List of abbreviations

AAV	Adeno-Associated Virus
Act	Activin
ActRIIA/B	Activin Receptor type II A/B
ALK4/5	Activin-Like Kinase-4/5
BMP	Bone Morphogenetic Factor
Cdk2	Cyclin-dependent kinase-2
cDNA	complementary DNA
DNA	Deoxyribonucleic Acid
ca	constitutively active
CHO cells	Chinese Hamster Ovary cells
CMV	Cytomegalovirus
CSA	Cross Sectional Area
Ctrl	Control
DAB	Diaminobenzidine
dn	dominant negative
4E-BP1	4E-Binding Protein-1
ERK	Extracellular signal-Regulated Kinase
FS	Follistatin
FLRG	Follistatin-Like Related Gene
FLRP	Follistatin-Like Related Protein
FSH	Follicle-Stimulating Hormone
FSTL3	Follistatin-Like-3
FOXO	Forhead box O
GASP-1	Growth and differentiation factor-Associated Serum Protein-1
GDF-8/11	Growth and Differentiation Factor-8/11
GH	Growth Hormone
GSK-3β	Glycogen Synthase Kinase-3β
HDAC	Histone Deacetylase
HS	Heparin Sulfate
IGF-I	Insulin like Growth Factor-I
IGF-II	Insulin like Growth Factor-II

IGFBP-3/5	IGF binding protein-3/5
JNK	c-Jun N-terminal Kinase
kDa	kiloDalton
KO	Knock Out
LAP	Latency Associated Peptide
MAPK	Mitogen-Activated Protein Kinase
MEF2	Myocyte Enhancer Factor-2
MHC	Myosin Heavy Chain
MRF	Myogenic Regulatory Factor
Mstn	Myostatin
mRNA	messenger Ribonucleic Acid
MuRF1	Muscle Ring Finger-1
NF-κB	Nuclear Factor-κB
NO	Nitric Oxide
ns	non significant
Pax-3/7	Paired box gene-3/7
p70S6K	protein 70 kDa ribosomal S6 Kinase
PI3kinase	Phosphoinositide kinase-3
rpS6	ribosomal protein S6
SC	Satellite Cell
TA	Tibialis Anterior
TGF-β	Transforming Growth Factor beta
TSA	Trichostatin A
WT	Wild-Type

Chapter 1: Introduction

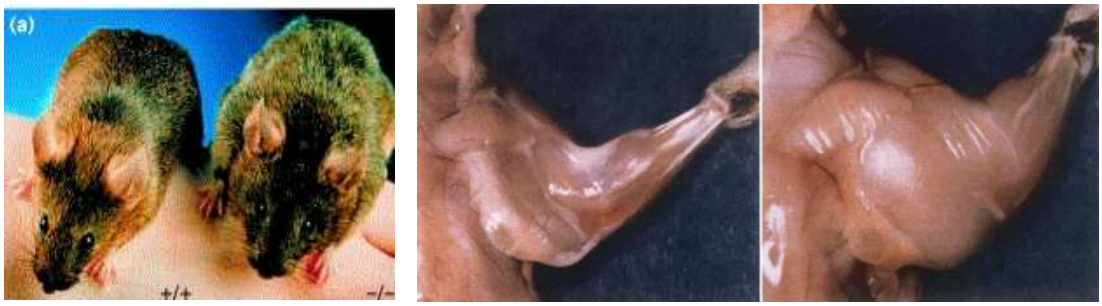
1. Regulation of muscle mass by myostatin

1.1. Generalities

1.1.1. Discovery of myostatin

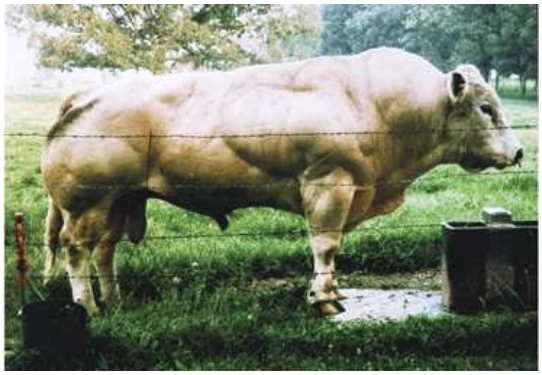
In an attempt to identify new members of the Transforming growth factor-beta (TGF-β) superfamily, McPherron *et al.* identified a new factor, Growth and differentiation factor 8 (GDF-8), which is specifically expressed in developing and adult skeletal muscle. The function of GDF-8 in skeletal muscle was established through gene targeting experiments in mice. The observation of a "double muscling" phenotype in GDF-8-null mice highlighted a negative role of this new TGF-β member in the regulation of muscle growth (1;1) (Fig 1a). Based on the phenotype of GDF-8-null mice and the tissue specificity of GDF-8 expression, GDF-8 was then referred as myostatin (Mstn). Then, in concert with this observation, the dramatic increase of muscle size in the Belgian Blue cattle was demonstrated to be due to a natural inactivating mutation of the Mstn gene (2;3) (Fig 1b). Later, the role of Mstn on muscle mass was confirmed by a transgenic mouse model in which Mstn overexpression was associated with lower muscle mass (4).

Fig 1a: Wild-type and Mstn-null mice, and their upper forelimb muscles



***McPherron AC** et al, Nature 387: 83–90, 1997*

Fig 1b: Belgian Blue cattle

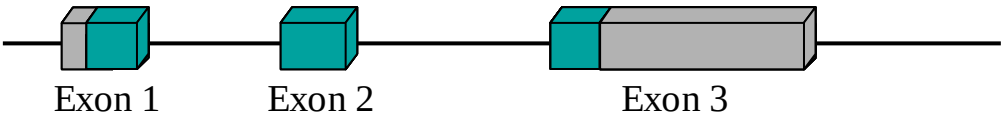


***McPherron AC** et al PNAS 94: 12457–12461, 1997*

1.1.2. Myostatin gene and protein structure

Mstn gene has been mapped in several vertebrate species, and is located on chromosome 2 in humans, cattle and sheep, 1 in mice and 9 in rats (5;6). In all vertebrates, the genomic organisation includes three exons separated by two introns (Fig 2). The mature Mstn peptide is encoded by the third exon (7).

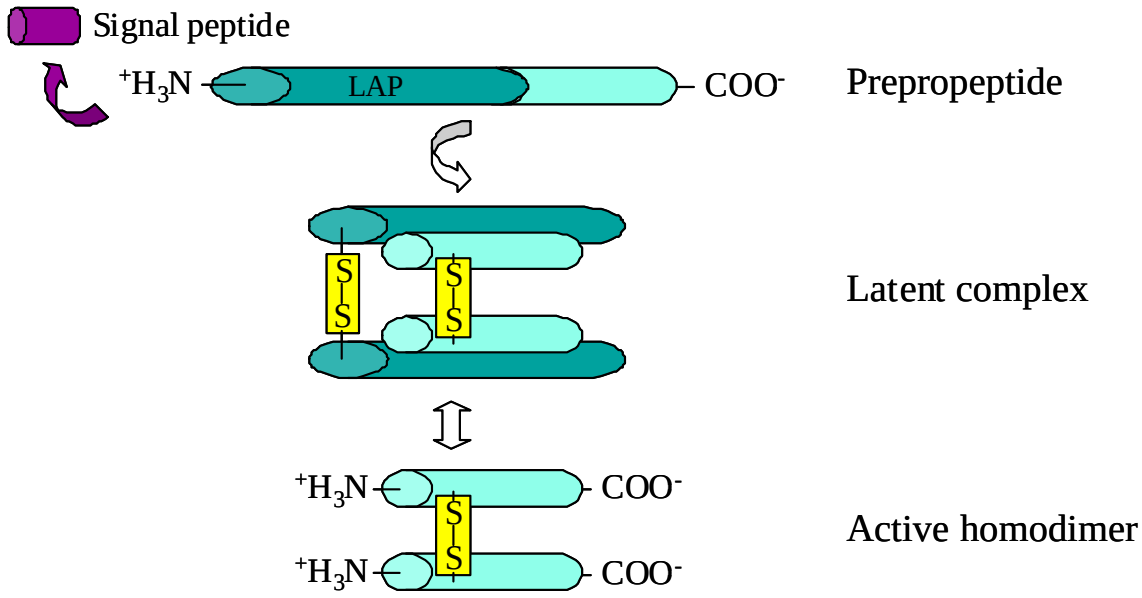
Fig 2: The Mstn gene (coding sequences)



The Mstn sequence has been highly conserved through evolution. Remarkably, the sequence of mature Mstn peptide is identical in human, rat, mouse, pig, turkey and chicken (3).

The Mstn protein is synthesised as a prepropeptide precursor containing 375 amino acids (52kDa). After release of the N-terminal signal peptide (24 amino acids), the precursor undergoes catalytic cleavage by a furin-like endoproteinase resulting in a 40kDa N-terminal propeptide (LAP, latency associated peptide) and a 12.5kDa C-terminal peptide (109 amino acids). A disulfide bond between two C-terminal peptides leads to the formation of the active homodimeric signalling molecule, the mature Mstn peptide (26kDa, 218 amino acids) (8). Once secreted from the cell, Mstn is held in an inactive conformation via noncovalent interactions with two propeptides (LAP) in a tetrameric complex that is disassembled following proteolytic cleavage by a BMP1/Tolloid family enzyme (9-11).

Fig 3: The Mstn protein synthesis



1.1.3. Tissue expression

Mstn is predominantly expressed in skeletal muscle throughout life, from the early stages of embryogenesis to late adulthood. At early stages of development, Mstn expression is restricted to developing somites of the myotome compartment (1). At later stages of development and in adult animals, Mstn is expressed almost exclusively in skeletal muscle (1). Remarkably, there is a positive correlation between fast type II fiber content and Mstn mRNA abundance in several skeletal muscles (12), which suggest that fast-twitch muscles could be more sensitive than slow-twitch muscles to changes in the Mstn expression.

In muscle cell culture, myogenesis is associated with an induction of Mstn mRNA. Indeed, Mstn expression seems undetectable during proliferation, peaks at the onset of fusion and decreases during terminal differentiation (13).

Finally, besides skeletal muscle, adipose tissue (12), heart tissue (cardiomyocytes and Purkinje fibers) (14) and lactating mammary gland (15) also express Mstn.

