Protein Kinase B/ Akt: A Nexus of Growth Factor and Cytokine Signaling in Determining Muscle Mass

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Although genetics fundamentally determines the boundaries of skeletal muscle size this dynamic tissue also demonstrates great plasticity in response to environmental and hormonal factors. Recent work indicates that contractile activity, nutrients, growth factors and cytokines all contribute to determining muscle mass. Muscle responds not only to endocrine hormones but also to the autocrine production of growth factors and cytokines. Skeletal muscle synthesizes anabolic growth factors such as insulin-like growth factor (IGF)-I and potentially inhibitory cytokines such as interleukin (IL)-6, tumor necrosis factor (TNF)-α, and myostatin. These self-regulating inputs in turn influence muscle metabolism including the utilization of nutrients such as glucose and amino acids. These changes are principally achieved by altering the activity of the protein kinase known as protein kinase B or Akt. Akt plays a central role in integrating anabolic and catabolic responses by transducing growth factor and cytokine signals via changes in the phosphorylation of its numerous substrates. Activation of Akt stimulates muscle hypertrophy and antagonizes the loss of muscle protein. Here we review the many signals that funnel through Akt to alter muscle mass.
INTRODUCTION

A combination of genetic and environmental factors determine skeletal muscle size (65, 86). As individuals, we are greatly influenced not only by our kin (i.e. inherited genes) but by our surroundings. This includes our access to food, the quality of our diet, and the ability of our bodies to optimally utilize ingested nutrients. Food intake and utilization are tightly controlled by hormones and growth factors and these same factors often act synergistically with nutrients to enlarge muscle mass (4). In contrast, exposure to trauma, environmental toxins, and pathogens all have the potential to negatively impact muscle mass either directly or secondarily through altered secretion of hormones and cytokines (33).

The above environmental and hormonal cues impinge on muscle at the cellular level by forcing individual muscle fibers to either make or destroy protein. Anabolic factors such as nutrients, growth hormones, anabolic steroids, and muscle contraction in due course increase protein synthesis and inhibit protein degradation. In contrast, factors such as malnutrition, stress hormones, cytokines, and inactivity induce an atrophic phenotype (110). These same factors may also expand or contract the pool of stem cells in muscle by either stimulating satellite cell replication or augmenting apoptosis, respectively (12, 97).

Growth factor receptors, nutrients and even muscle contraction all increase the activity of the protein kinase known as protein kinase B (PKB) or Akt (87, 90, 108). Akt is a serine(S)/threonine(T) kinase that signals via a wortmannin-sensitive pathway downstream of growth factor receptors by
activating phosphoinositide-3 (PI3) kinase. Akt is activated by phospholipid binding to the plextrin-homology domain which induces a conformational change allowing for phosphorylation of T308 by phosphoinositide-dependent kinase (PDK)-1. Akt is autophosphorylated on T72 and S246 and mutation of these sites dramatically inhibits the ability of insulin-like growth factor (IGF)-I to stimulate kinase activity (67). An additional site in the carboxy terminus (serine 473) is phosphorylated by the mammalian target of rapamycin (mTOR) and may provide substrate selectivity to the kinase (47).

Akt has the ability to phosphorylate and change the activity of many critical metabolic targets. Akt stimulates glucose uptake, glycogen synthesis, and protein synthesis by phosphorylating Akt substrate 160, glycogen synthase kinase-3\(\alpha/\beta\), and the tuberous sclerosis (TSC)-2 proteins in that order (18, 50, 54). Akt also inhibits apoptosis and protein degradation in skeletal muscle by promoting the phosphorylation and inactivation of the pro-apoptotic protein Bad and FoxO transcription factors, respectively. Phosphorylation of FoxO by Akt alters its subcellular localization and prevents the expression of FoxO-dependent atrophy related genes such as atrogin-1 and muscle ring-finger (MuRF)-1 (95, 98).

Akt is therefore situated at a critical juncture in muscle signaling where it responds to diverse anabolic and catabolic stimuli. Akt has the dual potential to drive the formation of new protein while preventing the proteolytic degradation of existing protein. As a result, Akt is a key metabolic control point in a number of diseases which are characterized by changes in muscle mass (15, 55, 85, 87,
Hence, because of its central role the purpose of this review is to summarize the molecular mechanisms by which Akt bi-directionally regulates signaling in muscle in response to growth factors, cytokines, and muscle contraction. Furthermore, the review will emphasize the factors which play a critical role in mediating muscle wasting in catabolic conditions.

