evidence suggests that improvements in insulin sensitivity and aerobic endurance in mice after short-term Mstn inhibition were as effective as four weeks of aerobic exercise training, suggesting a potential complementary role for myostatin antagonists in the treatment of obesity and type 2 diabetes (19). It is therefore plausible that exercise-associated decreases in muscle and plasma Mstn may contribute to improvements in whole-body insulin sensitivity.

We had previously hypothesized that increased muscle and plasma Mstn is a secondary response to acquired insulin resistance with physical inactivity (8). While the evidence presented in this study would appear to support this hypothesis it creates a minor logical

paradox, wherein increased circulating Mstn is both cause and effect of insulin resistance. The data presented in this and other exercise studies indicate that muscle activity levels primarily dictate Mstn expression levels and that the secondary effects are metabolic. In fact, in addition to muscle (7) Mstn signaling has been shown to play a role in the metabolic adaptation of liver (36), heart (31) and placenta (26) suggesting a broad role in metabolic cross-talk between organs. Although we found no evidence that Mstn altered circulating levels of hormones and cytokines known to influence insulin signaling (Table 2), to determine if myostatin signaling acts directly on the insulin signaling pathway and glucose uptake we plan to conduct follow-up studies using liver and muscle cells in culture.

Like most members of the TGF β family Myostatin is extensively regulated at the post-translational level, where it is cleaved into a chaperone-like pro-domain and an active peptide-dimer in the very stable cysteine knot conformation (23,39). This is why some confusion persists about the appropriate molecular weight of the immunoreactive myostatin and why some prefer to study the mRNA levels alone. In fact, studies of Mstn mRNA have provided important information about the timeframe for the suppression of Mstn expression with aerobic exercise. The exercise-induced suppression of Mstn mRNA and protein occurs in as little as 1 hour and persists as long as 24 hours after a single exercise session (3,11,20).

Like myostatin, insulin-like growth factor 1 (IGF-1) is an important component of the homeostatic mechanism linking muscle activity to hypertrophy and atrophy (4,16). Exercise-induced IGF-1 signaling is mediated in part by the PI3K/PTEN/AKT pathway that promotes cell proliferation and myogenesis in both the heart and skeletal muscle. Opposing this mechanism, myostatin has been shown reduce IGF-1 stimulated AKT phosphorylation in a dose-dependant manner by blocking both PI3 Kinase and activating PTEN (13). Interestingly, myostatin, IGF-1 and insulin all share the PI3K/Akt pathway, which is important for signaling a number of downstream substrates that play a role in cell growth and metabolism. In fact it has been demonstrated that the major growth inhibiting actions of myostatin are mediated through the attenuation of IGF-1 induced Akt phosphorylation (13). Finally it has been shown that both insulin and IGF-1 inhibit the Mstn-induced degradation of p300, an important transcriptional coactivator regulating the expression of cyclin D1 (13). Cumulatively, this suggests a plausible mechanism linking increased circulating myostatin with inactivity to the development of insulin resistance.

In summary, we found that Mstn protein levels decreased in matching muscle and plasma samples from insulin resistant men after six months of aerobic exercise training. Pre-training plasma myostatin correlated better with insulin sensitivity than lean or fat mass, suggesting a cause-effect relationship that was subsequently confirmed in myostatin-injected mice. The profound reduction in insulin sensitivity with modest increase in plasma myostatin levels was also characterized at the organ level where the insulin-stimulated phosphorylation of AKT was shown to be significantly reduced in both muscle and liver of myostatin treated mice. These unique observations imply a role for circulating Mstn in the pathophysiology of the insulin resistance syndromes. Finally, since moderate exercise is sufficient to both reduce circulating Mstn levels and improve insulin sensitivity, we believe that Mstn should be evaluated further as therapeutic benefit of exercise and a target for defining the optimal mode, dose and intensity of exercise for health benefits.

