Clinical manifestations, biology, and cellular & molecular mechanisms of muscle wasting and growth in metabolic and neuromuscular diseases.

**Cell size is determined by a balance between new protein accumulation and degradation of existing proteins.** Genetic studies in both dorsoptile and mammals have shown that pathways controlling protein synthesis and protein breakdown have an important role to determine cell size. The two processes are tightly regulated and interrelated. The first level of connection occurs during protein synthesis when the quality control of the cell degrades proteins that are not correctly folded. At a further level, protein degradation systems determine the half-life of protein and, in muscle, are required to replace sarcomeric proteins as a consequence of changes in muscle activity. Both systems need ATP, and muscle energy level is one of the cellular check points that decide either to promote growth and hypertrophy or activate protein breakdown and atrophy. Importantly, the proteolytic systems can produce alternative energy substrates that are used by the cell to maintain internal homeostasis in conditions of energy stress. Recent findings provide a new view, which considers the growth-promoting pathways and the proteolytic systems coordinately regulated. The following short review will focus mainly on in vivo studies and will be schematically divided into two parts: the first part will describe pathways controlling muscle hypertrophy, and the second part will be focused on signaling involved in muscle atrophy.

**Muscle Hypertrophy**

The growth of skeletal muscle mass, like the mass of any other tissue, depends on protein turnover and cell turnover (83). Cellular turnover plays a major role during muscle development in embryos. Moreover satellite cell incorporation into the growing fibers takes place during postnatal muscle growth (65) concomitantly with increased protein synthesis. The activation of satellite cells is important for maintaining a constant size of each nuclear domain (quantity of cytoplasm/number of nuclei within that cytoplasm). Unlike young muscle, the contribution of cellular turnover to homeostasis of adult fibers is minor, and its role in hypertrophy has even been recently debated (56, 73). In adult muscle, the physiological conditions promoting muscle growth, therefore, do so mainly by increasing protein synthesis and decreasing protein degradation. However satellite cells are activated in compensatory hypertrophy (65, 85), and addition of new nuclei to the growing fiber seems to be required for extreme hypertrophy. The pathways controlling cellular and protein turnover are different, and their contribution to muscle hypertrophy has to be considered during the interpretation of data resulting from studies with transgenic animals. Loss- and gain-of-function studies in which the transgene is perturbed early during postnatal growth might affect cellular turnover significantly more than protein synthesis. Results could be completely different if the same pathway is acutely perturbed in adult muscle age when the role of protein turnover is dominant (Figure 1).

**IGF1-AKT signaling and the control of muscle growth**

**IGF1.** IGF1 is among the best characterized muscle growth-promoting factors. In addition to circulating IGF1, mainly synthesized by the liver under GH control, local production by skeletal muscle of distinct IGF1 splicing products has recently raised considerable interest. A specific IGF1 splice product is important for load- and stretch-induced adaptations in skeletal muscle (25). Increased IGF1 gene expression has been demonstrated following functional overload induced by elimination of synergistic muscles (55). Muscle-specific overexpression in transgenic mice results in muscle hypertrophy (68) and, importantly, the growth of muscle mass matches with a physiological increase of muscle strength. Moreover even acute ectopic expression of IGF1 in adult muscles by electroporation is sufficient to increase muscle strength. Moreover even acute ectopic expression of IGF1 in adult muscles by electroporation is sufficient to increase muscle strength.

**Akt.** Akt is a substrate of phosphatidylinositol 3-kinase, a lipid kinase that is activated in response to a variety of extracellular stimuli such as growth factors, cytokines, insulin, and protein synthesis. Two distinct Akt isoforms (Akt1, Akt2) are expressed in the muscle (98). Akt activity is also controlled by metabolic feedback pathways, which dictate Akt phosphorylation in vivo and under certain conditions may promote muscle growth or atrophy (56). In adult muscle, the physiological conditions promoting muscle growth, therefore, do so mainly by increasing protein synthesis and decreasing protein degradation. However satellite cells are activated in compensatory hypertrophy (65, 85), and addition of new nuclei to the growing fiber seems to be required for extreme hypertrophy. The pathways controlling cellular and protein turnover are different, and their contribution to muscle hypertrophy has to be considered during the interpretation of data resulting from studies with transgenic animals. Loss- and gain-of-function studies in which the transgene is perturbed early during postnatal growth might affect cellular turnover significantly more than protein synthesis. Results could be completely different if the same pathway is acutely perturbed in adult muscle age when the role of protein turnover is dominant (Figure 1).

**Akt activation and the regulation of muscle growth**

Akt activation is involved in the regulation of muscle growth at multiple levels, including the control of cell growth and cell survival. Akt activation is also involved in the regulation of muscle growth at multiple levels, including the control of cell growth and cell survival. Akt is phosphorylated by phosphatidylinositol 3-kinase (PI3K), a lipid kinase that is activated in response to a variety of extracellular stimuli such as growth factors, cytokines, insulin, and protein synthesis. Akt activation is also controlled by metabolic feedback pathways, which dictate Akt phosphorylation in vivo and under certain conditions may promote muscle growth or atrophy (56). In adult muscle, the physiological conditions promoting muscle growth, therefore, do so mainly by increasing protein synthesis and decreasing protein degradation. However satellite cells are activated in compensatory hypertrophy (65, 85), and addition of new nuclei to the growing fiber seems to be required for extreme hypertrophy. The pathways controlling cellular and protein turnover are different, and their contribution to muscle hypertrophy has to be considered during the interpretation of data resulting from studies with transgenic animals. Loss- and gain-of-function studies in which the transgene is perturbed early during postnatal growth might affect cellular turnover significantly more than protein synthesis. Results could be completely different if the same pathway is acutely perturbed in adult muscle age when the role of protein turnover is dominant (Figure 1).