1.1.4. Myostatin binding proteins

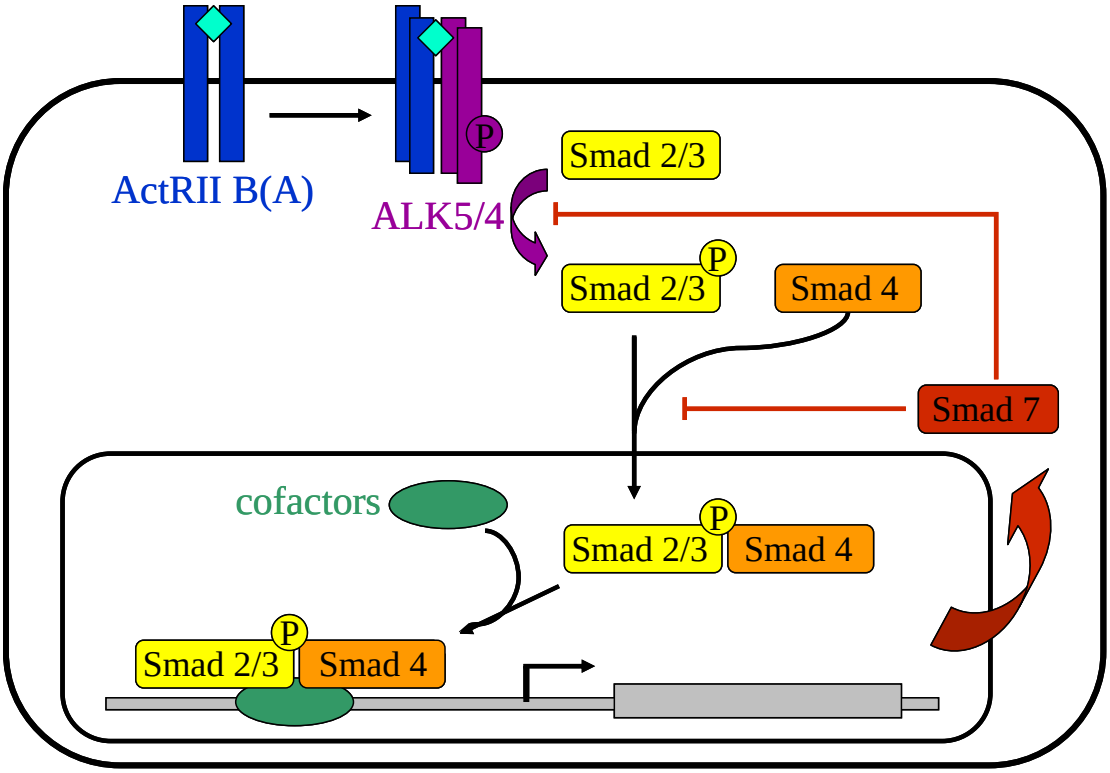
The availability of bioactive Mstn is tightly regulated through protein sequestration. In serum, Mstn circulates as a latent complex containing Mstn and other proteins including the N-terminal propeptide (LAP) that results from the proteolytic processing of Mstn precursor (8). Two molecules of propeptide monomer bind and inhibit the biological activity of one molecule of Mstn homodimer. The inhibitory domain of the propeptide is located in the region between amino acids 42 and 115 (16). The generation of mice carrying a mutation of the Mstn propeptide has showed that the proteolytic cleavage of the propeptide by the BMP-1/Tolloid proteases plays a critical role in the activation of latent Mstn *in vivo* (11). Other proteins can also bind and inhibit Mstn activity including Follistatin (FS) (8), Follistatin-Like Related Gene (FLRG; also known as Follistatin Like-3, FSTL-3, and Follistatin-Like Related Peptide or FLRP) (17), and Growth and differentiation factor-Associated Serum Protein 1 (GASP-1) which binds directly not only Mstn, but also its propeptide (18). Recently a matrix-associated protein called decorin has been described to bind Mstn with relatively high affinity and to prevent Mstn receptor binding and activation (19;20).

1.1.5. Myostatin receptor and signalling pathway

Mstn signals into the cell via a receptor complex composed of two type II and two type I receptors (21). Accumulating evidence indicates that Mstn shares its pair of receptors with

activin specifically Activin Receptor(ActR)IIB and ActRIIA. The Mstn initiates signalling through ActRIIB(A) which possess a constitutively active kinase domain. Binding of Mstn to ActRIIs recruits the corresponding type I receptor (Activin-like kinase-5 and 4 or ALK5/4) (21), forming an activated heterotetrameric receptor complex, which transphosphorylates the type I receptor on a Gly/Ser motif called the GS region. The activated receptor complex then phosphorylates the receptor-regulated Smad2/3 proteins that oligomerize with a common mediator Smad or co-Smad termed Smad4. The Smad complex translocates into the nucleus where it interacts with cofactors to regulate target gene transcription (22;23). These Smad binding partners regulate, in a cell type-specific manner, subsets of genes that are target genes of Mstn signalling. Another Smad, Smad7, stimulated by Mstn serves to abrogate Mstn signalling by establishing an autoinhibitory feedback loop (23;24). First, Smad7 inhibits Mstn gene expression (24). Second, Smad7 inhibits Smad2/3 phosphorylation by the type I receptors (23). Third, Smad7 interferes with the formation of a Smad 2/3 - Smad4 complex resulting in the inhibition of Mstn signalling (23).

Fig 4: The Mstn signalling pathway



1.2. General biological effects of myostatin

1.2.1. Muscle anti-anabolic action

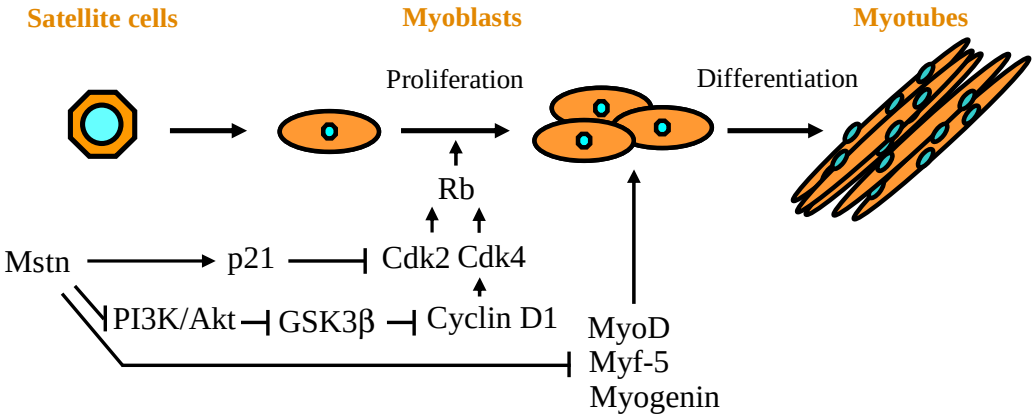
1.2.1.1. Inhibition of satellite cell activation, proliferation and self-renewal

Several observations indicate that Mstn inhibits muscle growth by different mechanisms. Mstn has been reported to maintain the quiescence of satellite cells. Satellite cells are mononuclear muscle stem cells that are intercalated between the basal lamina and the plasma membrane of myofibers (25). Although typically quiescent in normal adult muscle, they become activated and recruited to the cell cycle when there is a requirement to increase myonuclear number in growth, adaptation and regeneration. Satellite cells are the only source of proliferative muscle precursors and myonuclei, in normal growing muscle The descendants of activated satellite cells, called myoblasts, undergo multiple rounds of division prior to terminal differentiation and fusion to form multinucleated myofibers (25). Satellite cells isolated from skeletal muscle are characterized as having the gene expression profile: Pax-7+, CD34+, CD45-, Sca1- (25). The Mstn gene deletion is associated with an increased activation of the muscle satellite cells (26). Mstn is also an inhibitor of myogenic cell proliferation. Indeed, myoblasts derived from double-muscled cattle with absence of functional Mstn have a higher ability to proliferate than myoblasts derived from normal-muscled cattle (27). Mstn prevents the proliferation of satellite cells and myoblasts by inhibiting cell cycle progression from the G1- to S-phase. First, Mstn has been shown to up-regulate p21, decreasing therefore the levels and activity of Cdk2, resulting in the accumulation of hypophophorylated pRb which sequesters some transcription factors necessary for the S phase (26;28-30) (Fig 5). Secondly, Mstn repress the muscle cell proliferation by stimulating the cyclin D1 protein degradation through the ubiquitin-proteasome system (31). The Mstn-induced downregulation of cyclin D1 is mediated through inhibition of the PI3K/Akt/GSK-3β signaling cascade. Indeed, the effects of Mstn on cyclin D1 and cell proliferation can be reversed by treatment with IGF-1. Third, the upregulation of the IGF binding proteins (IGFBP) 3 and 5 could contribute also to the inhibitory effect of Mstn on muscle cell proliferation. Actually, Mstn is able to increase the expression of IGFBP-3 and -5, two IGF binding proteins which have been shown to suppress cell proliferation (32). Finally, Mstn inhibits the self-renewal of satellite cells (26), an effect mediated by decreased expression of Pax-7 (33).

1.2.1.2. Inhibition of cell differentiation

In addition to inhibiting proliferation, Mstn also impairs myogenic cell differentiation (30;34;35). Several mechanisms could explain the inhibitory effect of Mstn on myogenesis. First, Mstn downregulates the expression of myogenic regulatory factors (MRFs) including Pax-3 (36;37), Myf-5 (34;36), myogenin (29;30;34), and MyoD (27;29;34;36;37) which are directly responsible for muscle differentiation (Fig 5). Second, Mstn also inhibits the activity of MyoD (34), a crucial MRF for differentiation. Indeed, Smad3 activated by Mstn signalling is able to interact with MyoD and to prevent its translocation into the nucleus (34). Third, Mstn has been found to activate the $ERK^{1/2}$ cascade, a pathway known to suppress the myogenic cell differentiation, suggesting the contribution of ERK in the inhibitory effect of Mstn on myogenesis. Finally, Mstn could exert its negative effect on myogenesis through the activation of the c-Jun N-terminal kinase (JNK) signalling pathway. In fact, blocking the JNK activation prevents the Mstn's ability to inhibit differentiation (38).