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References

1. Allen DL, Cleary AS, Speaker KJ, et al. Myostatin, activin receptor IIb, and follistatin-like-3 gene expression are altered in adipose tissue and skeletal muscle of obese mice. *Am J Physiol Endocrinol Metab* 2008;294(5):E918–27. [PubMed: 18334608]
2. Bajpeyi S, Tanner CJ, Slentz CA, et al. Effect of exercise intensity and volume on persistence of insulin sensitivity during training cessation. *J Appl Physiol* 2009;106(4):1079–85. [PubMed: 19196913]
3. Callis TE, Pandya K, Seok HY, et al. MicroRNA-208a is a regulator of cardiac hypertrophy and conduction in mice. *J Clin Invest* 2009;119(9):2772–86. [PubMed: 19726871]
4. Gentile MA, Nantermet PV, Vogel RL, et al. Androgen mediated improvement of body composition and muscle function involves a novel early transcriptional program including IGF-1, MGF and induction of beta-catenin. *J Mol Endocrinol* 2010;44:55. [PubMed: 19726620]
5. Gonzalez-Cadavid NF, Bhasin S. Role of myostatin in metabolism. *Curr Opin Clin Nutr Metab Care* 2004;7(4):451–7. [PubMed: 15192449]
6. Gonzalez-Cadavid NF, Taylor WE, Yarasheski K, et al. Organization of the human myostatin gene and expression in healthy men and HIV-infected men with muscle wasting. *Proc Natl Acad Sci U S A* 1998;95(25):14938–43. [PubMed: 9843994]
7. Guo T, Jou W, Chanturiya T, Portas J, Gavrilova O, McPherron AC. Myostatin inhibition in muscle, but not adipose tissue, decreases fat mass and improves insulin sensitivity. *PLoS One* 2009;4(3):e4937. [PubMed: 19295913]
8. Hittel DS, Berggren JR, Shearer J, Boyle K, Houmard JA. Increased secretion and expression of myostatin in skeletal muscle from extremely obese women. *Diabetes* 2009;58(1):30–8. [PubMed: 18835929]
9. Houmard JA, Tanner CJ, Slentz CA, Duscha BD, McCartney JS, Kraus WE. Effect of the volume and intensity of exercise training on insulin sensitivity. *J Appl Physiol* 2004;96(1):101–6. [PubMed: 12972442]
10. Hulmi JJ, Kovanen V, Selanne H, Kraemer WJ, Hakkinen K, Mero AA. Acute and long-term effects of resistance exercise with or without protein ingestion on muscle hypertrophy and gene expression. *Amino Acids* 2009;37(2):297–308. [PubMed: 18661258]
11. Hulmi JJ, Tannerstedt J, Selanne H, Kainulainen H, Kovanen V, Mero AA. Resistance exercise with whey protein ingestion affects mTOR signaling pathway and myostatin in men. *J Appl Physiol* 2009;106(5):1720–9. [PubMed: 19299575]
12. Jespersen JG, Nedergaard A, Andersen LL, Schjerling P, Andersen JL. Myostatin expression during human muscle hypertrophy and subsequent atrophy: increased myostatin with detraining. *Scand J Med Sci Sports*. 2009 in press.
13. Ji M, Zhang Q, Ye J, Wang X, Yang W, Zhu D. Myostatin induces p300 degradation to silence cyclin D1 expression through the PI3K/PTEN/Akt pathway. *Cell Signal* 2008;20(8):1452–8. [PubMed: 18472397]
14. Kim JS, Cross JM, Bamman MM. Impact of resistance loading on myostatin expression and cell cycle regulation in young and older men and women. *Am J Physiol Endocrinol Metab* 2005;288(6):E1110–9. [PubMed: 15644458]
15. Kondapaka SB, Zarnowski M, Yver DR, Sausville EA, Cushman SW. 7-hydroxystaurosporine (UCN-01) inhibition of Akt Thr308 but not Ser473 phosphorylation: a basis for decreased insulin-stimulated glucose transport. *Clin Cancer Res* 2004;10(21):7192–8. [PubMed: 15534092]
16. Kopple JD, Cohen AH, Wang H, et al. Effect of exercise on mRNA levels for growth factors in skeletal muscle of hemodialysis patients. *J Ren Nutr* 2006;16(4):312–24. [PubMed: 17046615]
17. Kostek MA, Angelopoulos TJ, Clarkson PM, et al. Myostatin and follistatin polymorphisms interact with muscle phenotypes and ethnicity. *Med Sci Sports Exerc* 2009;41(5):1063–71. [PubMed: 19346981]