**IGF1-AKT signaling and the control of muscle growth**

**IGF1.** IGF1 is among the best characterized muscle growth-promoting factors. In addition to circulating IGF1, mainly synthesized by the liver under GH control, local production by skeletal muscle of distinct IGF1 splicing products has recently raised considerable interest. A specific IGF1 splice product is important for load- and stretch-induced adaptations in skeletal muscle (25). Increased IGF1 gene expression has been demonstrated following functional overload induced by elimination of synergistic muscles (55). Muscle-specific overexpression in transgenic mice results in muscle hypertrophy (68) and, importantly, the growth of muscle mass matches with a physiological increase of muscle strength. Moreover even acute ectopic expression of IGF1 in adult muscles by electroporation is sufficient to promote muscle hypertrophy (6). Although these results suggest an autocrine/paracrine role for local IGF1 in activity-dependent muscle plasticity,

**Akt.** Akt activity is also controlled by metabolic feedback pathways, which dictate Akt phosphorylation in vivo and under certain conditions may promote muscle growth or atrophy (56). In adult muscle, the physiological conditions promoting muscle growth, therefore, do so mainly by increasing protein synthesis and decreasing protein degradation. However satellite cells are activated in compensatory hypertrophy (65, 85), and addition of new nuclei to the growing fiber seems to be required for extreme hypertrophy. The pathways controlling cellular and protein turnover are different, and their contribution to muscle hypertrophy has to be considered during the interpretation of data resulting from studies with transgenic animals. Loss- and gain-of-function studies in which the transgene is perturbed early during postnatal growth might affect cellular turnover significantly more than protein synthesis. Results could be completely different if the same pathway is acutely perturbed in adult muscle age when the role of protein turnover is dominant (Figure 1).

**Akt activation and the regulation of muscle growth**

Akt activation is involved in the regulation of muscle growth at multiple levels, including the control of cell growth and cell survival. Akt activation is also involved in the regulation of muscle growth at multiple levels, including the control of cell growth and cell survival. Akt is phosphorylated by phosphatidylinositol 3-kinase (PI3K), a lipid kinase that is activated in response to a variety of extracellular stimuli such as growth factors, cytokines, insulin, and protein synthesis. Akt activation is also controlled by metabolic feedback pathways, which dictate Akt phosphorylation in vivo and under certain conditions may promote muscle growth or atrophy (56). In adult muscle, the physiological conditions promoting muscle growth, therefore, do so mainly by increasing protein synthesis and decreasing protein degradation. However satellite cells are activated in compensatory hypertrophy (65, 85), and addition of new nuclei to the growing fiber seems to be required for extreme hypertrophy. The pathways controlling cellular and protein turnover are different, and their contribution to muscle hypertrophy has to be considered during the interpretation of data resulting from studies with transgenic animals. Loss- and gain-of-function studies in which the transgene is perturbed early during postnatal growth might affect cellular turnover significantly more than protein synthesis. Results could be completely different if the same pathway is acutely perturbed in adult muscle age when the role of protein turnover is dominant (Figure 1).
Akt activation is induced by IGF1 and insulin through the generation of phosphatidylinositol-3,4,5-triphosphates produced by PDK1, which is opposed by the activity of the phosphatase PTE1 and SHIP2. Phosphatidylinositol-3,4,5-triphosphates recruit Akt to the plasma membrane by binding to its NH2-terminal pleckstrin homology domain. At the membrane, Akt is phosphorylated on separate residues by at least two distinct kinases, PDK1 and the mTOR-Rictor complex. The role of Akt in muscle growth was first suggested by the finding that an active Ras double mutant (RasV12C40) that selectively activates the Akt pathway through the phosphatidylinositol 3 kinase (PI3K) promotes muscle growth, thus opening new perspectives in the signaling of fiber size (67) (FIGURE 2). This observation was subsequently confirmed by overexpressing a constitutively active form of Akt in adult skeletal muscle (10, 72). Similar results were obtained by the generation of conditional transgenic mice in which Akt is expressed in adult skeletal muscles only after tamoxifen (41) or tetracycline (37) treatment.

In mammals, there are three Akt genes, Akt1 (PKBα), Akt2 (PKBβ), and Akt3 (PKBγ), which appear to have distinct functions. In skeletal muscle, Akt1 and Akt2 are expressed at higher levels compared with Akt3, which is mainly expressed in the brain. Targeted deletion experiments have shown that Akt1-null mice display growth retardation and muscle atrophy, whereas Akt2-null mice suffer from a Type 2 diabetes-like syndrome, and Akt3-null mice have impaired brain development (111).

Exercise in vivo is associated with activation of Akt1 but not Akt2 and Akt3 kinases in contracting muscles (99). Akt activity is increased in the rat plantaris after functional overload induced by elimination of synergistic muscles (10). Subsequent studies both in rats and humans confirmed that Akt activity is increased in response to muscle contractile activity (69, 78–80). Surprisingly, this effect was observed only in the fast EDL muscle and not in the slow soleus muscle (78, 79). The finding that passive stretch of the fast rat EDL muscle can also induce Akt activation has suggested that mechanical tension may be a part of the mechanism by which contraction activates Akt in fast-twitch muscles (79). However, it remains to be established how mechanical stress is converted to Akt activation. Akt activity is also increased in response to hormonal and growth factor stimulation, in particular insulin is known to activate Akt2, whereas IGF1 activates primarily Akt1. Taken together with other observations, these results suggest that Akt1 is a major mediator of skeletal muscle hypertrophy. Although it has been established that Akt plays a crucial role in muscle growth, the downstream targets involved in muscle hypertrophy remain to be defined.

mTOR-S6K and the control of protein synthesis

Two major downstream branches of the Akt pathway, which are relevant to muscle hypertrophy, are the mTOR pathway, which is activated by Akt, and glycogen synthase kinase 3β (GSK3β), which is blocked by Akt; both of them control protein synthesis. A third downstream target of Akt is FoxO pathway, which controls protein degradation and will be discussed below in the atrophy section. GSK3β is inhibited by Akt and in turn blocks the eukaryotic initiation factor 2B (eIF2B), which is involved in protein synthesis. Expression of a dominant negative kinase inactive form of GSK3β induces a dramatic hypertrophy in skeletal myotubes (75). However, it remains to be proven in vivo whether inhibiting the negative action of GSK3β on eIF2β is sufficient to promote muscle growth.