Fig 5: A model for the role of Mstn during muscle growth and differentiation

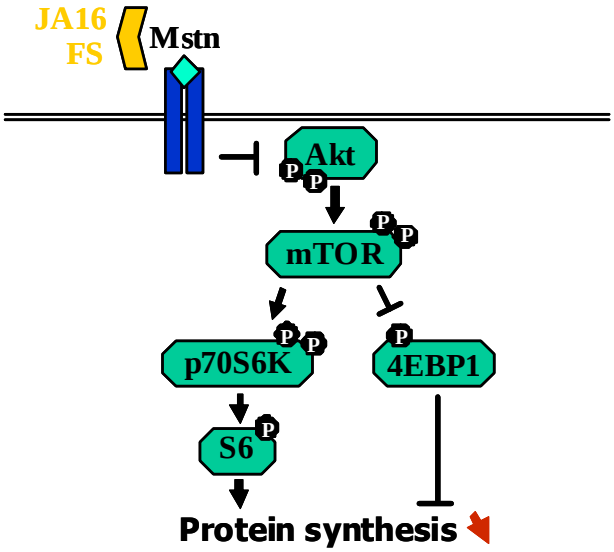


1.2.1.3. Inhibition of protein synthesis

A third mechanism by which Mstn inhibits muscle growth is the downregulation of the protein synthesis (39). Accordingly, a higher protein rate synthesis has been observed in muscles of Mstn KO mice compared to their control littermates (40). Furthermore, the postnatal inhibition of Mstn activity by anti-Mstn antibodies (JA16) or the binding protein FS stimulates the myofibrillar protein synthesis and the phosphorylation of p70 S6 kinase (S6K) and ribosomal protein S6 (rpS6), two factors involved in the regulation of the protein synthesis (41;42). In concert with these results, a recent study has provided further insights into the mechanisms by which Mstn negatively regulates protein synthesis. In this study, the muscle atrophy induced by Mstn overexpression was associated with a decreased

phosphorylation of Akt and two of its downstream targets: S6 and 4E-binding protein-1 (4E-BP1) (43). The data support therefore the conclusion that the down-regulation of Akt/mTOR/p70S6K signalling could mediate the negative effect of Mstn on protein synthesis.

Fig 6: A model for the role of Mstn in the regulation of protein synthesis

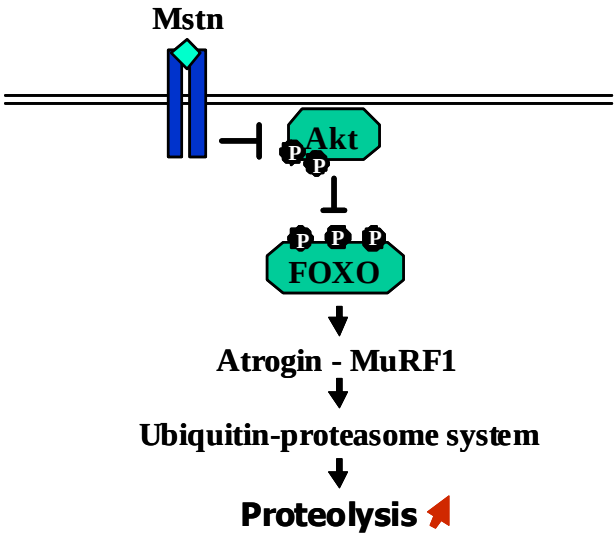


1.2.2. Muscle catabolic action

Muscle overexpression of Mstn either in transgenic animals or after gene electrotransfer results in skeletal muscle atrophy (4;44). However, the mechanism involved in the catabolic effect of Mstn is not well described. In addition to inhibit protein synthesis, Mstn might also stimulate muscle proteolysis. Indeed, treatment of C_2C_{12} myotubes with recombinant Mstn is associated with an upregulation of genes involved in the ubiquitin-proteasome pathway including Atrogin-1, MuRF-1 and $E2_{14kDa}$ (37). The regulation of these atrogens by Mstn could be mediated by an inhibition of the Akt pathway increasing the expression of atrophy-related genes through FOXO1 activation (37). However, the Mstn-induced muscle atrophy seems not to be systematically associated with an upregulation of atrogens. In fact, a study conducted on human myotubes shows that Mstn treatment at lower concentrations causes a decrease in myotube diameter together with a decline of Atrogin-1 and MuRF-1 mRNA levels (45). In the same way, Mstn overexpression in muscle by gene electrotransfer does not alter the ubiquitin-proteasome pathway despite muscle weight loss (43). Based on these inconsistent results, further works will be necessary to demonstrate an effect of Mstn on muscle proteolysis. Although it has been demonstrated that systemic administration of Mstn

induces cachexia in animals (37;46), the relevance of these observations for human physiology is questionable since the concentrations of circulating Mstn are much lower in humans than in rodents (47).

Fig 7: A model for the role of Mstn on the muscle proteolysis



1.2.3. Modification of the muscle fiber phenotype

Several observations suggest that Mstn could influence the muscle fiber phenotype. Indeed, the muscles of Mstn KO animals contain a larger proportion of fast type II fibers together with reduced proportion of slow type I fibers compared with wild-type animals (48-50). Thus, the absence of Mstn leads to an overall faster and more glycolytic muscle phenotype. It has been recently proposed that Mstn could regulate fiber-type composition by regulating the expression of myocyte enhancer factor 2 (MEF2) and MyoD during myogenesis (50). Indeed, the muscle of Mstn-null mice contains lower level of MEF2C, a transcription factor essential for the formation of slow type I fibers and increased level of MyoD, an factor important for the transcription of MHCIIb gene, characteristic of the fast type II fibers. However the effect of Mstn or its inhibition on the muscle phenotype is likely a consequence of developmental processes because Mstn inhibition in adult animals does not cause a transformation to a more fast and glycolytic phenotype. In fact, injections of Mstn antibody to adult mice have been shown to induce a muscle hypertrophy without any change of the fiber phenotype after 12 weeks of treatment (49).

1.2.4. Extra-muscular effects of myostatin

Cardiac muscle

Besides skeletal muscle, fetal and adult hearts also express Mstn. In heart, Mstn might function as a cardiac chalone. Indeed, Mstn expression is elevated in several models of cardiac hypertrophy, *in vivo* (Akt overexpression) (51) as well as *in vitro* (cyclic stretch) (52). In these models, Mstn is thought to provide a negative feedback mechanism to limit cardiac muscle growth.

Adipose tissue

Mstn is expressed at low level in adipose tissue. Its role in adipogenesis is suggested by the observation that the loss of Mstn function, in addition to increase muscle mass, also decreases body fat mass (1;53;54). However, whether Mstn regulates adipogenesis directly is unclear. *In vitro,* Mstn has been shown to influence the cellular physiology of three different adipocyte culture systems with conflicting results indicating that Mstn action is different during determination and differentiation steps. Indeed, while Mstn inhibits differentiation of preadipocytes (55-58), it promotes adipogenesis in a multipotent mesenchymal cell line (C3H10T1/2), suggesting that adipogenic determination may be stimulated by Mstn, but not differentiation. In concert with these data, while Mstn gene deletion results in a decreased body fat mass, the Mstn overexpression in adipose tissue results in small immature adipocytes without any change in body fat mass (59). Furthermore, it has been recently shown that decreased adiposity observed in Mstn-null mice is a consequence of alteration of skeletal muscle metabolism rather than a direct effect of the inhibition of Mstn signalling in adipose tissue. Indeed, Mstn inhibition by overexpression of a dnActRIIB in adipose tissue does not induce a change in the body composition, by contrast to the same deletion in the skeletal muscle (60). This result suggests that the decrease in adipose tissue in Mstn-null mice is most likely an indirect effect of the increased muscle mass.

Bone

It has been shown that Mstn KO mice are characterized by a significant increase in bone density compared to normal mice (61), and that the higher mineral content is mainly localized to regions where muscles attach (62). Although a direct role of Mstn on bone formation has not yet been clearly demonstrated, some *in vivo* studies suggest that Mstn could inhibit the

recruitment and proliferation of progenitor cells and then regulate cell differentiation in the early process of fracture healing (63;64).

1.3. Regulation of myostatin expression

1.3.1. Glucocorticoids

Dexamethasone, a glucocorticoid agonist, induces a dose-dependent marked induction of muscle Mstn mRNA and protein expression *in vitro* as *in vivo* (65-67). This effect results from an upregulation of Mstn gene transcription, possibly through a glucocorticoid receptor-mediated pathway. Indeed, the effect of dexamethasone on Mstn expression is prevented by coincubation with the glucocorticoid receptor antagonist RU-486 (68). *In vivo*, the upregulation of Mstn expression by dexamethasone in muscle seems to be transient. In fact, although the increase in Mstn protein content is pronounced after 5 days of treatment, it is not sustained when treatment is extended to 10 days (66). The stimulation of the Mstn expression by glucocorticoids suggests that Mstn could contribute to the muscle atrophic effect of glucocorticoids.

1.3.2. Growth hormone

In vitro, Mstn expression appears to be sensitive to GH. Indeed, exposure of C_2C_{12} myotubes to GH causes a dose-dependent reduction of the Mstn expression, suggesting that downregulation of Mstn could contribute to the anabolic action of GH (69). *In vivo*, the regulation of Mstn expression by GH is however more controversial. In GH-deficient individuals, the muscle hypertrophy induced by GH administration is associated with downregulation (human) (69) or no change (rat) (70) of the Mstn mRNA levels. By contrast, in healthy individuals, the muscle expression of Mstn increases (rat) (70) or remains stable (human) (71) following GH treatment. Further studies are then necessary to clarify the role of Mstn in the anabolic effect of GH on skeletal muscle.

1.3.3. IGF-I

In skeletal muscle, IGF-I seems to have no effect on Mstn expression. First, the Mstn mRNA and protein levels are similar in muscle of IGF-I-null mice compared to their wild-type controls, indicating that Mstn expression is not influenced by the absence of IGF-I (72). Second, the muscle mass increase induced by IGF-I overexpression in gastrocnemius of chicken embryos is not associated with changes in Mstn concentrations (73). By contrast, in

cardiac muscle, the Mstn expression seems to be regulated by IGF-I. In fact, mechanical stress increases the myocardial expression of Mstn (51;52;74), suggesting that Mstn could represent a negative feedback to limit the cardiac muscle growth. Interestingly, in this model, IGF-I has been shown to mediate the stretch-induced upregulation of Mstn (52). Altogether, these observations indicate that the Mstn expression is differently regulated by IGF-I in skeletal and cardiac muscles.