AKT SHARES ITS METABOLIC MISSION

Three separate genes code for Akt mRNAs and these mRNAs are translated into three distinct Akt proteins designated Akt-1, -2, and -3. Akt1 and -2 are highly expressed in insulin-sensitive tissues whereas the abundance of Akt3 is low in skeletal muscle (71). Constitutive expression of Akt1 in L6 myoblasts promotes glucose and amino acid transport into the cell as well as protein synthesis (42). In contrast, overexpression of Akt2, but not Akt1, in C2C12 cells stimulates this cell line to differentiate into myotubes suggesting that both isoforms are necessary for attaining optimal muscle mass (100).

Mice lacking Akt1 show only a subtle decrease in muscle size compared to their wild-type littermates whereas mice that lack Akt2 exhibit a diabetic phenotype (14, 37). Although whole body glucose disposal was impaired in Akt2 null mice insulin-stimulated glucose uptake was reduced in the EDL but not the soleus muscle and therefore displayed fiber type selective insulin resistance (15). The data are consistent with muscle glycogen being reduced in type IIa but not type I fibers from patients with diabetes mellitus and the concept that the intracellular concentration of glucose in type II fibers is regulated predominantly
at the level of glucose transport whereas in the soleus it is regulated predominantly at the level of glucose phosphorylation (43, 44). A similar fiber type selective loss of signaling downstream of Akt occurs in sepsis where the gastrocnemius but not the soleus exhibits reduced mTOR activity (59). Double knockout mice display multiple developmental defects including severe muscle atrophy implying both isoforms of Akt contribute to the determination of muscle size.

Intramuscular injection of an adenovirus carrying constitutively active Akt1 induced myofiber hypertrophy in the gastrocnemius which was associated with a greater recruitment of new blood vessels and perfusion of the muscle (103). Lai also demonstrated that expression of constitutively active Akt from a tamoxifen-inducible promoter increased the weight of the quadriceps by 73% in just 14 days (55). In addition these mice exhibited a 2- to 3-fold increase in the number of fibers in the tibialis anterior and gastrocnemius with cross-sectional areas greater than those of wild-type muscle. Because expression of Akt was induced postnataly rapid hypertrophy appears not to be due to changes in muscle development in-utero and therefore Akt may be amenable to pharmacological manipulation in disease states. Akt activation in this model also increased the phosphorylation of S6K1 on T421/S424 suggesting muscle hypertrophy results from downstream activation of the mTOR/S6K1 pathway and an increase in muscle protein synthesis. Later studies by Latres et al also showed that Akt phosphorylates FoxO transcription factors to inhibit the expression of atrophy-
related genes such as atrogin-1 and MuRF-1 and therefore Akt exerts its influence on both sides of the muscle protein balance equation (64, 92).

**PI3K SIGNALING REGULATES MUSCLE MASS VIA AKT**

One would predict that loss of PI3K, the upstream signal activating Akt in skeletal muscle, would significantly impair growth and alter the insulin responsiveness of this tissue. As the total loss of PI3K is embryonic lethal, Luo generated mice lacking the p85α/β regulatory subunits of PI3K specifically in skeletal muscle. As expected, the knockout mice exhibited a 30% decrease in the weight of the gastrocnemius and a dramatic decrease in the cross-sectional area of muscle fibers (69). Elimination of the p85 subunit was also associated with a reduction in the phosphorylation of Akt, ribosomal protein S6 and 4EBP-1 in fed mice consistent with a decrease in translation initiation in these animals. The mRNAs for the atrophy-related ubiquitin ligases atrogin-1 and MuRF-1 were not increased in the knockout mice relative to their fasted wild-type littermates making it unlikely that the decreased muscle size is due to an atrogene-induced muscle atrophy in the PI3K null mice.

The PI3 kinase/Akt signaling pathway is also negatively regulated by the phosphatase and tensin homolog deleted on chromosome 10 (PTEN). PTEN attenuates PI3K signaling by dephosphorylating the phosphatidylinositol 3,4,5-trisphosphate generated by PI3K. PTEN increases in rat muscle as they age and this has been proposed as one mechanism by which the protein synthetic response to insulin is blunted in more mature animals (101). Indeed muscle
specific deletion of PTEN protected mice from high fat diet-induced diabetes but the deletion of PTEN did not significantly alter the weight of either the soleus or EDL muscle (112). In addition, neither the basal level of Akt phosphorylation or insulin-stimulated Akt phosphorylation was enhanced by the loss of PTEN in skeletal muscle. These data suggest muscle may have a redundant system to compensate for the loss of PTEN but not for the loss of PI3K.