mTOR. The kinase mTOR (mammalian target of rapamycin) has recently emerged as a key regulator of cell growth that integrates signals from growth factors, nutrients, and energy status to control protein synthesis and other cell functions (32, 97). As the name implies, mTOR is selectively inhibited by rapamycin, a drug used as an immuno-suppressant in organ transplantation: rapamycin binds to members of the FK binding protein (FKBP) family and the complex rapamycin/FKBP binds to mTOR and blocks its activity. The role of mTOR in muscle growth was demonstrated by in vivo studies showing that rapamycin blocks overload hypertrophy and regenerating muscle growth (10, 72). Indeed in tetracycline-inducible Akt transgenic mice, rapamycin completely blunts Akt effects on muscle growth (37). The activation of mTOR by Akt is indirect and involves the phosphorylation and inhibition by Akt of tuberous sclerosis 2 (TSC2). TSC2 is a GTPase activating protein (GAP) that functions together with TSC1 to inactivate the small GTPase, PTEN; both of them control protein synthesis. A third downstream target of Akt is FoxO pathway, which controls protein degradation and will be discussed below in the atrophy section. GSK3β is inhibited by Akt and in turn blocks the eukaryotic initiation factor 2B (eIF2B), which is involved in protein synthesis. Expression of a dominant negative kinase inactive form of GSK3β induces a dramatic hypertrophy in skeletal myotubes (75). However, it remains to be proven in vivo whether inhibiting the negative action of GSK3β on eIF2β is sufficient to promote muscle growth.

mTOR-S6K and the control of protein synthesis

Two major downstream branches of the Akt pathway, which are relevant to muscle hypertrophy, are the mTOR pathway, which is activated by Akt, and glycogen synthase kinase 3β (GSK3β), which is blocked by Akt; both of them control protein synthesis. A third downstream target of Akt is FoxO pathway, which controls protein degradation and will be discussed below in the atrophy section. GSK3β is inhibited by Akt and in turn blocks the eukaryotic initiation factor 2B (eIF2B), which is involved in protein synthesis. Expression of a dominant negative kinase inactive form of GSK3β induces a dramatic hypertrophy in skeletal myotubes (75). However, it remains to be proven in vivo whether inhibiting the negative action of GSK3β on eIF2β is sufficient to promote muscle growth.

mTOR. The kinase mTOR (mammalian target of rapamycin) has recently emerged as a key regulator of cell growth that integrates signals from growth factors, nutrients, and energy status to control protein synthesis and other cell functions (32, 97). As the name implies, mTOR is selectively inhibited by rapamycin, a drug used as an immuno-suppressant in organ transplantation: rapamycin binds to members of the FK binding protein (FKBP) family and the complex rapamycin/FKBP binds to mTOR and blocks its activity. The role of mTOR in muscle growth was demonstrated by in vivo studies showing that rapamycin blocks overload hypertrophy and regenerating muscle growth (10, 72). Indeed in tetracycline-inducible Akt transgenic mice, rapamycin completely blunts Akt effects on muscle growth (37). The activation of mTOR by Akt is indirect and involves the phosphorylation and inhibition by Akt of tuberous sclerosis 2 (TSC2). TSC2 is a GTPase activating protein (GAP) that functions together with TSC1 to inactivate the small G

FIGURE 1. Schematic description of spectrum of developmental stages and the different contribution of cellular turnover and protein turnover to muscle growth.
protein Rheb that in turn activates mTORC1 in complex with the raptor adapter protein (mTOR-raptor or TORC1). Transgenic mice overexpressing TSC1 specifically in skeletal muscle show a defect in muscle growth (103).

**S6K1.** mTOR is part of two multiprotein complexes: mTORC1, which contains raptor and is rapamycin sensitive, is required for signaling to S6K and 4E-BP1; whereas mTORC2, which contains rictor, is required for signaling to Akt-FoxO (FIGURE 2). The effect of mTOR on the translation machinery and protein synthesis is mediated by TORC1-dependent phosphorylation of the ribosomal protein S6 kinases (S6K1 and 2) and of 4E-BP1, a repressor of the cap-binding protein eIF4E. S6K1 appears to be an important effector of the Akt pathway, since muscle fibers are smaller in S6K1-null mice, and their hypertrophic response to IGF1 and to activated Akt is blunted (71). However S6K1 knockout mice show no impairment in polysome formation, in protein synthesis, and in protein degradation (60). TORC1 complex also negatively regulates the IGF1 pathway via S6K1 (4, 100). Thus the two mTOR complexes, mTORC1 and mTORC2, may have opposite effects on Akt activity: TORC1 negatively regulates IGF1 signaling, whereas TORC2 enhances Akt activity (FIGURE 2). An additional factor that complicates the pathway is that long-term rapamycin treatment in vitro can inhibit not only TORC1 but also the mTORC2 complex and thus potentially affect, in some cell types, the Akt-FoxO signaling. This raises the possibility that, under certain circumstances, rapamycin may induce transcriptional regulation not through a direct effect, e.g., by blocking mTOR phosphorylation of downstream targets, but through an indirect transcriptional effect by inhibiting Akt and thus derepressing FoxO. It is therefore suggested that FoxO activity/localization should always be monitored in studies involving rapamycin treatment (44).

