

# The Myostatin Gene

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## Summary

**Myostatin is an important negative regulator of muscle growth. Natural mutations and knockouts in animals produce a “double-muscled” phenotype the basis of which is a dramatic increase in muscle mass. Therapeutics that target myostatin are currently in development. There are almost no documented side effects of suppression, and early research suggests that myostatin inhibition can restore function in cases of muscular dystrophy. Clinical trials are currently underway, although myostatin research has not taken advantage of some techniques like RNA interference. With the development of myostatin therapies and advances in gene therapy, the social and ethical implications of using these as performance enhancement strategies raise interesting questions about athleticism in general.**

## History of Discovery

For some time now, scientists have been characterizing the process by which muscle cells are formed by differentiation of myoblasts and subsequent fusion into long fibers with contractile properties. It is clear that muscle growth in humans and other animals is not unlimited; thus, there must be a biochemical signal for myoblasts to stop differentiation and proliferation. McPherron, Lawler, and Lee (1997) first identified the protein they called growth/differentiation factor-8 (GDF-8) using PCR with primers designed from conserved regions of the transforming growth factor-B (TGF-B) superfamily. The same group discovered that knockout of the myostatin gene in mice results in a doubling to tripling in the size of the mouse muscles.

Soon after, Grobet et al. (1997) identified an eleven base pair deletion in the myostatin gene in the Belgian Blue cattle breed (BBCB) expressing the well-known double-muscled phenotype for that species. The double-muscled BBCB was the first observed phenotype resulting from a natural myostatin mutation, and it had been known and intentionally bred into cattle populations for some time before the molecular basis and inheritance patterns were truly understood. More recently, the first documented case of myostatin in humans was published by Schuelke et al. (2004). This case describes a 4-year-old German boy who was extraordinarily muscular from birth. In the 11 years since its discovery, myostatin has become an intense area of focus because of its potential for alleviating muscle wasting, which is a common side effect of many diseases and treatments.

## Characterization of the Myostatin Gene and Expression

Gonzalez-Cadavid et al. (1998) first characterized the human myostatin gene by scanning the human EST data bank for orthologs to the well-known mouse myostatin gene. They found that the coding region is approximately 6.2kbp

long with a small intron (1.8kbp) and a larger intron (2.4kbp) separating three exons. This sequence resides on the 2q33.2 chromosomal region and is transcribed into a 3.1kbp mRNA, which is then translated into a 375 amino acid myostatin protein precursor of size 26-kDa. The precursor then undergoes further processing to form the mature myostatin protein of size 12-kDa. This mature form of the protein dimerizes to form the active myostatin ligand. Human serum and skeletal muscle were demonstrated to contain only mature myostatin by Western blotting, but other human organs that are composed of smooth muscle tissue such as the colon, intestine, stomach, bladder, and prostate did not show any myostatin precursor or mature protein. It was demonstrated by Sharma et al. (1999) that myostatin is expressed in both fetal and adult hearts and may play a role in the pathology of heart muscle. In mice models myostatin is also expressed in skeletal muscle tissue and is measurable in serum; to a lesser extent in heart muscle in both developing and adult animals (McPherron et al., 1997). As will become obvious, the role of myostatin in tissues outside of skeletal muscle is poorly understood, but this is changing with the development of drugs that target myostatin.

## Biochemical Pathways of Myostatin Action

Because myostatin clearly impacts the formation of muscle, it is necessary to have a basic picture of the process of muscle formation. Muscle fiber formation begins with multipotent cells descendent from the mesoderm. In the presence of the transcription factors MyoD or Myf5, which bind to DNA sites that activate muscle-specific genes, these cells commit to becoming mature muscle cells called myoblasts. Myoblasts proliferate until they leave the cell cycle. Then, multiple myoblasts line up together and fuse to form multinucleated myotubes. These myotubes express proteins that allow them to become the contractile units we know as skeletal muscle tissue (Gilbert, 2000).

With regard to the proliferation of myoblasts, Joulia et al. (2003) demonstrated, using flow cytometry that myostatin overexpression leads to an accumulation of myoblasts in the G0/G1 and G2 phases and thus are not available for differentiation into myotubes. They also inhibited myostatin by expressing a myostatin mRNA antisense gene and found that cells were influenced in the opposite way: to withdraw from the cell cycle and begin differentiation. Patterns of expression of several other genes were measured, and it was determined that myostatin exerts its effects by moderating myogenin and increasing the expression of p21 and p53, both of which are involved in cell-cycle control.