1.3.4. Sexual steroids

Androgens are strong positive effectors of skeletal muscle growth. For this reason, it has been hypothesised that Mstn downregulation could mediate the anabolic effect of these sexual steroids. In the levator ani muscle, which is particularly sensitive to the anabolic action of androgens, the Mstn mRNA and protein levels are increased following testosterone deprivation (castration), supporting the possibility that Mstn inhibition contributes to the anabolic effect of testosterone (75). In the gastrocnemius muscle, however, the regulation of Mstn expression by testosterone is less clear. Actually, treatment with testosterone induces a downregulation of the muscle Mstn expression in intact but not in hypophysectomised rats (70). In culture models, evidences do not sustain a negative effect of androgens on the Mstn expression. Indeed, the treatment of bovine satellite cells with various concentrations of trenbolone, a synthetic androgen, does not affect Mstn mRNA levels (76). Furthermore, dihydrotestosterone has been shown to increase surprisingly the Mstn mRNA levels in C_2C_{12} myoblasts, despite a stimulation of cell proliferation (77). In conclusion, the effect of androgens on Mstn expression is controversial and the results obtained seem to be dependent on the molecule used, the type of muscle studied and the differentiation state of the cell culture models.

Studies have also investigated the regulation of Mstn expression by estrogens. Estrogens have been shown to induce a transient and muscle specific upregulation of Mstn. In fact, estrogens replacement in ovariectomised rats attenuates muscle growth and increases the Mstn protein levels in the slow soleus muscle but not in the fast extensor digitorum longus and gastrocnemius muscles (78). The interpretation of these observations remains difficult.

1.3.5. Muscle un/up-loading

Several groups have investigated whether the muscle atrophy caused by disuse is accompanied by increased levels of Mstn. In humans, disuse atrophy is associated with an induction of Mstn mRNA in the vastus lateralis muscle (79). In rodents, hindlimb suspension and microgravity environment have been shown to induce a transient induction of Mstn expression. In fact, the muscle atrophy induced by unloading is associated with an increase of the Mstn mRNA and protein levels in the tibialis anterior, biceps femoris, quadriceps, gastrocnemius and plantaris muscles but surprinsingly not in the soleus muscle, the most sensitive muscle to disuse atrophy (12;80;81). The absence of Mstn induction in the soleus muscle could be explain by its lower content in fast type II fibers which have been demonstrated to express higher level of Mstn compared to the slow type I fibers (12). Furthermore, disuse atrophy is not prevented by the absence of Mstn (82). Taken together, these two observations suggest that Mstn does not play a major role in muscle disuse atrophy.

Conversely, the muscle weight gain induced by overload is coupled with lower Mstn mRNA and protein levels in the muscle of rodents (83;84). Altogether these data support the hypothesis that muscle loading is associated with modulation of the Mstn expression in skeletal muscle.

1.3.6. Muscle denervation

Several studies have investigated the Mstn expression profile following muscle denervation. The increase of Mstn expression levels in denervated muscle compared to control muscle suggests a possible role of Mstn in the denervation-induced muscle atrophy (85-89). The time course of the Mstn induction has been mostly studied in response to sciatic nerve crush. This model of denervation causes a quick and transient induction of Mstn. The peak of Mstn expression has been observed at days 3 (88), 14 (87) or 28 (86), depending on the study. Although different Mstn expression profiles have been described, most authors have found a negative linear correlation between muscle Mstn content and muscle atrophy degree (86;87).

1.3.7. Muscle regeneration

Since Mstn is known to play a role in myogenesis, its expression is expected to change during the muscle regeneration process. Indeed, the Mstn protein is present at high level in the necrotic fibers and connective tissue after injury, while a decreased Mstn content is observed

in survivor fibers (90). When measured in the whole muscle, the Mstn expression has been reported to decrease in the first 3 days after injury (84;91;92). In almost all studies, the Mstn protein level seems to be correlated with the Mstn mRNA transcript content. Only in the work of Mendler L et al an increased Mstn protein level has been observed in damaged muscle despite a decrease in the corresponding mRNA level (91).

1.3.8. Aging

The aging process is associated with a progressive decline in muscle mass also called sarcopenia. It has been hypothesised that a Mstn upregulation could contribute to the age-related sarcopenia but studies have led to conflicting results. In human males, although Welle S *et al* failed to highlight any change in the Mstn muscle mRNA content during aging (93), the recent work of Leger B *et al* described an increase of the Mstn mRNA and protein levels in the muscle of old men compared to young subjects (94). In rodents, no change in the Mstn protein level has been reported in the muscle of mice during aging (95) by contrast to studies conducted in rats that have described a decrease of Mstn mRNA and protein in the muscle of aged animals (85;96). In conclusion, the regulation of Mstn during aging is controversial and studies available do not allow to assign a role of Mstn in sarcopenia.

1.3.9. Exercise

Several studies have investigated the modulation of Mstn expression in response to exercise training. The data suggest an inverse correlation between exercise training and Mstn mRNA levels in a variety of exercise regimens. First, intense running exercise has been reported to decrease the muscle Mstn levels in humans (97). Second, short-term endurance training is able to cause a muscle-specific downregulation of the Mstn expression. In fact, the data show a reduction of the Mstn mRNA content in the gastrocnemius and vastus lateralis muscles but not in soleus (98). Third, resistance training has been shown to also repress Mstn expression. In humans as in rodents, both long-term (99;100) and acute resistance training (101-103) inhibit the Mstn expression in muscle. Only one study has observed opposited results. In this work, authors have observed an increase of the Mstn mRNA and protein levels in the muscle of young men after resistance training (104). The explanation for these conflicting data is uncertain. In conclusion, the regulation of the Mstn expression after exercise training suggests a role of Mstn in remodelling of skeletal muscle in response to training.

1.3.10. Sepsis and burning

The Mstn expression has been measured following injury to determine whether its muscle content was altered by burning or infection. Neither the injection of endotoxin nor the induction of peritonitis significantly alter the Mstn mRNA level in the muscle of adult rats (65). In contrast, the abundance of Mstn mRNA has been shown to increase in muscle after a 30% total body surface area burn injury. The burn-induced increase in Mstn is probably mediated by the enhanced endogenous secretion of glucocorticoids because glucocorticoid receptor antagonist RU486 is able to prevent the upregulation of Mstn mRNA after burn (65).

1.3.11. Cancer

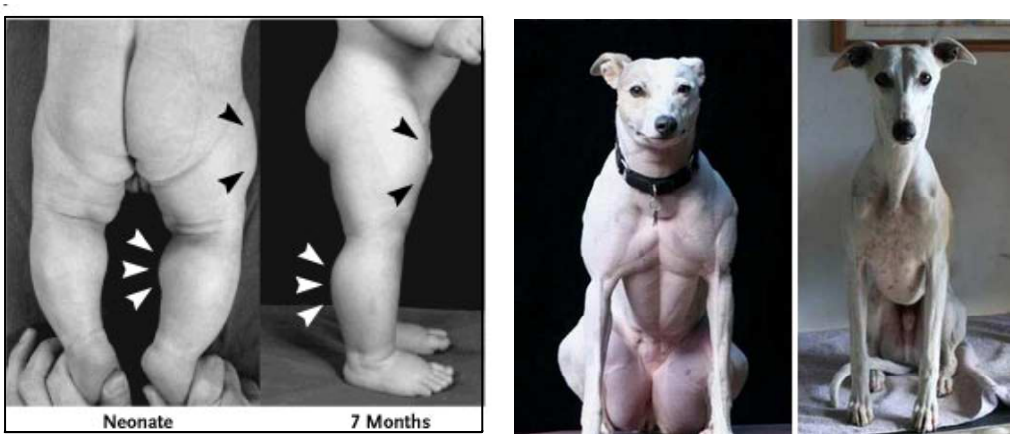
Cancer is associated with a cachexia syndrome characterized by profound skeletal muscle wasting and protein hypercatabolism. It has been shown that Mstn expression is increased in the skeletal muscle of tumour-bearing rodents suggesting that the Mstn upregulation could be involved in the pathogenesis of experimental cancer cachexia wasting syndrome (105;106).

1.4. Interest of myostatin inhibition in the treatment of muscle atrophy

1.4.1. Myostatin gene invalidation

Mice carrying a targeted deletion of the Mstn gene have a dramatic and widespread increase in skeletal muscle mass (1). Similarly, naturally occurring mutations in the Mstn gene in cattle, sheep, dogs, swine and humans also lead to a hypermuscular phenotype (47;107-109) (Fig 8). Remarkably, postnatal inactivation of the Mstn gene also leads to muscle hypertrophy demonstrating the role of Mstn on muscle growth not only during embryogenesis but throughout development (7;110). Interestingly, the muscle weight gain induced by Mstn gene deletion is due to muscle fiber hyperplasia and/or hypertrophy (1;7). The ability of Mstn inhibition to lead to muscle fiber hypertrophy seems to be dependent of the time of inactivation and the muscle type. Indeed, when deletion of the Mstn gene occurs prenatally, the muscle weight gain is mainly due to fiber hyperplasia (1), while postnatal inactivation of the Mstn gene results exclusively in fiber hypertrophy (7). Furthermore, in constitutive Mstn KO mice, the amplitude of increase in fiber cross sectional area is higher in gastrocnemius (+49%) than in tibialis cranialis (+14%) muscle (1).

Fig 8: Naturally occurring null mutations in the Mstn gene of human and whippets dogs



***Schuelke M** et al, N Engl J Med 2004* ***Mosher DS** et al, PLoS Genet 2007*

The muscle hypertrophy resulting from Mstn gene deletion is associated with increased muscle strength. Whippet dogs carrying at least one mutant Mstn allele display enhanced racing performance, confirming a functional benefit to loss of Mstn (111). Additionally, Mstn gene deletion has been shown to be potentially beneficial in aging muscle. In fact, the prolonged absence of Mstn in mice reduces the fiber atrophy associated with aging (112;113) and improves muscle healing after acute injury, even in senescent muscle (112-114). Finally, the Mstn loss of function leads to functional improvement in muscle degenerative diseases. Indeed, *mdx* mice, which represent a genetic model of Duchenne muscular dystrophy, crossed with Mstn-null mice have dramatic improvement in muscle mass, integrity and function compared with their *mdx* counterparts (115;116). Similarly, Mstn gene deletion in mice $scgd^{-/-}$, a model of limb-girdle muscular dystrophy, improves muscle mass, regeneration, and reduced fibrosis (117). However, the positive effects of Mstn inhibition seem to be limited in more severe diseases. Actually, crossing dy^{w}/dy^{w} mice, a laminin α2-deficient model of muscle dystrophy, with Mstn-nulls fails to prevent a severe disease state despite elevated muscle regeneration (117).

1.4.2. Myostatin interfering RNA

Short interfering RNAs targeting Mstn administrated locally in mouse skeletal muscles or intravenously cause a marked increase in the muscle mass within a few weeks after application (118;119). These data suggest that Mstn silencing may provide a therapeutic approach to increase muscle mass.