18. Lakshman KM, Bhasin S, Corcoran C, et al. Measurement of myostatin concentrations in human serum: Circulating concentrations in young and older men and effects of testosterone administration. *Mol Cell Endocrinol* 2009;302(1):26–32. [PubMed: 19356623]
19. LeBrasseur NK, Schelhorn TM, Bernardo BL, Cosgrove PG, Loria PM, Brown TA. Myostatin inhibition enhances the effects of exercise on performance and metabolic outcomes in aged mice. *J Gerontol A Biol Sci Med Sci* 2009;64(9):940–8. [PubMed: 19483181]
20. Louis E, Raue U, Yang Y, Jemiolo B, Trappe S. Time course of proteolytic, cytokine, and myostatin gene expression after acute exercise in human skeletal muscle. *J Appl Physiol* 2007;103(5):1744–51. [PubMed: 17823296]
21. Matsakas A, Bozzo C, Cacciani N, et al. Effect of swimming on myostatin expression in white and red gastrocnemius muscle and in cardiac muscle of rats. *Exp Physiol* 2006;91(6):983–94. [PubMed: 16873457]
22. Matsakas A, Friedel A, Hertrampf T, Diel P. Short-term endurance training results in a muscle-specific decrease of myostatin mRNA content in the rat. *Acta Physiol Scand* 2005;183(3):299–307. [PubMed: 15743390]
23. McFarlane C, Langley B, Thomas M, et al. Proteolytic processing of myostatin is auto-regulated during myogenesis. *Dev Biol* 2005;283(1):58–69. [PubMed: 15975431]
24. McPherron AC, Lawler AM, Lee SJ. Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. *Nature* 1997;387(6628):83–90. [PubMed: 9139826]
25. Milan G, Dalla Nora E, Pilon C, et al. Changes in muscle myostatin expression in obese subjects after weight loss. *J Clin Endocrinol Metab* 2004;89(6):2724–7. [PubMed: 15181048]
26. Mitchell MD, Osephook CC, Leung KC, McMahon CD, Bass JJ. Myostatin is a human placental product that regulates glucose uptake. *J Clin Endocrinol Metab* 2006;91(4):1434–7. [PubMed: 16464946]
27. Mukherjee A, Sidis Y, Mahan A, et al. FSTL3 deletion reveals roles for TGF-beta family ligands in glucose and fat homeostasis in adults. *Proc Natl Acad Sci U S A* 2007;104(4):1348–53. [PubMed: 17229845]
28. Park JJ, Berggren JR, Hulver MW, Houmard JA, Hoffman EP. GRB14, GPD1, and GDF8 as potential network collaborators in weight loss-induced improvements in insulin action in human skeletal muscle. *Physiol Genomics* 2006;27(2):114–21. [PubMed: 16849634]
29. Ricaud S, Vernus B, Duclos M, et al. Inhibition of autocrine secretion of myostatin enhances terminal differentiation in human rhabdomyosarcoma cells. *Oncogene* 2003;22(51):8221–32. [PubMed: 14614446]
30. Roth SM, Martel GF, Ferrell RE, Metter EJ, Hurley BF, Rogers MA. Myostatin gene expression is reduced in humans with heavy-resistance strength training: a brief communication. *Exp Biol Med* (Maywood) 2003;228(6):706–9. [PubMed: 12773702]
31. Shyu KG, Lu MJ, Wang BW, Sun HY, Chang H. Myostatin expression in ventricular myocardium in a rat model of volume-overload heart failure. *Eur J Clin Invest* 2006;36(10):713–9. [PubMed: 16968467]
32. Slentz CA, Aiken LB, Houmard JA, et al. Inactivity, exercise, and visceral fat. STRRIDE: a randomized, controlled study of exercise intensity and amount. *J Appl Physiol* 2005;99(4):1613–8. [PubMed: 16002776]
33. Slentz CA, Duscha BD, Johnson JL, et al. Effects of the amount of exercise on body weight, body composition, and measures of central obesity: STRRIDE--a randomized controlled study. *Arch Intern Med* 2004;164(1):31–9. [PubMed: 14718319]
34. Thomas M, Langley B, Berry C, et al. Myostatin, a negative regulator of muscle growth, functions by inhibiting myoblast proliferation. *J Biol Chem* 2000;275(51):40235–43. [PubMed: 10976104]
35. Walker KS, Kambadur R, Sharma M, Smith HK. Resistance training alters plasma myostatin but not IGF-1 in healthy men. *Med Sci Sports Exerc* 2004;36(5):787–93. [PubMed: 15126711]
36. Wilkes JJ, Lloyd DJ, Gekakis N. Loss-of-function mutation in myostatin reduces tumor necrosis factor alpha production and protects liver against obesity-induced insulin resistance. *Diabetes* 2009;58(5):1133–43. [PubMed: 19208906]

37. Willoughby DS. Effects of an alleged myostatin-binding supplement and heavy resistance training on serum myostatin, muscle strength and mass, and body composition. *Int J Sport Nutr Exerc Metab* 2004;14(4):461–72. [PubMed: 15467103]
38. Willoughby DS. Effects of heavy resistance training on myostatin mRNA and protein expression. *Med Sci Sports Exerc* 2004;36(4):574–82. [PubMed: 15064583]
39. Zhao B, Wall RJ, Yang J. Transgenic expression of myostatin propeptide prevents diet-induced obesity and insulin resistance. *Biochem Biophys Res Commun* 2005;337(1):248–55. [PubMed: 16182246]

Figure 1. Mature myostatin protein decreases with exercise training in skeletal muscle of insulin resistant male subjects

The mature myostatin 26 kDa peptide dimer was detected using Western blotting of muscle protein from each of 10 male subjects before and after 6 months of AE training. Expression levels were normalized to the abundance of alpha-actin $\pm$ SEM and a double asterisk (**) indicates significant differences ($p < 0.05$) using a paired t-test.