**Figure 1.** A normal whippet on the left and a whippet exhibiting the double-muscled phenotype on the right. ([http://www.dailymail.co.uk/pages/live/articles/news/news.html?in\\_article\\_id=467985&in\\_page\\_id=1770](http://www.dailymail.co.uk/pages/live/articles/news/news.html?in_article_id=467985&in_page_id=1770)).

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A step past proliferation of myoblasts on the way to muscle fibers is their differentiation, which appears to be affected by myostatin as well. Joulia et al. (2003) also found that the production of myotubes, which are the multinucleated fused form of maturing myoblasts, was inhibited in myostatin-overexpressing cells. Similarly, Langley et al. (2002) showed that adding myostatin to growth medium with bovine myoblasts inhibited the formation of myotubes, and that this inhibition increased as the dose of myostatin increased. Northern blots of the culture medium detected decreased expression of MyoD and myogenin, important determining factors in myogenesis. Rios, Carneiro, Arce, and Devesa (2002) came to the same conclusion by examining the overexpression of endogenous myostatin.

Myostatin seems to have some other peripheral effects. It negatively regulates apoptosis in muscle cells (Rios et al. 2001), while expression of myostatin antisense RNA has the opposite effect. This is an important result because it shows that while myostatin overexpression does not lead to excess muscle production, it does not achieve this by causing the death of muscle cells. This data, along with those showing that myostatin inhibits myoblast proliferation and differentiation, demonstrates how at the biochemical level myostatin mutations can lead to the observed double muscled phenotypes.

### Physiological Influences of Myostatin

Myostatin is well characterized in terms of its physiological effects on muscle mass through both hyperplasia and hypertrophy. Muscle hyperplasia refers to an increase in the number of muscle fibers present while muscle hypertrophy refers to an increase in the volume of muscle fibers already present. Hyperplasia is usually regulated at the level of myoblast differentiation and proliferation while hypertrophy is regulated in mature skeletal muscle fibers.

The original study by McPherron et al. (1997) produced a lot of information regarding gross anatomical changes in homozygous myostatin knockout mice. These mice were almost 30% larger than controls and the mass was distributed throughout the body in increased skeletal muscle. The amount of weight increase in individual muscles correlated well with levels of myostatin expression in those muscles. Histological analysis revealed that the increase in muscle mass was due to both hyperplasia and hypertrophy, with the number of fibers increased by 86% and fiber size increased by 49%. Every study done since this original has documented similar increases in muscle size, such as that done by Mosher et al. (2007) examining whippets heterozygous for a myostatin mutation. Amthor, Otto, Macharia, McKinnell, and Patel (2006) noted increased muscle mass as well as some histological changes in muscle fiber composition in a myostatin deficient mouse line. Zebrafish also have a myostatin gene that results in increased muscle mass when knocked out (Xu, Wu, Zohar, and Du, 2003). Although the original studies in mice suggested an increase in muscle mass through both hyperplasia and hypertrophy, Yang et al. (2001) noted only an increase in hypertrophy with no significant hyperplasia. McCroskery, Thomas, Maxwell, Sharma, and Kambadur (2003) showed that myostatin decreased muscle stem cell activation, consistent with the hypothesis that myostatin deficiency leads to hyperplasia. A novel drug-inducible knockout was used to study the effects of post-developmental myostatin knockout by Welle, Bhatt, Pinkert, Tawil, and Thornton (2006). They engineered transgenic mice with a drug-inducible gene that expresses Cre recombinase and a form of the myostatin gene with loxP sites flanking one of the exons. After the mice reached

adulthood, they were given the drug, which knocked out the myostatin gene. The result was mice with 25% greater muscle mass after 3 months of near-complete myostatin suppression. The researchers measured fiber size and claimed that the increase in fiber size (hypertrophy alone) was enough to account for the gains in overall mass. This is somewhat unsatisfying; a complete analysis would have included a fiber count to rule out hyperplasia. Nevertheless, at this point it seems that myostatin knockout during development leads to hyperplasia and hypertrophy, while post-developmental knockout leads primarily to hypertrophy.

Interestingly, myostatin also has a significant effect on adipose tissue. Artaza et al. (2005) used a marker of adipogenesis with pluripotent cells and either endogenous myostatin or myostatin antibodies to demonstrate that myostatin promotes adipogenesis and myostatin antibodies suppress adipogenesis. Lin et al. (2002) showed that after 12 weeks myostatin deficiency led to decreased fat pad mass, and this age-dependent fat mass decrease has been echoed in other studies such as those done by McPherron and Lee (2002). Feldman, Streeter, Farese, and Yamamoto (2006) demonstrated that myostatin can determine the fate of multipotent stem cells, but not preadipocytes, and that this effect leads to adipocytes with increased insulin sensitivity. Molecularly then, myostatin has favorable effects on the development of both skeletal muscle and adipose tissue.