1.4.3. Myostatin antibodies

Mstn inhibition may also be achieved by neutralizing antibodies. Injection of Mstn antibodies leads to increased muscle mass and function into either wild-type or *mdx* mice (116;120-122). In *mdx* mice, long-term administration of Mstn antibodies not only enhances myofiber regeneration, but also reduces the muscle fibrosis observed in these animals (116). However, mitigated results obtained in models of limb-girdle muscular dystrophy and amyotrophic lateral sclerosis suggest that Mstn blockade in severely affected or late-stage disease may be ineffective (117;123;124).

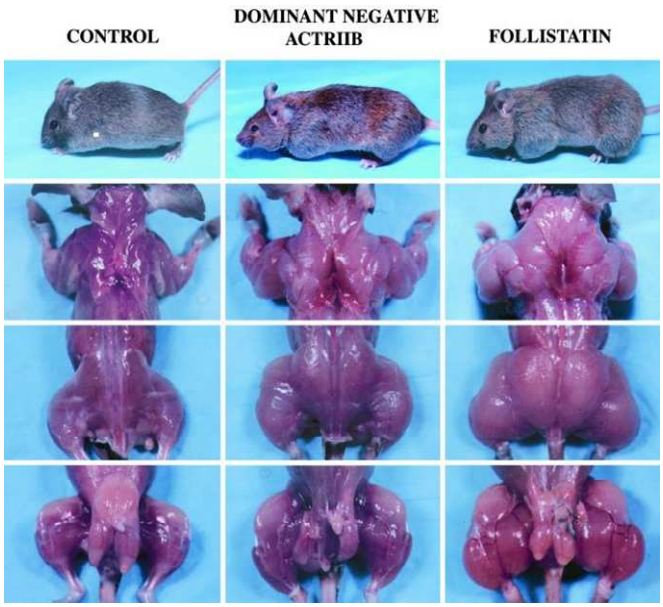
1.4.4. Myostatin propeptide and truncated form of ActRIIB receptor

Mstn blockade using Mstn propeptide increases muscle mass in wild-type mice and improves pathophysiology in *mdx* mice (10;125). Furthermore, functional benefits, evidenced by specific force improvement, exceed those obtained with Mstn antibodies (116).

Similarly, a soluble form of the ActRIIB receptor can cause significant increase in muscle mass when injected to wild-type mice. In addition, treatment with soluble ActRIIB improves function in a mouse model of amyotrophic lateral sclerosis, by increasing the muscle weight and strength (126). Besides muscle weight gain, the transgenic expression of a dominant-negative form of ActRIIB has further beneficial effect in *mdx* mice, as it improves the success of myoblast transplantation, resulting in more abundant and bigger dystrophin positive fibers in the muscle of these animals (127).

Fig 9:

Increased muscling in mice overexpressing a dominant-negative form of ActRIIB or FS



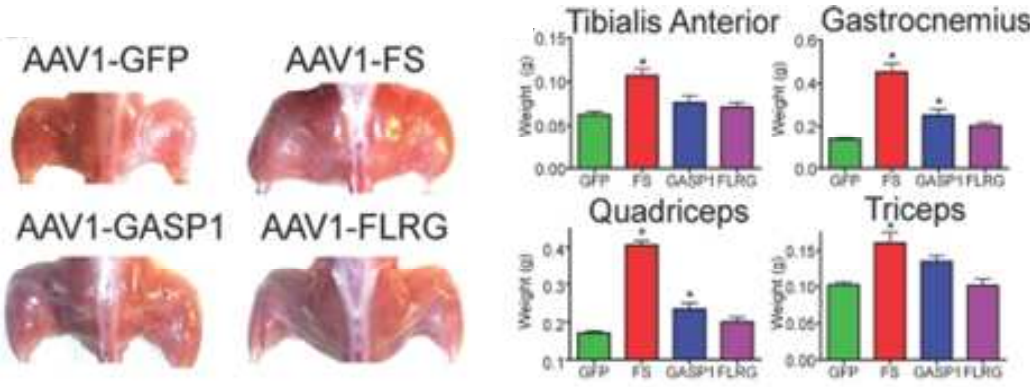
***Lee SJ** et al, PNAS 31; 98(16): 9306-11, 2001*

1.4.5. Myostatin binding proteins

In addition to propeptide, other binding proteins are able to regulate Mstn activity and then muscle growth. Indeed, postnatal intramuscular injection of adeno-associated virus (AAV) encoding Mstn-inhibitor-proteins (FS, FLRG, GASP-1) results in long-term improvement of muscle size and strength in wild-type animals (128). Interestingly, the most dramatic effect on muscle mass is obtained by using the FS construct (128) suggesting that FS is the most efficient Mstn-inhibitor-protein to stimulate muscle growth. The next chapter covers this interesting Mstn binding protein.

Fig 10:

Skeletal muscle hypertrophy induced by gene administration of Mstn-inhibitor-proteins



***Haidet AM** et al, PNAS 18; 105(11): 4318-22, 2008*

Finally, the gene transfer of decorin, another Mstn binding protein, has been shown to promote skeletal muscle regeneration and accelerated muscle healing after injury (129).

Altogether, these data suggest that Mstn inhibition represents a novel strategy for the treatment of muscle-wasting disorders such as muscular dystrophy, cachexia and sarcopenia. Among Mstn blockers, FS seems to be the most promising target, because of its powerful effects on skeletal muscle.

2. Regulation of muscle mass by follistatin

FS was originally identified as a single chain polypeptide able to suppress the follicle-stimulating hormone (FSH) secretion from anterior pituitary cells (130). Later, it was established that this inhibitory effect results from the binding by FS of Activin, a strong hormonal stimulus of FSH secretion (131-133).

2.1. The follistatin gene and protein structure

The FS gene is composed of six exons each encoding distinct functional regions of the protein: a signal peptide, a N-terminal domain, three FS domains (FSI, II, III) and a C-terminal extremity (134;135). Through an alternative splicing, two mRNAs are produced that encode for a preprotein of 317 or 344 amino acids. The removal of the signal peptide (29 amino acids) results in polypeptides of 288 or 315 amino acids with a molecular weight ranging from 31 to 42 kDa (136) (Fig 11). In addition, some evidence suggests that the FS315 can be cleaved *in vivo* at the C-terminal to give an intermediate isoform of 303 amino acids which may be the predominant form present in follicular fluid (137). The two major isoforms of FS (FS288 and 315) differ in their C-terminal sequences (138) and in their ability to bind heparin sulphates (HS). The binding site for HS is located in the FSI domain, at residues 72-86, which is a region rich in basic amino acids (139;140). In the longest form FS315, the acidic tail hides the basic region of the FSI domain and makes the FS unable to bind HS (Fig 12). FS315 is therefore the primary soluble FS form observed in serum. By contrast, the shortest form, FS288, locates to cellular surfaces thanks to its binding to HS of the extracellular matrix (35;138;141).

Fig 11: The FS gene (coding sequences) and protein

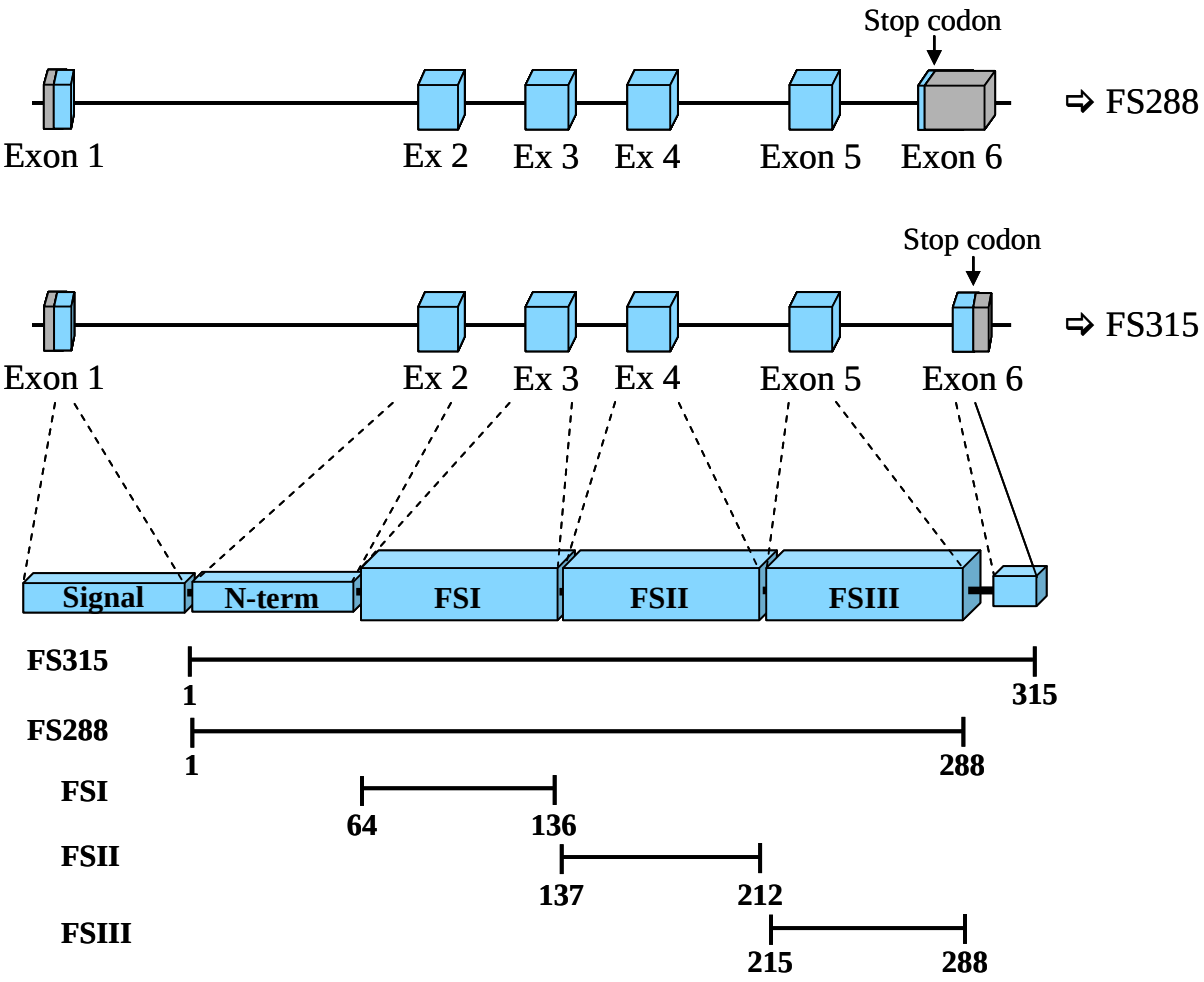
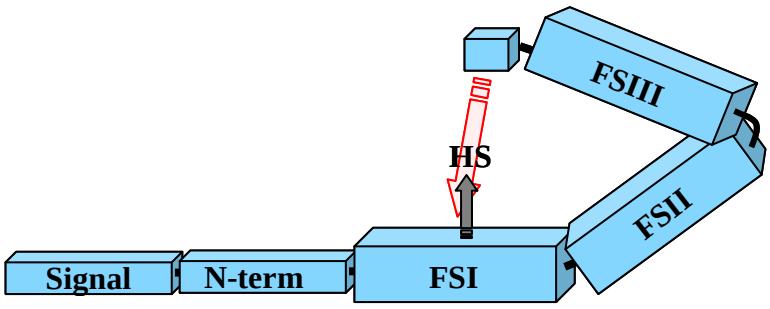


Fig 12: The acidic tail of the FS315 inhibits the binding of FSI to heparin sulphate



Remarkably, the FS protein sequence is highly conserved in mammals. As an example, the sequence of the human FS protein has 98% homology with the mouse sequence.