Figure 2. Plasma myostatin levels correlate with Si in insulin resistant male subjects before and after exercise training

Linear regression analyses were conducted using the least squares method to compare plasma myostatin levels to insulin sensitivity (Si) before and after 6 months of AE training. Significant associations ($p < 0.05$) were found between circulating myostatin and Si.

Figure 3. Myostatin-treatment induces significant insulin resistance in male C57BL6 mice
 Insulin (0.75 U/kg) was administered to mice by intraperitoneal injection and tail vein blood glucose measured from 15–90 minutes in myostatin-treated (25 ug/kg for 7 days; circles) compared to PBS-treated (diamonds) animals. Data represent percent of blood glucose level at time 0 $\pm$ SEM with n=10 mice per treatment group double asterisk (**) indicates significant differences (MYO vs PBS) using ANOVA.

Figure 4A

Figure 4B

Figure 4. Myostatin-treatment attenuates insulin-stimulated AKT-phosphorylation in muscle and liver of male C57BL6 mice

PBS and MSTN treated mice were either given an injection of saline or insulin. **A)** Western blots of two separate muscle types and liver revealed significant reductions in insulin-stimulated AKT (Thr³⁰⁸) phosphorylation suggesting a mechanism for myostatin-induced whole-body insulin resistance. **B)** Insulin-stimulated Akt phosphorylation was quantified and normalized to the abundance of total Akt $\pm$ SEM and a double asterisk (**) indicates significant differences ($p < 0.05$) using a t-test. Blots are representative of three independent experiments.

Table 1Baseline and Exercise-Trained Characteristics of Male Subjects (Mean $\pm$ SEM).

Characteristic	Baseline (n=10)	Exercise -Trained (n=10)
Age (years)	53.1 $\pm$ 5.5	+ 9 Months
Weight	95.3 $\pm$ 6.2	95.2 $\pm$ 6.7
Lean Mass (kg)	65.4 $\pm$ 8.4	64.9 $\pm$ 9.0
Fat Mass (%)	30.3 $\pm$ 6.3	29.4 $\pm$ 5.5
BMI (kg/m ²)	30.1 $\pm$ 1.1	30.2 $\pm$ 1.3
Fasting Insulin (uU/ml)	16.2 $\pm$ 4.2	13.8 $\pm$ 3.6 **
Plasma Myostatin (ng/ml)	28.7 $\pm$ 2.6	22.8 $\pm$ 2.7 **
Fasting Glucose (mg/dl)	96.4 $\pm$ 4.6	95.4 $\pm$ 3.2
VO ₂ Max (l/kg/min)	29.8 $\pm$ 3.1	32.0 $\pm$ 3.3 **
Si	2.0 $\pm$ 0.6	2.8 $\pm$ 0.6 **

Significant differences (**) are P< 0.05, pre vs post exercise training using a paired t-test.

Table 2

Characteristics of sham treated (PBS) and myostatin treated (MSTN) mice (Mean $\pm$ SEM).

Characteristic	PBS Treated (n=10)	MSTN Treated (n=10)
Mouse Weight (g)	22.1 $\pm$ 0.3	21.9 $\pm$ 0.3
Gastroc mass (mg)	138.6 $\pm$ 6.4	133.1 $\pm$ 9.2
Soleus mass (mg)	7.1 $\pm$ 0.7	7.5 $\pm$ 1.0
Glucose (mM)	7.7 $\pm$ 0.2	8.1 $\pm$ 0.2
Insulin (pg/ml)	399.9 $\pm$ 56.4	475.0 $\pm$ 69.5
Mstn (ng/ml)	15.3 $\pm$ 1.7	22.1 $\pm$ 2.4 ** (P=0.06) **
Leptin (ng/ml)	3.8 $\pm$ 1.1	3.3 $\pm$ 0.5
Resistin (ng/ml)	0.9 $\pm$ 0.1	1.3 $\pm$ 0.2
Adiponectin (ng/ml)	8.9 $\pm$ 0.8	10.6 $\pm$ 0.7
TNF α (pg/ml)	29.7 $\pm$ 2.3	28.6 $\pm$ 2.1

No significant differences were observed between treatment groups.