**AKT LOCALIZATION DETERMINES ITS SUBSTRATES AND ACTIVITY**

The sub-cellular localization of Akt greatly influences its ability to stimulate different pathways. For example, constitutively active Akt was sufficient to increase the phosphorylation of 4EBP-1 whereas S6K1 phosphorylation required Akt to not only be catalytically active but also to be targeted to the cell membrane (26). Akt also binds to a scaffold protein called periplakin and overexpression of the carboxy-terminal portion of periplakin localizes Akt to intermediate filaments while excluding Akt from the nucleus (107). Nuclear exclusion of Akt ameliorates its ability to phosphorylate FoxO proteins while concomitantly inhibiting FoxO-mediated transcription of atrophy associated genes.

Nuclear bodies first described in promyelocytic leukemia and referred to as Pml bodies, have also been shown to inactivate nuclear Akt and prevent FoxO3 phosphorylation. Pml brings protein phosphatase 2a (PP2a) subunits and Akt in close proximity in nuclear bodies to dephosphorylate T308 thereby inactivating the enzyme (104). Borgotti et al reported Akt binds strongly to the nuclear matrix protein nucleolin and PP2A. PP2A binding was strongest in nuclei
with weak Akt activity (7). Dephosphorylation of Akt via PP2a would be expected to yet again activate FoxO3-dependent genes such as atrogin-1 and MuRF-1 as well as the pro-apoptotic protein Bim (106).

In a similar series of studies Bernardi found Pml also binds to and favors the nuclear accumulation of mTOR (5). The physical interaction between mTOR and Pml in the nucleus negatively regulates the association of mTOR with the small GTPase Rheb and its ability to phosphorylate downstream substrates. Rheb is normally distributed to membranous structures in the cytoplasm with characteristics of the Golgi apparatus (9). Failure of mTOR and Rheb to attach to these membrane structures impedes Rheb signaling to S6K1. Thus Pml may orchestrate the loss of muscle mass at multiple levels including the inhibition of protein synthesis, enhanced ubiquitin ligase-dependent protein degradation, and the enhanced expression of the pro-apoptotic protein Bim that is normally repressed by Akt.

Although not specifically demonstrated in skeletal muscle, Akt also translocates from the cytosol to mitochondria in cardiac muscle. Activation of mitochondrial ATP-sensitive potassium channels with diazoxide provides cardioprotection and triggers the binding of Akt to mitochondria in a PI3K-dependent manner (1). Akt may therefore play an important role in energy and protein balance in skeletal muscle as outlined below.
AKT ENERGIZES SKELETAL MUSCLE WITH ATP

Recently Akt has unexpectedly been shown to regulate cellular ATP content. Addition of serum to control cells nearly doubles ATP levels whereas Akt1/2 double knockout cells have reduced ATP and a marked increase in the AMP/ATP ratio (41). Because protein synthesis is a highly ATP demanding process eukaryotic organisms have developed exquisitely sensitive mechanisms to conserve ATP and restrain translation. ATP depletion increases AMP-activated protein kinase (AMPK) activity and attenuates the ability of activated Akt and growth factors to stimulate mTOR activity (41). These data are consistent with studies demonstrating AMPK activation with 5-aminoimidazole-4-carboxamide 1-beta-d-ribonucleoside decreases muscle protein synthesis by blocking mTOR activity (6, 113). Akt therefore functions at two levels, first it phosphorylates TSC2 on T1462 to inactivate the TSC1/2 complex and secondly Akt raises ATP levels to inhibit AMPK and relieve the negative effect of AMPK on mTOR.

TSC1 and -2 appear to play a critical role in regulating basal mTOR activity in skeletal muscle because over expression of TSC-1 leads to a reduction in muscle fiber cross-sectional area. Expression of the TSC1 transgene mediates its affect by stabilizing and increasing the level of TSC2. Despite the negative influence of excess TSC1/TSC2 on muscle mass TSC1/TSC2 does not antagonize insulin-stimulated mTOR signaling (111). Therefore, pharmacological levels of insulin and/or IGF-I would be expected to restore muscle mass in pathophysiological conditions characterized by an excess of the TSC1/TSC2
complex. An exception to this rule may occur in patients that are treated chronically with the immunosuppressant rapamycin for 6 mo. Insulin fails to stimulate Akt phosphorylation on S473 under these conditions while increasing basal IRS-1 activation (24).