2.2. Tissue expression of follistatin

FS was first identified in ovarian follicular fluid (142). In fact, FS expression is broadly distributed in adult tissues and is not confined solely to those associated with reproduction. In the muscular system, FS is abundantly expressed in skeletal muscle and in a lesser extent in heart (143). Remarkably, the majority of FS found in the circulation is likely secreted from the walls of blood vessels (133).

Table 1: Tissue expression (mRNA) of FS in human fetal tissues

Neural tissues	
Cerebrum	+
Cerebellum	++
Spinal cord	+
Muscular tissues	
Heart	+
Skeletal muscle	+++
Stomach (smooth m.)	+
Hemopoetic tissues	
Bone marrow	-
Spleen	-
Thymus	-
Exocrine glandular tissues	
Kidney	+++
Salivary	+
Pancreas	-
Liver	+++
Steroidogenic tissues	
Adrenal	+
Testis	**n.d.**
Ovary	**n.d.**

+++, strong expression

++, moderate expression

+, weak expression

-, no expression

n.d., not detected

***Tuuri T** et al, J Clin Endocrinol Metab, 1994*

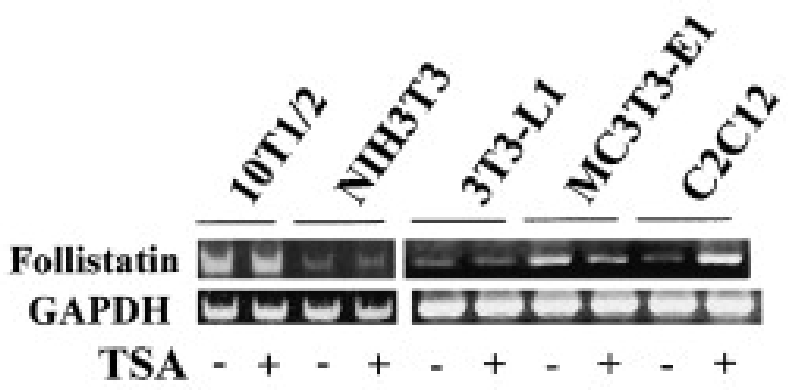
2.3. Regulation of follistatin expression in skeletal muscle

2.3.1. Histone deacetylase inhibitors

It has been demonstrated that histone deacetylase (HDAC) inhibitors like trichostatin A, valproic acid and sodium butyrate, are able to upregulate FS expression in a tissue-specific manner. In fact, these drugs activate FS expression only in skeletal muscle cells (Fig 13). For this reason, it is thought that some of the muscle effects of these drugs are mediated by the stimulation of FS production (144;145).

Fig 13:

FS expression is upregulated by trichostatin A (TSA) specifically in skeletal muscle cells



***Mineti GC** et al, Nat Med 12(10): 1147-50, 2006*

Indeed, the administration of HDAC inhibitors has shown beneficial effects on muscle growth and pathophysiology of dystrophic muscles. In fact, *mdx* and α-sarcoglycan-deficient mice treated with trichostatin A have been reported to display muscle mass enhancement and functional improvement attributed to FS upregulation (145).

2.3.2. Testosterone

Androgens are known to promote myogenic differentiation. It has been hypothesized that modulation of FS expression by androgens could contribute to their muscle anabolic effect. In agreement with this hypothesis, the myogenic differentiation induced by testosterone in mesenchymal multipotent C3H 10T1/2 cells is associated with an upregulation of the FS expression (146). Furthermore, the testosterone-induced upregulation of MyoD and MHCII proteins in the C3H 10T1/2 cells is abolished by simultaneous addition of anti-FS antibody (146). In the same way, testosterone supplementation in castrated mice upregulates FS mRNA expression in androgen responsive *levator ani* muscle (146). Conversely, lowering of

testosterone levels in mice by orchiectomy leads to a significant decrease in FS expression (146). Altogether, these data suggest that FS may act as a mediator of the testosterone effects on myogenic differentiation.

2.3.3. Nitric oxide

Recent data have demonstrated that Nitric oxide (NO) generation is required for myoblast fusion both in embryonic myoblasts and in satellite cells, and that FS upregulation mediates the action of NO on myogenesis (147).

2.3.4. Muscle regeneration

As FS promotes muscle development, it has been suggested that FS upregulation could contribute to the differentiation process in regenerating muscle. Actually, FS transcript is detected in activated satellite cells as early as the first stages of regeneration after cardiotoxin injury, and is transiently expressed in forming myotubes (148). These results suggest that FS could contribute to the differentiation process stimulated by regeneration.

2.3.5. Sepsis and inflammation

Inflammatory reaction as observed in patients with septicaemia (149;150) or as induced in animals by endotoxin injection is associated with a profound and long-lasting increase of circulating FS concentrations (151-154). However, the origin of FS production during inflammation has not yet been established.

2.4. Biological actions on skeletal muscle

Up to date, there is no evidence for the existence of a FS receptor. Indeed, the only known biological activity of FS is the neutralization of TGF-β superfamily ligands.

FS binds Activin (Act) A with a very high affinity (50 pM), the closely related ActB isoform with 10 fold lower affinity (500 pM), and many BMPs (2, 4, 6, 7, and 15) with lower affinities ranging from 200 to 80.000 pM. FS binds also Mstn and GDF-11, with affinities intermediate between ActA and BMP ligands (500 pM) (8;36;132;155-157). Since FS can bind to Mstn with a higher affinity than its receptor ActRIIB, FS is proposed to block Mstn signalling and action (8;35).

To better understand the functional role of FS, some genetically modified mice models have been developed. First, FS-null mice exhibit growth retardation and defects in skeletal muscle development causing death by asphyxia shortly after birth (156;158). This model pinpoints an important role for FS on skeletal muscle development. Second, FS transgenic and gene transfection animal models are characterized by dramatic increase in muscle mass, establishing FS as a potent inducer of muscle growth (see above) (8;128). Remarkably, the muscle hypertrophy observed in FS transgenic mice is even greater than observed in Mstn-null animals, suggesting that at least part of the FS effect results from impact on another ligand independent of Mstn inhibition. This hypothesis is reinforced by the observation of a quadrupling of muscle mass in Mstn-null mice carrying a FS transgene, compared with the doubling of muscle mass induced by lack of Mstn alone (159).

The mechanism by which FS promotes skeletal muscle growth includes at least a stimulation of the protein synthesis. In fact, it has been demonstrated that FS infusion stimulates protein synthesis in skeletal muscle of neonatal rats by increasing the phosphorylation of the translation initiation factors, S6K1 and rpS6 (41). As Mstn has been shown to inhibit muscle protein synthesis (39), these data are consistent with the notion that FS blocks the Mstn signalling. Furthermore, FS has been shown to rescue the muscle differentiation inhibited by Mstn. In chick limb bud, FS prevents the Mstn-mediated downregulation of Pax-3 and MyoD, two regulators of myogenesis (35), and in a mesenchymal cell culture model, FS antagonises the inhibitory effect of Mstn on myogenic differentiation (35). Finally, *in vitro*, FS promotes muscle development as measured by an increase of the creatine phosphokinase activity and a higher number of nuclei found in MHC-immunoreactive cells (160).

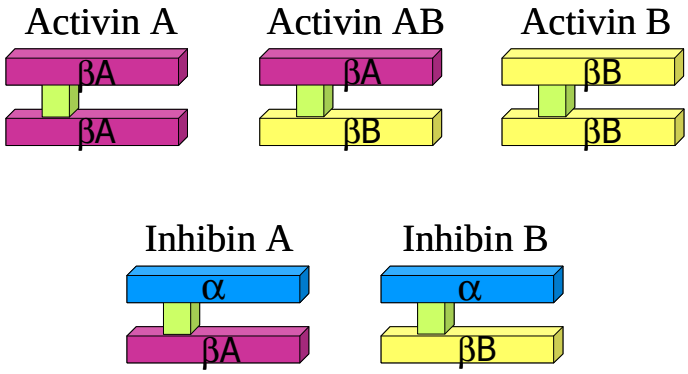
The data obtained in Mstn KO mice overexpressing FS has highlighted a potential role of other FS ligands which could regulate the skeletal muscle size in concert with Mstn. However, further studies will be necessary to determine the ligands involved in the anabolic effect of FS.

2.5. High affinity follistatin ligands

2.5.1. Activin/Inhibin

Act and Inhibin are structurally related and form a distinct group of the TGF-β superfamily. Inhibin is a heterodimer composed of a α subunit linked to a β subunit (βA or βB), whereas Act is a dimer of β chains (161;162) (Fig 14). FS binds with high affinity to the β-subunits and neutralizes the activity of Act and, in a lesser extent, Inhibin (153).

Fig 14: Structure of the Act and Inhibin proteins



Inhibin is secreted by granulosa cells of ovary in the female and Sertoli cells of the testes in the male. The role of Inhibin is to regulate the gametogenesis via a negative feedback mechanism on the production of follicle stimulating hormone (FSH) by the pituitary gland. It has also a local paracrine action in the gonads (163). Moreover, Inhibin acts also as negative regulator of Act activity, by binding to betaglycan and reducing the capacity of Act to bind its receptor ActRII (164).