### Myostatin-based Therapeutics

Myostatin seems to be the ideal candidate for a therapeutic target in diseases that involve muscle wasting. When Gonzalez-Cadavid et al. (1998) mapped the myostatin gene they also correlated serum myostatin concentrations with muscle wasting in HIV-infected males. The more muscle wasting was observed, the higher the myostatin concentration. The side effects of myostatin mutations, if not nonexistent, are undocumented in many animal studies, and the known natural mutations in humans thus far have not demonstrated any pathological risks. A common concern is that myostatin may influence heart muscle, but Artaza et al. (2007) found that even though myostatin expression is related to left ventricular mass in mice, heart performance is not impacted as measured by the ejection fraction.

Some studies have suggested that myostatin treatments may be effective in ameliorating diseases. It has been found that patients with muscular atrophy have increased levels of myostatin (Wojcik, Nogalska, Engel, & Askanas, 2008). Independently, Gilson et al. (2007) showed that myostatin completely alleviated atrophy associated with glucocorticoid use. Bogdanovich et al. (2002) observed both increases in muscular size and functional improvements in the mdx mouse model of Duchenne Muscular Dystrophy (DMD) using a myostatin antibody. The same group later found that using the myostatin propeptide fused to an antibody was a more effective strategy for increasing functional markers of the disease progression in the mdx mouse (Bogdanovich, Perkins, Krag, Whittemore, & Khurana, 2005). In another situation, Zhao, Wall, and Yang (2005) also used propeptide-mediated inhibition of myostatin to prevent a high-fat diet from inducing insulin resistance and obesity in mice, suggesting that myostatin-based treatments may be effective in treating diabetes and other metabolic disorders characterized by insulin resistance.

Myostatin inhibition does not seem to affect physiological parameters besides muscle mass in mice (Whittemore et al. 2003). Furthermore, there are many diseases in which muscle wasting or lack of muscular development is either the cause or side effect of the disease, so the potential to help patients seems enormous. With this in mind, recent myostatin research has focused on

developing effective inhibition strategies. The most common methods for knocking out myostatin have been either genetically engineered animals with myostatin mutations, animals expressing a myostatin antisense RNA, or anti-myostatin antibodies. In fact, a phase I/II clinical trial with a myostatin-targeting antibody administered to muscular dystrophy patients was recently completed and although not yet published, preliminary reports verify the safety of the drug (Wagner et al. 2008).

In my opinion, exploiting RNA interference (RNAi) should be the future goal of myostatin therapeutics, although this approach has only been used in a few myostatin-related studies to date. Acosta, Carpio, Borroto, Gonzalez, and Estrada (2005) used double stranded RNA (dsRNA) to suppress myostatin in zebrafish, leading to the double muscled phenotype for that species. Magee et al (2006) published the most impressive report of small interfering RNA (siRNA) being able to suppress myostatin mRNA by 27% leading to a 10% increase in skeletal muscle mass. This is modest compared to other forms of knockout, thus more research needs to be done. Because the RNAi system offers the possibility of tightly controlled and efficient silencing as demonstrated in other studies, this system should be explored as a method of myostatin silencing (Novina & Sharp, 2004).

### Social and Ethical Considerations with Myostatin-blocking Treatments

Clearly treatments that target myostatin have the potential to alleviate the suffering of many patients with a plurality of diseases. This type of therapy, however, may also attract the interest of people on the other end of the health spectrum: athletes. A recent study by Amthor et al. (2007) shows that myostatin deficient mice display impaired force production. This study stands in contrast to the findings by Mosher et al. (2007) mentioned earlier, that states that heterozygote whippets not only show increased muscle mass but also enhanced racing performance. Clearly racing performance is dependent on the ability to produce force rapidly. The difference may be that the first study examined animals homozygous for myostatin mutations and the second examined heterozygotes, or it may be that there is an interspecies difference. The fact that lack of myostatin to some degree can enhance performance might make myostatin-based therapeutics an attractive option for elite athletes looking to gain an edge in competition. Traditionally, athletes have turned to other performance enhancing drugs like steroids and growth hormone for strength sports, or EPO for endurance sports. Myostatin treatment, because it may prove to have significantly less side effects than other performance enhancers, will certainly be considered by some a viable and safer method. Even if there are side effects, athletes have proven their willingness to gamble with their health for the sake of greater performance.