As noted above mTOR not only phosphorylates down stream substrates such as S6K1 and 4EBP-1 but also phosphorylates the upstream kinase Akt on S473. As a result mTOR participates in a positive feedback loop for the preservation of mTOR activity. The TORC2 complex binds to Rictor as well as a protein known as stress activated protein kinase interacting protein (Sin)-1 (51). Knockdown of Sin1 in Drosophila and mammalian cells decreases Akt activity in vitro and Jacinto found Akt to be only singly phosphorylated on T308 in Sin1-negative cells (93, 116). These studies defined mTOR as the kinase responsible for S473- Akt phosphorylation. Although Akt retained only minimal activity in Sin1 negative cells it continued to normally phosphorylate substrates such as GSK3 and TSC2. Moreover, phosphorylation of S6K1 and 4EBP-1 were normal. In contrast to most Akt substrates, Sin1 deficiency strongly inhibited the phosphorylation of FoxO3a at T24/T32 whereas S256 phosphorylation was normal. These data define an explicit linkage between mTOR, Akt, and FoxO3-dependent genes that does not affect other Akt substrates. Insufficient Sin1 may therefore accelerate muscle atrophy as activated FoxO3a upregulates the genetic program for atrophy-related atrogenes in skeletal muscle (91).

The mammalian target of rapamycin is also a classical nutrient sensor that responds to excursions in plasma amino acid levels. The amino acid leucine in
particular strongly stimulates mTOR both *in vitro* and *in vivo*. In L6 muscle cells leucine stimulated mTOR activation is PI3 kinase-dependent but Akt-independent whereas *in vivo* leucine fails to stimulate Akt phosphorylation on T308 in skeletal muscle (58, 83). Amino acids such as leucine may stimulate mTOR activity by promoting the binding of other proteins such as the Rheb GTPase to mTOR and its substrates although these functions still remain largely unknown (3, 53).

Akt can also directly affect energy metabolism by binding to outer membrane sites on mitochondria. Here Akt interacts with the mitochondrial hexokinase and cooperates with the voltage-dependent anion channel (VDAC) and the inner mitochondrial membrane adenine nucleotide translocator. These two membrane complexes are critical for the passage of metabolites and adenine nucleotides in and out of the mitochondria. Hexokinase inhibitors such as 3-bromopyruvate inhibit mitochondrial adenine nucleotide transport (36). Majewski has demonstrated activated Akt increases the association of hexokinase with mitochondria to facilitate energy homeostasis and prevent the release of cytochrome C and apoptosis (70). Akt is also important in insulin-mediated maintenance of mitochondrial oxidative phosphorylation enzymes, mitochondriogenesis, and maximizing the generation of ATP (80).

Like Akt the mTOR-raptor complex can also be purified in the mitochondrial fraction implying the mTOR pathway may mediate and/or facilitate some of the affects of Akt on energy production. Treatment of cells with rapamycin disrupts the interaction of mTOR and raptor and lowers mitochondrial membrane potential and their capacity to resynthesize ATP (94). Interestingly,
rapamycin does not alter protein expression in mitochondria but has a dramatic affect on the mobility (and potential phosphorylation) of mitochondrial proteins including pyruvate decarboxylase and VDAC (94). Given the functional ability of mTOR to assist in the selection of Akt substrates it will be interesting to know whether the phosphorylation of mitochondrial proteins is altered by Akt, mTOR or both kinases.

ATP and Akt may also have other positive affects on skeletal muscle. ATP stimulates mouse embryonic stem cell proliferation and this can be inhibited by the PI3K inhibitor wortmannin (46). It is possible that Akt-dependent elevations in ATP may expand the pool of satellite cells in skeletal muscle and aid in the restoration of muscle mass after injury. Therefore, Akt, mTOR, and the FoxO transcription factors may also affect the genesis of new muscle (48).

**AKT MEDIATES THE POSITIVE EFFECTS OF IGF-I IN MUSCLE**

Growth hormone and IGF-I are potent regulators of muscle mass as evidenced by the dramatic enlargement of muscle in transgenic mice overexpressing these proteins (16, 78, 79). In general this increase is characterized by a hypertrophic response due to cell volume expansion in existing myocytes similar to that described above for Akt overexpression (55). Indeed cells from Akt1/2 double knockout mice are resistant to the stimulatory effect of serum growth factors on Akt and 4EBP-1 phosphorylation and conversely dominant negative forms of Akt negate the positive effects of IGF-I on cell survival (81, 109).
It is likely that insulin receptor substrate (IRS)-1 transmits the insulin/IGF-I anabolic signal in skeletal muscle because IRS-1 deficient mice exhibit reduced insulin-stimulated Akt phosphorylation, S6K1 activity, and protein synthesis (89, 115). Huang demonstrated that IRS-1 signals to both Akt1 and -2 in myotubes whereas IRS2 only signals to Akt2. This selectivity in signaling translates into major differences in physiological endpoints where siRNA against IRS-1 but not IRS-2 decreased insulin-stimulated glucose uptake in muscle cells (49). Therefore, it is likely IRS-1 mediates the majority of signaling to Akt and mTOR to elicit muscle hypertrophy.