By contrast, Act is a positive regulator of the FSH secretion by the pituitary gland (130;165). Unlike Inhibin, Act is expressed in a wide variety of tissues, extending its biological roles to the regulation of developmental decisions for many cell types. In fact, Act influences reproduction (163), wound healing (166), bone formation (167), immune responses (168) and pancreatic β-cell proliferation (169). Its role in skeletal muscle has not been clearly described, but its expression has been detected in skeletal muscle (143) and some *in vitro* data suggest a negative effect of Act on muscle growth. Indeed, ActA inhibits the differentiation of limb muscle precursors of chick embryos (170) and blocks the development of muscle cells in primary cell culture (160).

The best characterized Act is ActA, which is composed of two βA subunits. In addition to its role in modulating FSH release from the pituitary, ActA has been shown to be involved in erythroid differentiation, mesoderm induction, neuron survival and plasmacytoma cell inhibition (164;171). These functions indicate that ActA has widespread actions beyond reproduction.

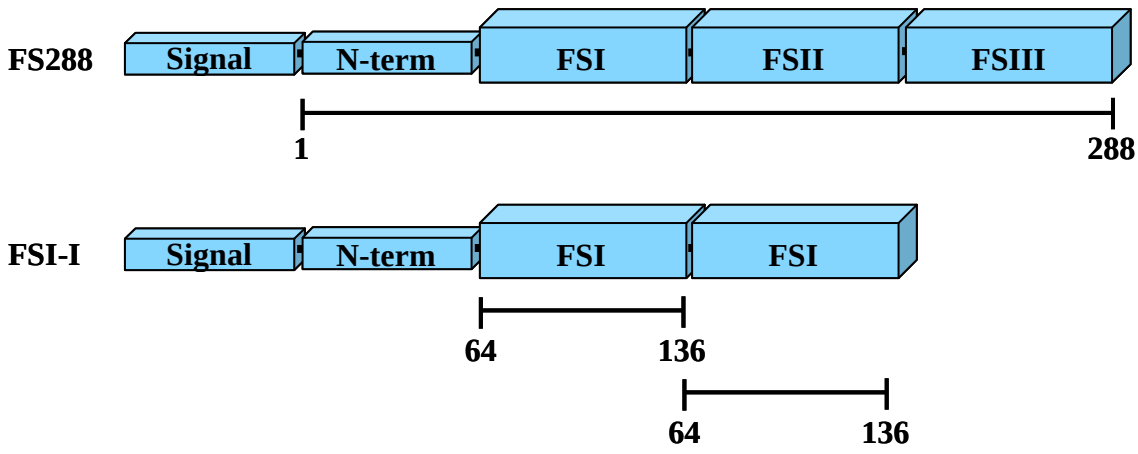
2.5.2. GDF-11

GDF-11 is a TGF-β family member closely related to Mstn with 90% amino acid identity. Like Act and Mstn, GDF-11 (or BMP-11) can bind ActRIIB (172). GDF-11-null mice present dramatic abnormalities of the axial skeleton, kidney agenesis, and an increase in progenitor cell number in several tissues (173). But, in contrast to Mstn, the GDF-11 gene global deletion does not affect muscle mass (174). These observations suggest that despite a high degree of sequence identity with Mstn, GDF-11 affects distinct biological processes. Nevertheless, some data support a role of GDF-11 in the regulation of myogenesis. Indeed, GDF-11 has been shown to inhibit the myogenic cell differentiation in the developing chick limb (175), and in a C_2C_{12} model (172). However, it has been recently demonstrated that gene deletion of GDF-11 specifically in skeletal muscle does not affect muscle size, fiber number, or fiber type (176).

2.6. Interest of follistatin mutants

Because Act has a variety of functions in tissues other than skeletal muscle, its inhibition by FS could lead to potentially diverse effects including on reproductive performance and fertility. Consequently, FS mutants displaying minimal Act-inhibiting activities have been developed. In fact, key residues for Act binding are found in the second FS domain, while Mstn binding is more dependent on FSI domain (177;178). The deletion of FSII domain has then created Mstn antagonists with greatly reduced Act binding (179;180) (Fig 15). The results obtained with the FS mutants containing two copies of FSI domain (FSI-I) indicate that Mstn blockade by FSI-I has a therapeutic potential for muscle wasting. Indeed, transgenic expression of FSI-I have been shown to increase muscle mass and strength in healthy as in *mdx* animals (179).

Fig 15: Structure of FSI-I compared with FS288



3. Mechanisms of glucocorticoid-induced muscle atrophy

3.1. Generalities

Glucocorticoids are known to cause skeletal muscle atrophy. Indeed, administration of high doses of glucocorticoids induces decreased muscle mass and strength, attributed to a decrease protein synthesis and a stimulation of muscle proteolysis (181;182). Furthermore, hypercortisolism has been shown to play a major role in skeletal muscle atrophy associated with several catabolic conditions (183;184). Typically, skeletal muscle atrophy caused by glucocorticoids is characterized by a decrease in the size of fast-twitch, or type II, muscle fibers (particularly IIx and IIb) with little to no impact observed in type I fibers (185). As a result, fast-twitch, glycolytic muscles (i.e., tibialis anterior) are more susceptible than oxidative muscles (i.e., soleus) to glucocorticoid-induced muscle atrophy.

3.2. Mechanisms of the glucocorticoid-induced muscle atrophy

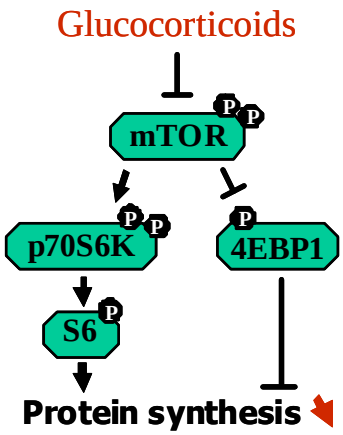
3.2.1. Anti-anabolic action of glucocorticoids

3.2.1.1. Inhibition of protein synthesis

Glucocorticoids exert an inhibitory effect on protein synthesis. First, glucocorticoids inhibit the transport of amino acids into the muscle, limiting protein synthesis (186). Second, glucocorticoids prevent the stimulatory action of insulin, IGF-I and amino acids (in particular, leucine) on the phosphorylation of the eIF4E-binding protein 1 (4E-BP1) and the p70 ribosomal protein S6 kinase (p70S6K), two key factors that control the initiation step of mRNA translation (187-189). The inhibition of protein synthesis by glucocorticoids results primarily from the inhibition of mTOR, the kinase responsible for the phosphorylation of 4E-BP1 and p70S6K. Repression of mTOR signaling results in a reduction in the initiation phase of mRNA translation with downregulation of protein synthesis.

Fig 16:

Glucocorticoids inhibit muscle protein synthesis through downregulation of mTOR



3.2.1.2. Inhibition of cell differentiation

There is also some evidence that glucocorticoids can cause muscle atrophy by inhibiting myogenesis. Indeed, glucocorticoids decrease the expression of Myogenin (190) and increase the degradation of MyoD (191), two transcription factors required for differentiation of satellite cells into muscle fibers.

3.2.2. Catabolic action of glucocorticoids

3.2.2.1. Stimulation of proteolysis

The stimulation of muscle protein degradation by glucocorticoids is mainly mediated by the activation of the ubiquitin-proteasome and lysosomal pathways. In particular, glucocorticoids stimulate the expression of several components of the ubiquitin-proteasome system including the muscle-specific E3-ligases, Atrogin-1 and muscle ring finger 1 (MuRF1) (192) and several subunits of the proteasome (193). This leads to an increased rate of protein ubiquitination and increased proteolytic activities of the proteasome (194). Furthermore, glucocorticoids upregulate the expression of the lysosomal enzyme Cathepsin L, which has been shown to be the earliest mediator of skeletal muscle proteolysis induced by glucocorticoids (195). In addition to these proteolytic pathways, the calcium-dependent system (calpain) could be involved in the glucocorticoids catabolic effects. In fact, since the proteasome is not able to degrade intact myofibrills, it is thought that myofilaments need to be dissociated, probably by calpains, before they can be degraded by the ubiquitin-proteasome system (196). Finally, some *in vivo* data suggest that caspase-3 might be implicated in the muscle proteolysis

induced by glucocorticoids. Indeed, in glucocorticoid-dependent muscle-wasting models such as insulinopenia and chronic renal failure, the caspase-3 activity is stimulated by glucocorticoids, and its inhibition suppresses the accelerated muscle proteolysis (197). However, the role of glucocorticoids in the upregulation of caspase-3 has not yet been explored in these catabolic models.

Fig 17: The major proteolytic systems implicated in muscle atrophy

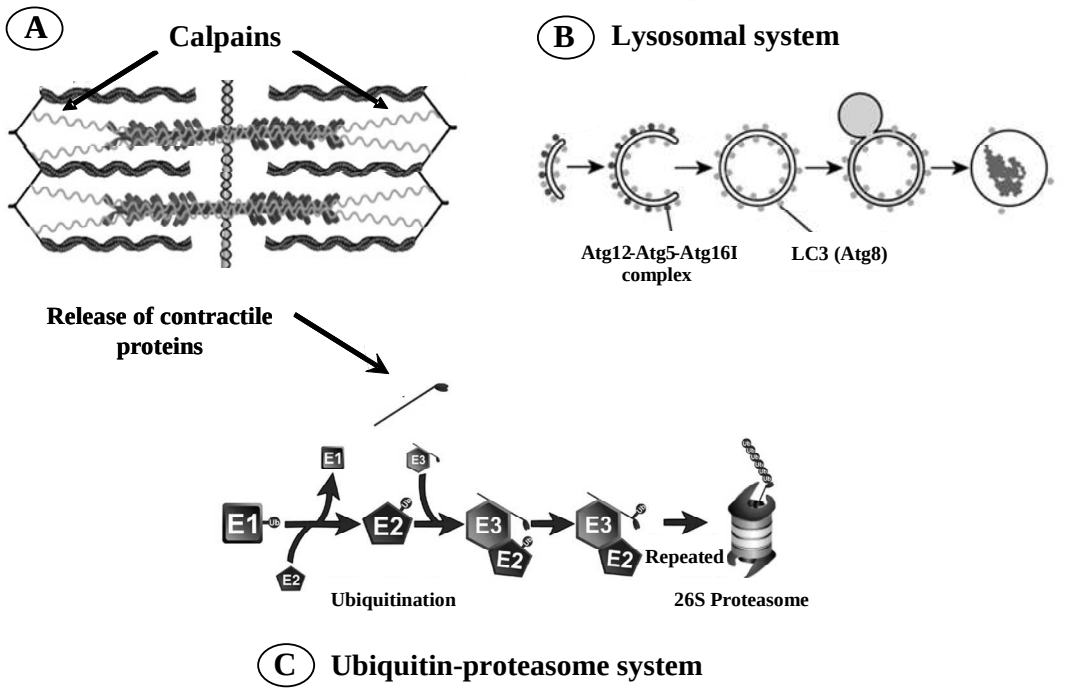
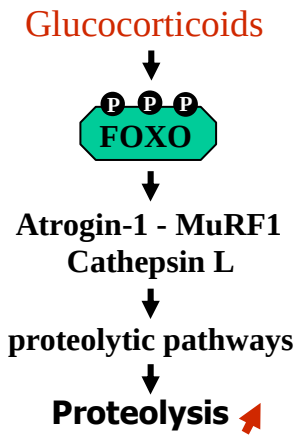