**Myostatin and the cellular turnover**

Myostatin, a member of the TGF-β family, is expressed and secreted predominantly by skeletal muscle and functions as a negative regulator of muscle growth. Mutations of the myostatin gene lead to a hypertrophic phenotype in mice, sheep, and cattle, and a loss-of-function mutation in the human myostatin gene was also found to induce increased muscle mass (14, 47, 59, 87). The increase in muscle mass is a consequence of hyperplasia, which is an increase in cell number, and hypertrophy, which is an increase in cell size. The hyperplasia suggests an activation of muscle stem cells, and, in fact, the myostatin pathway influences Pax 7, MyoD, and myogenin expression inhibiting satellite cell activation and differentiation (36, 57, 58). Only a few studies explore the effect of myostatin inhibition in adult muscle. Treating 24-wk-old mice with an anti-myostatin antibody for 5 wk induces a 12% increase in muscle mass (109). Furthermore, when tamoxifen-inducible Cre recombinase expressing mice were crossed with a myostatin null line, mutation of myostatin only in skeletal muscle (Skel-Null) mice increased muscle mass by nearly 30% (108). The increase in muscle mass is primarily the result of increased muscle cell size, and the increase in muscle cell size is due to increases in protein synthesis and inactivation of the myostatin pathway, which decreases muscle cell differentiation.

**Beta adrenergic**

Among the hormones that regulate muscle hypertrophy, the acute elevations of free norepinephrine, mediated by the β-adrenergic system, are of particular interest, first, as the most powerful system to influence skeletal muscle hypertrophy in vivo and, second, as the only system that is not influenced by dietary protein intake. Furthermore, the sympathetic nervous system appears to be a key regulator of muscle mass. As such, it is a powerful variable that can be modified in the setting of therapeutic interventions (50). In the skeletal muscle, the activation of the β-adrenergic system may induce myostatin mRNA expression, which may contribute to muscle mass loss.

**Conclusion**

Thus, the activation of the myostatin pathway may contribute to the loss of muscle mass and function with aging. However, the role of myostatin in muscle mass regulation is far from clear, and future studies should explore the role of myostatin in muscle mass regulation in more detail.
muscle growth. The amount of muscle mass and size increases as a consequence of the accumulation of satellite cells, which are thought to be responsible for muscle growth (46). There are few studies and conflicting results on the effect of exercise on muscle mass (14, 47, 59). Only a few studies have shown that exercise can increase muscle mass, the specific muscle force has not been measured in these two studies. Overexpression of follistatin, an inhibitor of myostatin (47), promotes a great increase in muscle mass. Interestingly, mice resulting from follistatin transgenic and myostatin knockout mice show tremendous increases in muscle mass supporting the concept that other myostatin-like molecules are present and relevant for muscle growth (46). There are few studies and conflicting results on the effect of exercise on myostatin gene expression in skeletal muscle (15). Furthermore, the increase of muscle mass of myostatin-null mice does not correlate with an increase in muscle force (7). Ultrastructural observations reveal an accumulation of tubular aggregates in type IIB fibers. This evidence is also supported by the decrease in specific force generation of hypertrophic ski transgenic mice (12).

Beta adrenergic and mechanical sensors

Beta-agonists such as clenbuterol, acting through β2-adrenoreceptors, are known to cause muscle hypertrophy and a slow-to-fast fiber-type switch. Interestingly, some effects of catecholamines could be mediated by local production of IGF-I and IGF-II by skeletal muscle (8, 9). Indeed the growth effect of beta-agonists is, at least partially, mediated by AKT-mTOR pathway since rapamycin almost completely blocks the hypertrophic effects of clenbuterol (40). Activation of beta receptors is known to also increase intracellular cAMP levels and activates protein kinase A (PKA), which may also activate the AKT pathway, and the transcription factor CREB; however, this pathway has not been explored in contracting muscle. An attractive emerging concept in muscle biology is that signals dependent on muscle activity, and specifically on mechanical load, may arise in the sarcomere, the basic unit of the contractile machinery of striated muscles, and from there transmitted to the nucleus to affect gene expression (43). The giant elastic protein titin, which spans half the sarcomere extending from the Z disk to the M band and interacts with a large number of muscle proteins, provides an exciting example of a sarcomeric activity-dependent signaling complex (signalosome) (42). A unique property of titin is the presence in the M-band region of a serine/threonine kinase domain that can be induced to acquire an open active conformation by stretch and contraction (28). In active muscle cells, the titin kinase domain (TK) is linked through two zinc-finger scaffolding proteins, nbr1 and p62, to a member of the muscle-specific RING-finger proteins, MURF2 (43). In the absence of mechanical activity, the signalosome is dissociated, and MURF2 translocates to the nucleus where it can interact with the serum response transcription factor SRF, leading to nuclear export of SRF and loss of SRF-dependent gene expression. This pathway may thus control muscle growth because SRF is known to regulate muscle gene expression, and conditional deletion of the SRF gene causes severe skeletal muscle hypoplasia during the perinatal period (51). SRF regulates muscle gene expression by binding serum response elements (SRE) in target genes and seems to integrate different growth promoting pathways: for example, SRF is a target of Akt signaling in Hela cells (107) and can recruit the androgen receptor to muscle gene promoters (105). The titin kinase-SRF pathway described above is probably just one of several links between the sarcomere and the nucleus that are only now beginning to emerge (43).

Muscle Atrophy

Atrophy is a decrease in cell size mainly caused by loss of organelles, cytoplasm, and proteins. This concept is important when a genetic approach is used to dissect the contribution of certain pathways to muscle loss. As mentioned above, the mass of a tissue is controlled by cellular turnover and protein turnover (FIGURES 1 AND 2). Genetic modifications that interfere with embryonic and postnatal growth result in smaller muscles in adults. This reduction in muscle size is caused by failure/inhibition of growth and not by a real atrophy process. Keeping in mind the above definition, another important concept is that protein turnover is dominant over cellular turnover during acute phases of muscle wasting when sarcomeric proteins are rapidly lost such as during fasting, disease, and denervation. Evidence that blocking satellite cells is sufficient to trigger muscle atrophy in adult muscle has never been determined. Furthermore, myonuclei are normally reduced during muscle atrophy to keep rather constant the size of the nuclear domain (16, 22). Thus the current understanding suggests that inhibiting cellular turnover would not influence protein...
breakdown and muscle weakness and would not affect
nuclear domain maintenance, but it might be impor-
tant for the replacement of damaged myofibers or
myonuclei. The importance of cellular turnover for
long-term muscle atrophy, e.g., long-term denerva-
tion, has yet to be addressed.