Classical IGF-I signaling via the above pathways may not be the sole means by which IGF-I increases muscle mass. In response to growth hormone, muscle damage, and muscle contraction IGF-I mRNA can also be alternatively spliced to generate a protein referred to as mechano-growth factor (MGF) (40). Exon 5 of MGF codes for an E-domain which precludes MGF from binding to IGF binding proteins and it has been speculated that MGF may be more potent than IGF-I derived from the circulation (40). Yang and Goldspink observed that MGF expands the pool of satellite/stem cells and whereas an IGF-I receptor antibody could block the affects of recombinant IGF-I the antibody could not block the affect of MGF. These data suggest MGF may interact with a unique receptor in muscle where it stimulates the proliferation and migration of myogenic precursor cells to restore muscle mass (73). Although the findings on MGF are provocative more progress needs to be made in identifying the MGF receptor and how it integrates with known signaling pathways.
Malnutrition, critical illness, and sepsis are all associated with a dramatic reduction in the overall pool of IGF-I contributed from the circulation as an endocrine hormone as well as autocrine IGF-I produce by the muscle itself. The fall in muscle IGF-I content in these conditions is associated with muscle atrophy. Consequently, growth hormone has been used clinically to increase lean body mass in patients with muscle wasting (8, 32). The development of growth hormone resistance in disease states has limited the use of growth hormone clinically whereas the risk of hypoglycemia has restricted the use of IGF-I (61). IGF-I complexed to insulin-like growth factor binding protein-3 (IPLEX<sup>TM</sup>, Insmed Therapeutic Proteins, Boulder, CO) which is now approved for the treatment of children with primary IGF-I deficiency, reverses muscle wasting in animal models of sepsis and alcoholic myopathy with little evidence of side-effects (60, 102). The IGF-I/IGF binding protein-3 complex has also been reported to be more efficacious than IGF-I alone at stimulating Akt phosphorylation in non-obese diabetic mice suggesting this complex may be useful in both growth hormone and insulin resistant conditions (13).

Although the effects of sepsis, critical illness, and endotoxin on the systemic growth hormone/IGF axis are profound we have focused our attention on the local synthesis of IGF-I in skeletal muscle <i>per se</i>. The rationale for this decision was threefold: 1) growth hormone stimulates IGF-I expression not only in the liver but also in muscle (105), 2) specific deletion of the IGF-I gene from the liver decreases circulating IGF-I levels by 80% but does not decrease muscle size (114), and 3) the local production of IGF-I is likely to contribute substantially
to the biologically active pool of IGF-I in the local milieu of the myofiber as evidenced by mice that specifically overexpress IGF-I in skeletal muscle (78).

Lipopolysaccharide (LPS) a Toll-like receptor-4 ligand, tumor necrosis factor (TNF)α, and interleukin (IL)-1 all decrease the local content of IGF-I in skeletal muscle (28, 29, 56). The decrease in IGF-I peptide is paralleled by a concomitant decrease in IGF-I mRNA. Furthermore, the LPS-induced decrease in muscle IGF-I mRNA only occurs in mice with a functional LPS receptor. Intraperitoneal injection of LPS into Toll-like receptor-4 signaling deficient C3H/HeJ mice maintained muscle IGF-I mRNA levels similar to those seen in wild-type mice injected with saline (31). The decrease in skeletal muscle IGF-I expression after LPS is likely mediated by cytokines such as TNFα and IL-1β because LPS failed to decrease IGF-I mRNA in skeletal muscle of rats that were pretreated with either a TNF neutralizing antibody or an IL-1 receptor antagonist (56).