Figure adapted from the thesis of O. Shackman (2009)

Several intracellular pathways mediate the stimulation of these proteolytic systems by glucocorticoids. The transcription factor FOXO is considered as one of them for several reasons. First, the FOXO expression is increased in myotubes exposed to glucocorticoids (198). Second, *in vitro* as well *in vivo*, FOXO overexpression causes muscle cell atrophy (199;200) associated with an induction of several atrogenes such as Atrogin-1, MuRF-1 and Cathepsin-L (199;201). Finally, overexpression of a dominant negative form of FOXO prevents the muscle cell atrophy and the induction of Atrogin-1 caused by glucocorticoids (199). Since FOXO, but not Atrogin-1, overexpression is sufficient to induce muscle atrophy, it is thought that FOXO activates several genes, in addition to Atrogin-1, that lead to atrophy. Altogether, these data indicate that upregulation of FOXO by glucocorticoids activates a gene transcriptional program responsible for triggering muscle atrophy.

Fig 18: Glucocorticoids stimulate muscle proteolysis through FOXO upregulation



In addition to FOXO, the glycogen synthase kinase 3β (GSK-3β) may also be involved in the catabolic effect of glucocorticoids. Indeed, *in vitro* data show that GSK-3β inhibition by overexpression of a dominant negative form or by pharmacologic inhibitors prevents the muscle proteolysis and cell atrophy caused by glucocorticoids (202-205). Furthermore, overexpression of a dominant negative GSK-3β prevents the glucocorticoid-induced muscle atrophy *in vivo* (206). The mechanism by which GSK-3β inhibition prevents the muscle protein degradation caused by glucocorticoids is not known. However, the inhibition of muscle proteolysis by GSK-3β inhibitors is associated with blockade of the upregulation of several atrogenes such as Atrogin-1 and MuRF-1, suggesting that this reduction in muscle proteolysis is mediated, at least in part, by inhibition of the ubiquitin-proteasome system (205). Alternatively, one of the targets of GSK-3β, β-catenin, which is down-regulated by glucocorticoids and reversed by dnGSK-3β overexpression might also be responsible for the anti-atrophic effect of GSK-3β inhibition, as its overexpression is sufficient by itself to prevent glucocorticoid-induced muscle atrophy *in vivo* (206).

3.2.2.2. Stimulation of apoptosis

Glucocorticoids can promote apoptotic cell death of differentiated skeletal muscle. This effect is mediated through the stimulation of the caspase-8 proapoptotic protein (207) and the decrease in the muscle expression of IGF-I, an anti-apoptotic factor towards skeletal muscle (208).

3.2.3. Regulation of local growth factors

Finally, some evidence pinpoints the role of local growth factors in the glucocorticoids-induced skeletal muscle atrophy. First, glucocorticoids decrease the expression of IGF-I. The reduced IGF-I levels in the muscle could contribute to the muscle atrophy caused by glucocorticoids. Indeed, it has been demonstrated that activation of the phosphatidylinositol-3-kinase/Akt pathway by IGF-I is able to prevent the glucocorticoid-induced muscle atrophy (206). Second, glucocorticoids stimulate the production of Mstn in the muscle (66;68). As Mstn overexpression is known to cause muscle atrophy (44), the upregulation of Mstn could contribute to the muscle loss induced by glucocorticoids. However, the role of Mstn upregulation in the glucocorticoid-induced muscle atrophy has not yet been demonstrated.

Fig 19:

Glucocorticoids regulate the muscle expression of IGF-I and Mstn, two growth factors exhibiting opposite effects on muscle development

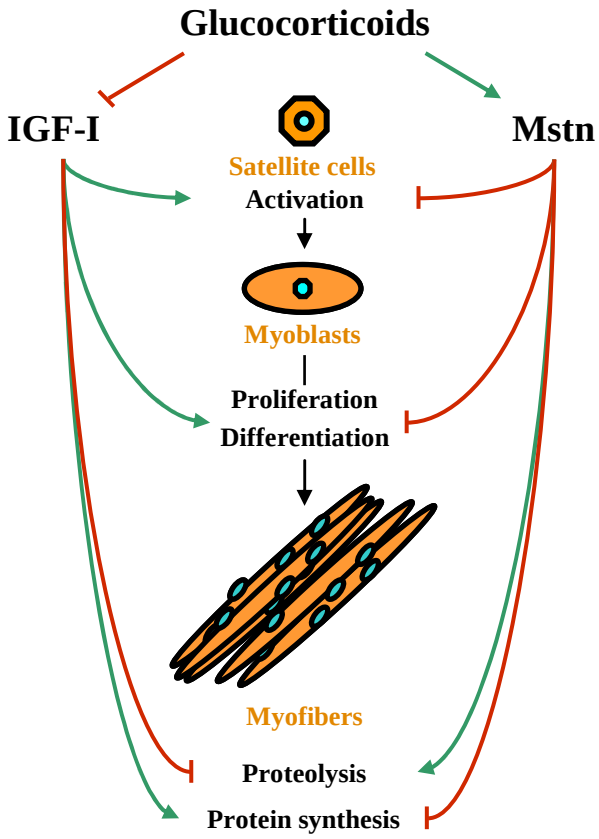


Fig 20: Signalling pathways involved in the glucocorticoid-induced muscle atrophy

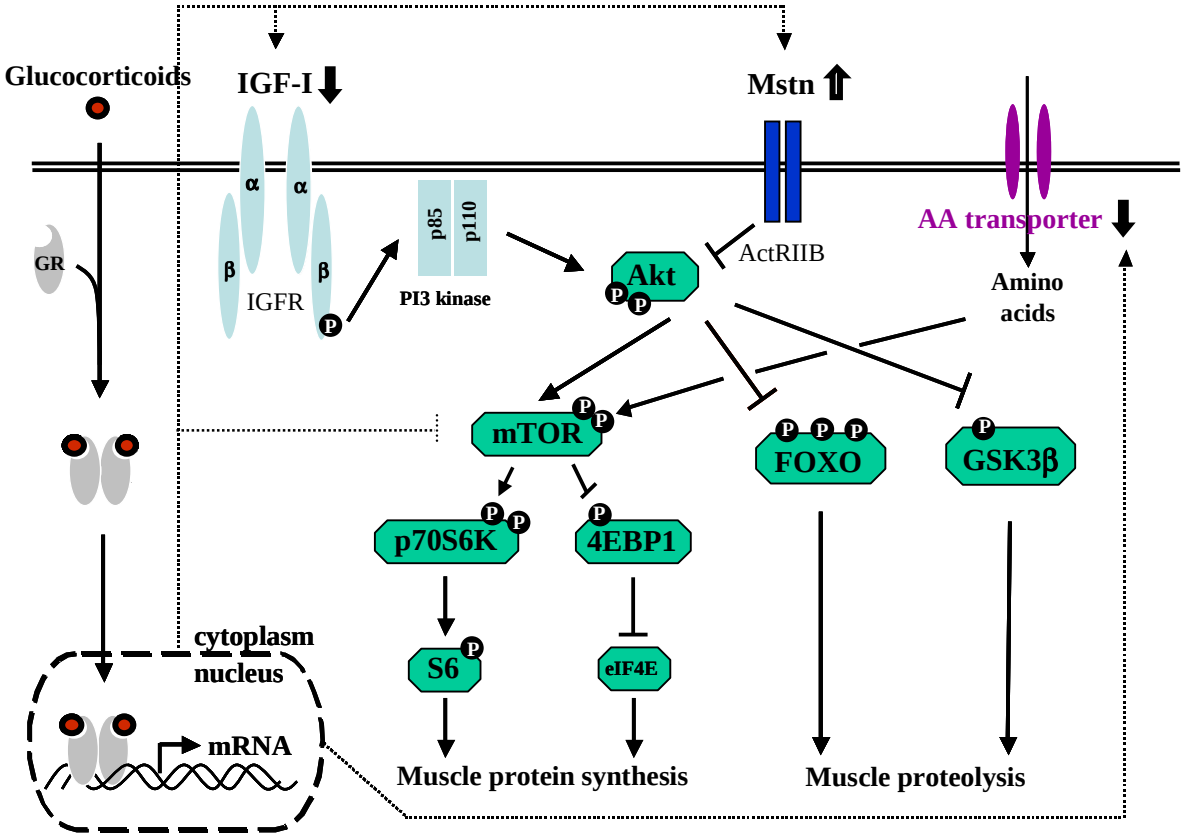


Figure adapted from the review of O. Schakman 2009

3.3. Role of glucocorticoids in muscle atrophy in catabolic states

In many catabolic conditions (sepsis, cachexia, starvation, metabolic acidosis, severe insulinopenia, etc), the muscle atrophy is associated with increased circulating levels of glucocorticoids (209), suggesting that these hormones could trigger the muscle atrophy observed in these situations. Accordingly, adrenalectomy or treatment with a glucocorticoid receptor antagonist (RU-486) attenuate the muscle atrophy observed in sepsis, cachexia, starvation and severe insulinopenia, indicating that glucocorticoids are in part responsible for this muscle loss. In addition to glucocorticoid excess, other factors including poor nutrition, cytokines and bed resting may contribute to muscle atrophy observed in these wasting conditions (183;209). In contrast, glucocorticoids seem not to be required for disuse atrophy (210) but may clearly exacerbate the deleterious effects of disuse on skeletal muscle mass (211). Therefore, glucocorticoids are involved in the muscle atrophy observed in many clinical situations.

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