Atrophy: an active process that requires transcrip-
tional regulation
A major contribution in understanding muscle atrophy
comes from the pioneering studies on gene expression
profiling performed independently by groups of
Goldberg and Glass (9, 26). The idea to compare gene
expression in different models of muscle atrophy leads
to the identification of a subset of genes that are com-
monly up- or downregulated in atrophy-promoting
muscles. Since all the diseases used for the experiments
of microarray (i.e., diabetes, cancer cachexia, chronic
renal failure, fasting, and denervation) have muscle
atrophy in common, the commonly up or down genes
are believed to regulate the loss of muscle components
and are called atrophy-related genes or atrogens (76).
Together, these findings indicate that muscle atrophy is
an active process controlled by specific signaling path-
ways and transcriptional programs. Furthermore, the
two most induced genes are two novel muscle-specific
ubiquitin ligases, atrogin-1/MAFbx and MuRF1, that
are upregulated in different models of muscle atrophy
and are responsible for the increased protein degrada-
tion through the ubiquitin-proteasome system (9, 26).
In fact, knockout mice for either atrogin-1/MAFbx or
MuRF1 are partially resistant to denervation atrophy (9).
Importantly, recent findings suggest that myocardial
heavy chains are ubiquitinated and degraded by
MuRF1 (13, 23). Thus, up to now, these two genes are
actually the best markers for muscle atrophy and could
be considered as master genes for muscle wasting.
However, several other genes among the atrophy-relat-
ed genes are of potential interest, including genes
coding for lysosomal protease, transcription factors,
regulators of protein synthesis, and enzymes of meta-
bolism, and therefore their specific role in muscle wasting
has to be defined in the near future.

IGF1-AKT-FoxO signaling
A subsequent crucial step was the identification of the
signaling pathways that regulate the expression of the
two muscle-specific ubiquitin ligases. Previous studies
have shown that IGF1/insulin signaling, while pro-
moting muscle growth, is able to suppress protein
breakdown (77). Furthermore, IGFl transgenic mice
are resistant to muscle atrophy induced either by
angiotensin treatment or in a mouse model of cardiac
cachexia (88, 91), and local IGFl injection is sufficient
to block muscle atrophy (93). In these models of mus-
cle loss, IGFl completely suppressed the induction of
the two critical ubiquitin-ligases. Further data sup-
porting the role of this pathway in regulating muscle
atrophy come from experiments of Akt transfection in
adult mice. Electroporation of constitutively active Akt
in adult myofibers completely blocked muscle atrophy
induced by denervation (14). These findings are
important to elaborate the various contributions of the
different signaling pathways during muscle atrophy.

The upregulation of atrogin-1/MAFbx and MuRF1 is
normally blocked by Akt functioning through negative
regulation of the FoxO family of transcription factors
(49, 82, 93). The FoxO family in skeletal muscle is com-
prised of three isoforms: FoxO1, FoxO3, and FoxO4.
Akt phosphorylates FoxO1 and FoxO3, but FoxO1 is
able to suppress protein breakdown and muscle atro-
phy when transfected in skeletal muscles in vivo (82).
Accordingly, FoxO1 transgenic mice showed markedly
reduced muscle mass and fiber atrophy, futh-
er supporting the notion that FoxO is sufficient to
promote muscle loss (39, 92) (FIGURE 2). On the
other hand, the knockdown of FoxO expression by
RNAi is able to block the upregulation of atrogin-
1/MAFbx expression and muscle atrophy when transfected in skeletal muscles in vivo (82). Thus, when AKT in active protein breakdown is sup-
pressed but when FoxO is induced, protein synthesis is
further suppressed. This is not trivial since FoxO activ-
ity is regulated by different posttranslational modifica-
tions, which include phosphorylation, acetylation,
and mono- and poly-ubiquitination (34) (FIGURE 3).
Most of these regulatory mechanisms are AKT inde-
dependent and may play a role in muscle atrophy
induced by oxidative or energy stress (see below).

Inflammatory cytokines and NF-κB signaling
The NF-κB transcription factors, which play a major
role as mediators of immunity and inflammation, are
also expressed in skeletal muscle and appear to medi-
ate the effect of inflammatory cytokines, in particular
TNF-α, on muscle wasting and cachexia. In the inac-
active state, NF-κB is sequestered in the cytoplasm by
a family of inhibitory proteins called IκB. In response
to TNF-α, the IκB kinase (IKK) complex phosphorylates
IκB, resulting in its ubiquitination and proteasomal
degradation; this leads to nuclear translocation of
NF-κB and activation of NF-κB-mediated gene transcrip-
tion. Muscle-specific overexpression of IKKε in trans-
genic mice leads to severe muscle wasting
mediated, at least in part, by the ubiquitin-ligase
MuRF1, but not by FoxO (94).

On the other
hand, AKT is unable to
block disuse atrophy (93). In these models of mus-
cle atrophy, particularly reduced under the
inflammatory and suppressive
action of TNF-α. Therefore, AKT is considered as
an active process controlled by specific signaling path-
ways and transcriptional programs. Furthermore, the
proteasomal degradation of FoxOs from the nucleus to the cytoplasm. As predict-
ed, the reduction in the activity of the Akt pathway
observed in different models of muscle atrophy results
in decreased levels of phosphorylated FoxO in the
cytoplasm and a marked increase of nuclear FoxO pro-
tein. The translocation and activity of FoxO members
is required for the upregulation of atrogin-1/MAFbx
and MuRF1, and FoxO3 was found to be sufficient to
promote atrogin-1/MAFbx expression and muscle
atrophy when transfected in skeletal muscles in vivo
(82).