This leads directly into a discussion of what we as a society consider athleticism. We watch sporting events and are thrilled to see world records continuously being broken, but we shun professional athletes who achieve these heights by what we consider cheating. Surely taking myostatin inhibitors would be cheating just as much as taking steroids is. But what about boys such as the one described above that have a natural myostatin mutation? Surely he should not be barred from Olympic competitions, but then why should we persecute people who simply try to level the playing field with genetic wonders like this by taking performance enhancers? These are issues that are important to society and also to me. Having been a competitive athlete for much of my life, I find discussions like

this compelling. With the first myostatin inhibitors having already completed phase II clinical trials, the day when we have to deal with this issue is upon us. It is no surprise that here, like in other cases, biomedical technology has advanced far beyond what biomedical ethics has considered.

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### References

- Acosta, J., Carpio, Y., Borroto, I., Gonzalez, O., & Estrada, M. P. (2005). Myostatin gene silenced by RNAi show a zebrafish giant phenotype. *Journal of Biotechnology*, 119(4), 324-331.
- Amthor, H., Macharia, R., Navarrete, R., Schuelke, M., Brown, S. C., Otto, A., et al. (2007). Lack of myostatin results in excessive muscle growth but impaired force generation. *Proceedings of the National Academy of Sciences of the United States of America*, 104(6), 1835-1840.
- Amthor, H., Otto, A., Macharia, R., McKinnell, I., & Patel, K. (2006). Myostatin imposes reversible quiescence on embryonic muscle precursors. *Developmental Dynamics : An Official Publication of the American Association of Anatomists*, 235(3), 672-680.
- Artaza, J. N., Bhasin, S., Magee, T. R., Reisz-Porszasz, S., Shen, R., Groome, N. P., et al. (2005). Myostatin inhibits myogenesis and promotes adipogenesis in C3H 10T(1/2) mesenchymal multipotent cells. *Endocrinology*, 146(8), 3547-3557.
- Artaza, J. N., Reisz-Porszasz, S., Dow, J. S., Kloner, R. A., Tsao, J., Bhasin, S., et al. (2007). Alterations in myostatin expression are associated with changes in cardiac left ventricular mass but not ejection fraction in the mouse. *The Journal of Endocrinology*, 194(1), 63-76.
- Bogdanovich, S., Krag, T. O., Barton, E. R., Morris, L. D., Whittemore, L. A., Ahima, R. S., et al. (2002). Functional improvement of dystrophic muscle by myostatin blockade. *Nature*, 420(6914), 418-421.
- Bogdanovich, S., Perkins, K. J., Krag, T. O., Whittemore, L. A., & Khurana, T. S. (2005). Myostatin propeptide-mediated amelioration of dystrophic pathophysiology. *The FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, 19(6), 543-549.
- Feldman, B. J., Streeper, R. S., Farese, R. V., Jr, & Yamamoto, K. R. (2006). Myostatin modulates adipogenesis to generate adipocytes with favorable metabolic effects. *Proceedings of the National Academy of Sciences of the United States of America*, 103(42), 15675-15680.
- Gilbert, S. F. (2000). Myogenesis: The Development of Muscle. In *Developmental biology* (3.14). Sunderland, MA: Sinauer Associates. Retrieved March 10, 2007, from <http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=dbio.section.3475>
- Gilson, H., Schakman, O., Combaret, L., Lause, P., Grobet, L., Attaix, D., et al. (2007). Myostatin gene deletion prevents glucocorticoid-induced muscle atrophy. *Endocrinology*, 148(1), 452-460.
- Gonzalez-Cadavid, N. F., Taylor, W. E., Yarasheski, K., Sinha-Hikim, I., Ma, K., Ezzat, S., et al. (1998). Organization of the human myostatin gene and expression in healthy men and HIV-infected men with muscle wasting. *Proceedings of the National Academy of Sciences of the United States of America*, 95(25), 14938-14943.
- Grobet, L., Martin, L. J., Poncelet, D., Pirotin, D., Brouwers, B., Riquet, J., et al. (1997). A deletion in the bovine myostatin gene