Dehoux et al found that fasting and diabetes increased the expression of atrogin-1 in skeletal muscle and that normalization of plasma IGF-I levels blunted its expression (20). Yet, these investigators recently found that although IGF-I could inhibit the induction of atrogin-1 by a mixture of TNFα and interferon (IFN)-γ that IGF-I could not protect C2C12 myotubes from a TNFα/IFNγ-induced loss of myosin heavy chain, MyoD, and a decrease in myotube diameter (21). Therefore, muscle atrophy can occur independently of atrogin-1 expression. It should also be noted that although atrogin-1 mRNA is elevated in a number of
studies very few investigations have reported whether there is a concomitant rise in atrogin-1 protein.

NEGATIVE AFFECTS OF CYTOKINES AND NFκB ACTIVATION ON MUSCLE PROTEIN

Muscle damage due to eccentric exercise induces TNFα and this cytokine dramatically impairs insulin stimulation of IRS-1, PI3-kinase, and Akt (22). TNFα is thought to produce insulin resistance in skeletal muscle by activation of the inhibitor of kappa B kinase (IKK). In this regard, IKK phosphorylates IRS-1 on S307 and thereby precludes subsequent tyrosine phosphorylation of IRS-1 and activation of PI3 kinase and Akt (19, 25). In agreement with this hypothesis, targeted ablation of IKK2 increased skeletal muscle strength and protected against atrophy by increasing signaling by means of protein synthetic pathways and decreasing activation of protein degradative pathways (77). Deletion of IKK2 dramatically upregulated the phosphorylation of Akt and S6K1 and this was maintained even after muscle denervation suggesting inhibition of this kinase provides protection from muscle atrophy. IKK2 deletion also blunted the rise in MuRF-1 but not atrogin-1 mRNA in response to denervation.

NFκB plays an essential role mediating muscle atrophy produced by unloading. Judge et al found overexpression of an IKBα super-repressor in the soleus inhibited unloading-induced atrophy by 40%. The super-repressor also blunted the expression of atrophy related genes (52).

We and others have found that sepsis, LPS, inflammatory cytokines as well as the over production of glucocorticoids all increase the expression of the
muscle atrophy genes atrogin-1 and MuRF-1 (34). Although sepsis increases both atrogenes only atrogin-1 expression is sustained up to 72 h. In addition, only atrogin-1 expression was suppressed in septic rats treated with a binary complex of IGF-I and IGF binding protein-3 a combination we have previously shown to reverse sepsis-induced changes in the weight and protein content of the gastrocnemius and the efficiency of protein to be synthesized from a given amount of RNA (102). It is particularly noteworthy that the IGF-I/IGF binding protein-3 complex was efficacious when given after the initial septic insult a time point at which anti-cytokine therapies have failed (74).

The most direct evidence for a role of NFκB in the etiology of muscle wasting comes from studies of transgenic mice engineered to express a constitutively active IKK2 exclusively in skeletal muscle. IKK2 overexpressing mice displayed greater activation of NFκB than control animals and severe muscle wasting was confirmed by a 50% decrease in fiber diameter (10). This resulted in a 75% decrease in the maximal force generated by single muscle fibers from the IKK overexpressing mice. Because the wasting phenotype was blocked by cross breeding these mice with mice overexpressing the IκB super-repressor it is highly likely the wasting phenotype is due to NFκB activation and not activation of other pathways.

Muscle from the IKK over expressing mice released 2.5-fold more tyrosine than wild-type muscle when incubated ex vivo suggesting that the muscle wasting is largely due to muscle protein breakdown. At least 50% of the change in muscle mass could be attributed to NFκB activation of the ubiquitin
ligase MuRF-1 because mating the IKK2 overexpressing mice and MuRF-1 knockout mice generated offspring in which the IKK-induced drop in muscle mass was ameliorated (10). The above findings are consistent with the ability of salicylate (an IKK inhibitor) to block atrophy in IKK overexpressing mice and an IKK2 inhibitor to ameliorate muscle wasting in a murine model of human acquired immune deficiency syndrome (45).