The upregulation of atrogin-1/MAFbx and MuRF1 is
downregulated in atrophy-related genes or atrogens (76).
Interestingly, our results show hyperphosphoryla-
tion of NF-κB, which seems to affect the expres-
several other pathways
and that the
thus, the other pathway
and myostatin, we
seems to affect the
muscle atrophy relative
mechanism of
(58). In fact, the
PKB-AKT pathway is
increased expression
between the two
genesis of fibrosis whose
atrogin-1 (58)
abrogates physio-
Downloaded from http://physiologyonline.org by 10.209.164.14 on September 14, 2017
Muscle atrophy and inflammation are two major negative factors that contribute to muscle wasting. In this regard, Akt pathway activity is essential to maintain muscle mass and function. Akt pathway activity is perturbed in conditions such as muscle atrophy, where Akt inhibition can significantly contribute to muscle atrophy. This concept is supported by results obtained with conditional knockout mice for IKKβ, which are resistant to muscle atrophy but show hyperphosphorylation of Akt (66). Thus, the amount by which muscle atrophy is reduced by IKKβ ablation and the degree to which it is Akt dependent is unclear. The cross talk between the two pathways is nevertheless important, and future studies should establish the contribution of IKK-NF-κB pathways versus Akt-FoxO pathways in relation to muscle atrophy. Interestingly, modulation of NF-κB signaling may affect the severity of muscle wasting in vivo. However, in muscle cell cultures, Akt inhibition can substantially contribute to muscle atrophy, further confirming the importance of Akt in muscle wasting (2). On the expression by myostatin of atrogin-1 and muscle loss in vivo, myostatin expression is controlled by FoxO1, supporting the notion that the myostatin pathway is synergistic with FoxO pathways (58). In fact, myostatin treatment blocks the IGF1-pathway and activates FoxO1, allowing the increased expression of atrogin-1 (58). Similarly, in cardiac cells, myostatin augments phenylephrine hypertrophic effects through inhibition of Akt (64). A further level of complexity is that myostatin expression is controlled by FoxO1, supporting the notion that the myostatin pathway is synergistic to Akt-FoxO signaling (5). However, despite the evidence of cross talk between Akt and TGF-β pathways, it is still unclear whether Smads, the transcription factors downstream of TGF-β signaling, are mediating some of the myostatin effects in muscle. Furthermore, Smads can recognize the DNA sequence CAGAC, but their affinity seems too low to support unsustained binding to DNA. In addition, if their affinity for the simple sequence were higher, Smads would decorate the entire chromosome. Therefore, activated Smads proteins must associate with different DNA-binding cofactors for the recognition and regulation of specific target genes (54). Interestingly, members of the forkhead box O family (FoxO1, FoxO3, and FoxO4) play such a role (27). To further confuse the role of Smads during muscle wasting is the presence of TGFβ, an inhibitor of Smads, among the upregulated atrophy-related genes (76). Thus the mystery of why an inhibitor of Smads is activated early, before muscle loss becomes evident, must be addressed. Dissecting the roles of Smads and identifying the co-factors that mediate Smad transcriptional activity in adult muscle is intriguing questions that require attention in the coming years.

**Myostatin and the enigma of the downstream targets**

Despite the hypertrophic effect of myostatin inhibition, the opposite situation of myostatin activation and its role in triggering muscle atrophy is not obvious. The first report that sustains this notion described a severe atrophy when CHO cells, engineered to express myostatin, were injected in skeletal muscles (113). The atrophy in these mice was so severe that some animals died. However, these findings were not confirmed by transgenic mice. Expressing myostatin specifically in skeletal muscles leads to only 20% of atrophy in males and no phenotype in females (74). Electroporation experiments show that myostatin expression in adult muscle induces a degree of atrophy comparable to that observed in transgenic mice (21). These findings suggest that CHO cells substantially contribute to muscle atrophy by secreting some cachectic factors and that the myostatin pathway is synergistic with other pathways. No report clearly shows whether myostatin is sufficient to trigger atrogene expression and protein breakdown in vivo. However, in muscle cell culture, myostatin is reported to upregulate the critical atrophy-related ubiquitin ligases, and interestingly the mechanism of such regulation is FoxO dependent (58). In fact, myostatin treatment blocks the IGF1-PI3K-AKT pathway and activates FoxO1, allowing the increased expression of atrogin-1 (58). Similarly, in cardiac cells, myostatin augments phenylephrine hypertrophic effects through inhibition of Akt (64). A further level of complexity is that myostatin expression is controlled by FoxO1, supporting the notion that the myostatin pathway is synergistic to Akt-FoxO signaling (5). However, despite the evidence of cross talk between Akt and TGF-β pathways, it is still unclear whether Smads, the transcription factors downstream of TGF-β signaling, are mediating some of the myostatin effects in muscle. Furthermore, Smads can recognize the DNA sequence CAGAC, but their affinity seems too low to support unsustained binding to DNA. In addition, if their affinity for the simple sequence were higher, Smads would decorate the entire chromosome. Therefore, activated Smads proteins must associate with different DNA-binding cofactors for the recognition and regulation of specific target genes (54). Interestingly, members of the forkhead box O family (FoxO1, FoxO3, and FoxO4) play such a role (27). To further confuse the role of Smads during muscle wasting is the presence of TGFβ, an inhibitor of Smads, among the upregulated atrophy-related genes (76). Thus the mystery of why an inhibitor of Smads is activated early, before muscle loss becomes evident, must be addressed. Dissecting the roles of Smads and identifying the co-factors that mediate Smad transcriptional activity in adult muscle is intriguing questions that require attention in the coming years.

**Rediscovering lysosomes under a new light: autophagy-mediated protein breakdown**

Lysosomes are the cellular system charged with the removal of organelles and protein aggregates. Indeed, autophagy is constitutively active in skeletal muscle, as shown by the accumulation of autophagosomes seen in human myopathies caused by a genetic deficiency of lysosomal proteins, e.g., Pompe’s and Danon’s disease, or by pharmacological inhibition of lysosomal function, as in chloroquine myopathy (89). Different studies have shown that cathepsin-L, a lysosomal protease, is upregulated in different models of muscle wasting (18, 45). The role of cathepsin-L could not be ignored.