- causes the double-muscled phenotype in cattle. *Nature Genetics*, 17(1), 71-74.
- Joulia, D., Bernardi, H., Garandel, V., Rabenoelina, F., Vernus, B., & Cabello, G. (2003). Mechanisms involved in the inhibition of myoblast proliferation and differentiation by myostatin. *Experimental Cell Research*, 286(2), 263-275.
- Langley, B., Thomas, M., Bishop, A., Sharma, M., Gilmour, S., & Kambadur, R. (2002). Myostatin inhibits myoblast differentiation by down-regulating MyoD expression. *The Journal of Biological Chemistry*, 277(51), 49831-49840.
- Lin, J., Arnold, H. B., Della-Fera, M. A., Azain, M. J., Hartzell, D. L., & Baile, C. A. (2002). Myostatin knockout in mice increases myogenesis and decreases adipogenesis. *Biochemical and Biophysical Research Communications*, 291(3), 701-706.
- Magee, T. R., Artaza, J. N., Ferrini, M. G., Vernet, D., Zuniga, F. I., Cantini, L., et al. (2006). Myostatin short interfering hairpin RNA gene transfer increases skeletal muscle mass. *The Journal of Gene Medicine*, 8(9), 1171-1181.
- McCroskery, S., Thomas, M., Maxwell, L., Sharma, M., & Kambadur, R. (2003). Myostatin negatively regulates satellite cell activation and self-renewal. *The Journal of Cell Biology*, 162(6), 1135-1147.
- McPherron, A. C., Lawler, A. M., & Lee, S. J. (1997). Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. *Nature*, 387(6628), 83-90.
- McPherron, A. C., & Lee, S. J. (2002). Suppression of body fat accumulation in myostatin-deficient mice. *The Journal of Clinical Investigation*, 109(5), 595-601.
- Mosher, D. S., Quignon, P., Bustamante, C. D., Sutter, N. B., Mellersh, C. S., Parker, H. G., et al. (2007). A mutation in the myostatin gene increases muscle mass and enhances racing performance in heterozygote dogs. *PLoS Genetics*, 3(5), e79.
- Novina, C. D., & Sharp, P. A. (2004). The RNAi revolution. *Nature*, 430(6996), 161-164.
- Rios, R., Carneiro, I., Arce, V. M., & Devesa, J. (2001). Myostatin regulates cell survival during C2C12 myogenesis. *Biochemical and Biophysical Research Communications*, 280(2), 561-566.
- Rios, R., Carneiro, I., Arce, V. M., & Devesa, J. (2002). Myostatin is an inhibitor of myogenic differentiation. *American Journal of Physiology. Cell Physiology*, 282(5), C993-9.
- Schuelke, M., Wagner, K. R., Stolz, L. E., Hubner, C., Riebel, T., Komen, W., et al. (2004). Myostatin mutation associated with gross muscle hypertrophy in a child. *The New England Journal of Medicine*, 350(26), 2682-2688.
- Sharma, M., Kambadur, R., Matthews, K. G., Somers, W. G., Devlin, G. P., Conaglen, J. V., et al. (1999). Myostatin, a transforming growth factor-beta superfamily member, is expressed in heart muscle and is upregulated in cardiomyocytes after infarct. *Journal of Cellular Physiology*, 180(1), 1-9.
- Wagner, K. R., Fleckenstein, J. L., Amato, A. A., Barohn, R. J., Bushby, K., Escolar, D. M., et al. (2008). A phase I/II trial of MYO-029 in adult subjects with muscular dystrophy. *Annals of Neurology*, in process.
- Welle, S., Bhatt, K., Pinkert, C. A., Tawil, R., & Thornton, C. A. (2007). Muscle growth after postdevelopmental myostatin gene knockout. *American Journal of Physiology. Endocrinology and Metabolism*, 292(4), E985-91.
- Whittemore, L. A., Song, K., Li, X., Aghajanian, J., Davies, M., Gigenrath, S., et al. (2003). Inhibition of myostatin in adult mice increases skeletal muscle mass and strength. *Biochemical and Biophysical Research Communications*, 300(4), 965-971.
- Wojcik, S., Nogalska, A., Engel, W. K., & Askanas, V. (2008). Myostatin and its precursor protein are increased in the skeletal muscle of patients with type-II muscle fibre atrophy. *Folia Morphologica*, 67(1), 8-14.
- Xu, C., Wu, G., Zohar, Y., & Du, S. J. (2003). Analysis of myostatin gene structure, expression and function in zebrafish. *The Journal of Experimental Biology*, 206(Pt 22), 4067-4079.
- Yang, J., Ratovitski, T., Brady, J. P., Solomon, M. B., Wells, K. D., & Wall, R. J. (2001). Expression of myostatin pro domain results in muscular transgenic mice. *Molecular Reproduction and Development*, 60(3), 351-361.
- Zhao, B., Wall, R. J., & Yang, J. (2005). Transgenic expression of myostatin propeptide prevents diet-induced obesity and insulin resistance. *Biochemical and Biophysical Research Communications*, 337(1), 248-255.