Sandri et al have recently shown the transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator (PGC)-1α protects skeletal muscle from atrophy due to denervation and fasting (91). PGC-1α strongly inhibited the expression of atrogin-1, MuRF-1 and cathepsin L consistent with its ability to prevent a loss in muscle fiber cross-sectional area and mass. PGC-1α was also a strong inhibitor of both a FoxO reporter plasmid as well as the atrogin-1 promoter in vivo. Muscle PGC-1α is downregulated in diabetic and uremic mice as well as mice with cancer cachexia and this response may explain the propensity of these animals to lose muscle mass (91). Loss of PGC-1α may also explain the muscle atrophy in mice overexpressing IKK because compounds which stimulate NFκB (e.g. LPS and palmitate) decrease PGC-1α (17, 84). Yet, rats injected with LPS exhibit a rapid but transient increase (not decrease) in PGC-1 mRNA (118). Collectively, these data suggest the ability of NFκB to decrease PGC-1α mRNA and induce muscle wasting is dependent on a more sustained activation of NFκB as would be delivered by constitutively active IKK or a protracted septic insult.
In contrast to the findings of Sandri, Miura et al found that overexpression of PGC-1α increased the oxidative capacity of skeletal muscle by increasing mitochondrial biogenesis in 13-15 week old mice (75). Although mitochondria from PGC-1α overexpressing mice exhibited a 2-3 fold increase in oxygen consumption respiration was uncoupled from ATP synthesis and ATP content was markedly decreased in PGC-1α transgenic mice (76). When PGC-1α overexpressing mice were followed for longer periods of time (25 weeks) the mice developed selective atrophy of the quadriceps and gastrocnemius (fast glycolytic type-2B fibers) but not in the soleus and these muscles exhibited increased infiltration of adipocytes (76). The dramatically different conclusion that can be reached by both studies may be due to differences in the age at which the mice were studied, differences in the promoters used to drive PGC-1α expression (muscle creatine kinase vs the human alpha-skeletal actin), or even the particular strain that was chosen for the studies. In the original work of Lin, for example, strain 23 expressed very high levels of PGC-1α and showed distinct weight loss (68).

Southgate et al have also provided evidence that PGC-1α is downregulated by Akt-mediated phosphorylation of FoxO1 in insulin-stimulated skeletal muscle. The PGC-1α promoter contains FoxO1 binding sites and insulin-stimulated phosphorylation of Akt and phosphorylation of FoxO1 restricts the transcription factor to the cytoplasm limiting PGC-1α promoter activity. This accounts for the negative regulation of PGC-1α mRNA by insulin in control subjects. In contrast in type II diabetics insulin failed to phosphorylate Akt and
FoxO1 as efficiently and did not diminish PGC-1α mRNA levels (96). Akt2 null mice were also insulin resistant at the level of FoxO1 phosphorylation and insulin failed to repress PGC-1α mRNA expression. It is not known whether the loss of adipose tissue described in Akt2 null mice is redistributed to skeletal muscle or if these changes contribute to muscle wasting (37, 96).

Interestingly, endurance training induces a fast-to-slow twitch phenotype which can be mimicked by low frequency stimulation of isolated rat muscles. Atherton et al demonstrated that low frequency stimulation increases PGC-1α in the soleus (2). These authors proposed that low frequency stimulation increases the AMP/ATP ratio in skeletal muscle and as a result activates AMPK. Indeed, Lee demonstrated that activation of AMPK with 5-aminimidazole-4-carboxamide 1-beta-d-ribonucleoside increased PGC-1α in C2C12 cells in vitro and C57BL/J6 mice in vivo (66). Endurance exercise may therefore mediate its anti-inflammatory and anti-atrophy effects by many routes including upregulating PGC-1α in muscle, reduced NFκB binding to DNA, downregulation of Toll-like receptors, and enhanced release of IL-6 resulting in inhibition of TNF production (27, 39, 82). It should also be noted that although muscle contraction limits atrophy this occurs despite elevated circulating concentrations of glucocorticoids which would normally be expected to promote protein breakdown. Atherton’s studies suggest short bursts of exercise and high frequency electrical stimulation compliment the above mentioned effects by robustly increasing Akt phosphorylation, markers of translation initiation, and protein synthesis in skeletal muscle (2).
MUSCLE CYTOKINES AND MYOSTATIN NEGATIVELY REGULATE AKT

Accumulating evidence from rodent studies suggests LPS and sepsis alter translation initiation in skeletal muscle by disrupting mTOR signaling (57, 58). Recently we examined the affects of LPS and IFNγ on mTOR signaling in C2C12 skeletal muscle cells in culture. The combination of LPS and IFNγ decreased the autophosphorylation of mTOR and its substrates S6K1 and 4EBP-1. A comparable reduction in the phosphorylation of ribosomal protein S6 and protein synthesis was also observed suggesting S6K1 activity is decreased in the presence of LPS and IFNγ. LPS alone could not in and by itself diminish mTOR signaling or protein synthesis in this cell type but it did induce the expression of IL-6 and nitric oxide synthase (NOS)-2 (35). A combination of LPS and IFNγ is necessary to produce the decline in mTOR phosphorylation and protein synthesis. We speculate that the dependency of this decrease on IFNγ may be due to the ability of this cytokine to dramatically enhance the magnitude and duration of LPS-stimulated NOS2 expression by stabilizing NOS2 mRNA (23). Hence, the elevated levels of NO expressed for a protracted period of time causes a greater damage to regulatory proteins in growth signaling pathways via nitrosylation. Indeed we observed that NOS inhibitors prevented both the LPS/IFNγ-induced decrease in protein synthesis and changes in mTOR signaling (Frost and Lang, submitted).