**FIGURE 3.** Scheme illustrating the regulation of FoxO transcription factors and their potential role in the atrophy program. FoxO is activated by growth factors and by physical activity and phosphorylates FoxO, reducing its translocation from the nucleus to the cytoplasm. Conversely, AMPK phosphorylates FoxO, increasing its transcriptional activity. Various other activators of FoxO are also indicated, including oxidative stress, which induces acetylation of peculiar lysine residues, and monoubiquitination. Polyubiquitination occurs when FoxO is relocalized in the cytoplasm triggering its proteasomal degradation.
induction is still unclear, but recent evidence suggests that the autophagy-lysosome system is activated during atrophy. Mizushima et al. (62) generated transgenic mice expressing LC3 fused with GFP. LC3 is the mammalian homolog of Atg8 gene and is critical for membrane commitment and growth to engulf organelles, cytoplasm, glycogen, and protein aggregates. Morphological analyses documented the activation of the autophagy system during fasting in skeletal muscle (62). Indeed, muscle cell culture confirmed that the autophagy-lysosome system is the major protein degradation pathway implicated in nutrient-dependent proteolysis (63). Further experiments lend insight into the signaling pathways involved and identified an mTOR-independent but PI3KIII-beclin-dependent proteolysis.

Further studies have shown that autophagy is activated also in denervation atrophy (24, 86). However, denervation-induced atrophy shows a slower pace of autophagy when compared with fasted-meditated atrophy. This effect is mediated by Runx1, which is upregulated during denervation and is required to preserve muscle mass. Lack of Runx1 resulted in myofibrillar disorganization and excessive autophagy in denervated muscles (105). Runx1 knockout mice show double- or triple-head myotubes (64). Moreover, with atheros, the master regulatory gene for mitochondria biogenesis, is downregulated in different models of muscle wasting. Furthermore, when the levels of PGC-1α are maintained, either by use of transgenic mice or by transfecting adult muscle fibers, expression of such genes is not sufficient and required to activate lysosomal-dependent protein breakdown in cell culture and in vivo. Interestingly, the role of mTOR signaling for the regulation of autophagy is irrelevant in skeletal muscles. However, the contribution of the autophagy-lysosome and ubiquitin-proteasome systems to organelle remodeling, protein breakdown, and, finally, muscle atrophy remains to be investigated.

**Mitochondrial homeostasis and energy balance: the metabolic control of cell size**

Several metabolic adaptations occur in atrophying muscles. In many forms of muscle wasting, the metabolic control of cell size is lost: the metabolic control of cell size is lost. Moreover, the contribution of the autophagy-lysosome and ubiquitin-proteasome systems to organelle remodeling, protein breakdown, and, finally, muscle atrophy remains to be investigated.

**Mechanical adaptations**

The dystrophic state of the muscle fibers is associated with a reduction in muscle mass caused by both mechanical (atrophy) and microvascular (hypertrophy) alterations. The dystrophic muscle fiber is characterized by myofibrillar disorganization, cell shrinkage, and increased metabolic demand. These changes are mediated by several factors, including hyperglycemia, hyperinsulinemia, and hyperlipidemia. The dystrophic state of the muscle fibers is associated with a reduction in muscle mass caused by both mechanical (atrophy) and microvascular (hypertrophy) alterations. The dystrophic muscle fiber is characterized by myofibrillar disorganization, cell shrinkage, and increased metabolic demand. These changes are mediated by several factors, including hyperglycemia, hyperinsulinemia, and hyperlipidemia.

**Regulation of autophagy**

The regulation of autophagy is complex and involves multiple signaling pathways. The mTOR pathway is a major regulator of autophagy in response to amino acid availability and energy status. Activation of mTOR leads to the inhibition of autophagy and the promotion of protein synthesis. On the other hand, starvation and caloric restriction activate the AMPK pathway, which stimulates autophagy and inhibits mTOR activity. Other factors, such as growth factors, cytokines, and hormones, can also modulate autophagy through the mTOR and AMPK pathways.

**Autophagy inhibitors**

Several pharmacological agents can inhibit autophagy and promote muscle atrophy. These include rapamycin, which selectively inhibits mTOR signaling, and 3-methyladenine, which blocks the elongation of autophagosomes. Other autophagy inhibitors include chloroquine, which blocks the lysosomal degradation of autophagosomes, and bafilomycin A1, which inhibits the activity of the vacuolar proton pump.

**Autophagy activators**

Several compounds can activate autophagy and promote muscle regeneration. These include starvation, calorie restriction, and exercise. The exposing to other stressors, such as cold, heat, and hypoxia, can also stimulate autophagy. Additionally, several drugs, such as metformin and rapamycin, have been shown to activate autophagy in muscle cells.

**Conclusion**

The regulation of autophagy is complex and involves multiple signaling pathways. The mTOR pathway is a major regulator of autophagy in response to amino acid availability and energy status. Activation of mTOR leads to the inhibition of autophagy and the promotion of protein synthesis. On the other hand, starvation and caloric restriction activate the AMPK pathway, which stimulates autophagy and inhibits mTOR activity. Other factors, such as growth factors, cytokines, and hormones, can also modulate autophagy through the mTOR and AMPK pathways.
muscles are protected from the atrophy induced by denervation, fasting, or expression of FoxO3 (81). Treatment with statins induces FoxO3-mediated atrogin-1 activation and related muscle alterations. In cell culture and in zebrafish, statins cause muscle atrophy, disorganization of myofibrils, and mitochondrial dysfunction (31). These changes are completely aborted by either the deletion of atrogin-1 or by PGC-1α overexpression. These results may explain why oxidative fibers tend to be resistant to atrophy compared with glycolytic fibers and suggest that metabolic changes are crucial for muscle atrophy (50). Among the different energy-dependent pathways, AMPK acts as the cellular sensor of energy balance. Indeed, stressing energy in muscle cell culture either by oligomycin treatment or by replacing glucose with 2-deoxyglucose triggers myotube atrophy (4). Interestingly, increased levels of AMP, activation of AMPK, lipid consumption, and smaller muscles have all been observed in S6K1 and S6K2 knockout mice. Importantly, AMPK inhibition rescues skeletal muscle growth in these mice, confirming that S6K1/2 deletion alters energy balance (4) and suggesting that AMPK activity is a key factor for myotube size. Interestingly, these mice show normal muscle formation and polyomomal profiles as well as autophagy and upregulation of atrogin-1/MAFbx and MuRF1 (60). Altogether, these findings suggest a connection between AMPK and FoxO3. AMPK phosphorylates several AKT-independent sites of FoxO3, stimulating its transcriptional activity on target genes (29, 30). Indeed, treating muscle cell culture with AICAR, an activator of AMPK, causes an increase of protein breakdown and atrogin-1/MAFbx expression via the FoxO3 family (70). The physiological relevance of such important control over protein breakdown and muscle atrophy in vivo remains to be explored.

Within the myotube, mitochondria are crucial in regulating metabolism and might be a potential source of catabolic signals. Insulin resistance and diabetes have been reported to trigger caspase-mediated actin cleavage, which would release myofibrils from the sarcomere for subsequent degradation via the proteasome (20). Caspase-3 activation is mediated by Bax, which disrupts the mitochondrial external membrane, causing cytochrome-c release and apoptosis formation (49). Furthermore, inhibition of caspases by XIAP overexpression protects adult skeletal muscle from atrophy in an animal model of diabetes (106). However, the contribution of caspases to muscle atrophy should be further investigated using loss of function approaches.

**Mechanical sensor of inactivity**

The dystrophin glycoprotein complex (DGC) anchors the muscle cytoskeleton to the cell membrane via dystrophin and its binding partners. This complex is important for correct transduction of myosin movements on actin to the extracellular matrix. Indeed, lack of dystrophin exacerbates mechanical stress on the myotube plasma membrane, causing muscle damage and degeneration. This classical view considers a purely structural function of DGC proteins. However, an attractive role of dystrophin in transduction of mechanical signals to the nucleus has recently been proposed. In fact, dystrophin is lost from the cell membrane under atrophic conditions, causing a loss of continuity between the cell membrane of the myotube and the extracellular matrix (1). This decreased interaction between membrane and matrix is required for muscle loss since forced dystrophin expression in transgenic mice counteracts both cachexia-induced muscle wasting and upregulation of atrogin-1/MAFbx and MuRF1. Further support for the DGC acting as a mechanical sensor has recently been published. Neuronal NOS (nNOS) is normally bound to the DGC, but when the DGC is disrupted, as occurs in dystrophic muscle, its localization is disturbed and it is no longer at the sarcolemma. A similar nNOS dysregulation has recently been described during disuse-induced muscle atrophy. Free nNOS dissociates to the cytoplasm where it enhances FoxO3-mediated transcription and upregulation of atrogin-1 and MuRF1 (95). Interestingly, the NF-κB pathway is not involved in nNOS-mediated muscle atrophy (95). Since nNOS generates NO, an important player in protein nitrosylation and in reactive oxygen species generation, the role of oxidative stress in muscle atrophy is an important aspect to be analyzed in future studies.

**Glucocorticoids-induced muscle atrophy**

Glucocorticoids are elevated in many pathological conditions associated with muscle loss. Moreover, glucocorticoid treatment induces atrogin-1 and MuRF1 expression and muscle wasting both in cell culture and in vivo [10, 13, 77, 82, 84]. Importantly, adrenalectomy or treatment with a glucocorticoid receptor antagonist (RU-486) attenuates muscle loss in some diseases (84). However, the mechanisms of glucocorticoid-mediated muscle atrophy are unclear. In fact, none of the atrophy-related genes have been found to be directly regulated by glucocorticoids, and no glucocorticoid response elements on atrogenes promoters have been identified as critical for their expression (45). Thus most of the glucocorticoid effects are indirect, mainly affecting pathways previously described to be crucial for muscle growth or loss. Glucocorticoids are reported to decrease IGF1 production and increase myostatin secretion. Other effects like downregulation of the anabolic transcription factor ATF4, activation of p380/ERK, or upregulation of REDD1, an inhibitor of mTOR signaling, are described, but no obvious mechanism can explain the
direct involvement of corticosteroids in regulation of atrophy-related genes (3, 84, 104, 110). Even less clear is the recent description of dexamethasone-dependent Mst3 and Mst1 degradation by Nlrp, terminal ubiquitination pathway, especially if we consider that Myod is described to be an atrogin-1 substrate (94, 98). How Myod loss can contribute to weakness and muscle atrophy remains unclear. Loss and gain of function experiments on glucocorticoid receptor should be performed to address the direct role of this pathway in muscle loss.

Conclusions

Over the last few years, the mechanisms controlling muscle loss have attracted the attention of the scientific community due to their relevance in various fields of study such as aging, prognosis of many diseases, quality of life, and sports medicine. From the recent findings, a new scenario emerges that considers the size of the myofiber and muscle performance as a result of not a single pathway but of a network of signaling (FIGURES 2 AND 4). Intriguingly, the different pathways cross talk and modulate one another at different levels, coordinating protein synthesis and degradation simultaneously. Findings of the last few years offer new and exciting perspectives to the field and introduce a series of new stimulating questions to the community, setting the base for future studies, which should enable us to identify new therapeutic targets and drugs.

The critical reading of Kenneth Dyar is gratefully acknowledged. I apologize to colleagues whose studies were not cited owing to space limitations.
involves increased muscle mass, down-regulated type I (slow twitch) myosin heavy chain, and increased type II (fast twitch) myosin heavy chain expression, suggesting a crucial regulatory role of dystrophic muscles in the skeletal muscle proteostatic network.}