Akt is a confirmed target for NO action. NO donors rapidly inactivate Akt by S-nitrosylation and mutation of cysteine 224 to serine restores Akt activity (117). Although originally described as a mechanism of insulin resistance in
diabetes it is likely nitrosylation and inactivation of Akt decreases mTOR signaling in myotubes exposed to LPS and IFNγ. Indeed, LPS increases the nitrosylation of the insulin receptor beta subunit, IRS-1, and Akt in skeletal muscle of wild-type mice but not NOS2 negative mice. Consequently, NOS2 knockout mice fail to develop LPS-induced insulin resistance (11).

Myostatin, an endogenous inhibitor of muscle mass is also highly expressed in select models of muscle atrophy. Gillson delineated the importance of myostatin in glucocorticoid-induced atrophy by showing myostatin knockout mice fail to lose muscle mass in response to the synthetic glucocorticoid dexamethasone (38). The myostatin knockout mice are also resistant to dexamethasone-induced atrogin-1 and MuRF-1 expression suggesting one or more of the atrogenes mediate the negative affects of myostatin on muscle mass. Burn injury increased myostatin expression 4-fold and this was inhibited by RU486 (63). Notwithstanding, Lang et al found that the rise in atrogin-1 and MuRF-1 after thermal injury was glucocorticoid-independent but inhibitable by IGF-I (62). These data suggest burn injury may induce two signals to stimulate atrogene expression and muscle atrophy: one mediated by glucocorticoids and myostatin and a second signal which alters basal but not IGF-I responsive Akt activity towards FoxO-dependent genes (30, 99).

McFarlane et al recently demonstrated myostatin induces cachexia by activating the ubiquitin proteolytic system through a FoxO1-dependent mechanism (72). Addition of myostatin to C2C12 cells decreased the phosphorylation of Akt on S473 and FoxO1 on S256 consistent with reduced
mTOR signaling. The enhanced translocation of FoxO1 into the nucleus and the stimulation of FoxO1-inducible genes and muscle wasting by myostatin is consistent with the muscle wasting phenotype of FoxO1 overexpressing mice (72).

SUMMARY

A plethora of studies in recent years have eloquently examined the mechanisms by which muscle grows or atrophies. Indeed muscle is largely self-sufficient in its ability to respond to varied inputs. The protein kinase Akt has emerged as a key intermediary in the response of muscle to activity, nutrients growth factors, and cytokines (Figure 1). Akt sits at a nexus of intracellular signaling in which it phosphorylates substrates such as TSC2 to increase protein synthesis and FoxO transcription factors to inhibit protein degradation. Akt monitors and responds to the flow of energy in the cell via its ability to interact with mitochondria and AMPK. Cytokines dramatically decrease the magnitude of growth factor signals such as IGF-I and attenuate the ability of nutrients to signal via mTOR. The generation of excess cytokine-induced NO decreases translational signaling and protein synthesis in vitro. Myostatin also decreases Akt phosphorylation and signals through FoxO transcription factors to induce atrophy. Muscle contraction, via signaling through many of these same pathways provides beneficial anti-inflammatory affects which limit atrophy and provide a strong stimulus to increase muscle mass.
FIGURE LEGENDS

**Figure 1.** Numerous environmental and hormonal cues impinge on skeletal muscle to stimulate its growth or bring about its atrophy. These cues include positive inputs such as exercise, IGFs, and nutrients and negative inputs such as cytokines, glucocorticoids, and myostatin. Many of these signals are self-regulating in that they are made in an autocrine fashion by muscle itself. The protein kinase Akt acts at a nexus of intracellular signaling by translating these signals into changes in the phosphorylation and activity of other metabolically important enzymes (mTOR) and transcription factors (FoxO). In a simplistic account mTOR activates protein translation and protein synthesis via S6K1 and 4EBP-1 phosphorylation to stimulate hypertrophy. In contrast, a reduction in Akt signaling allows for the nuclear accumulation of FoxO transcription factors and the transcription of atrogenes such as atrogin-1 and other components of the proteasome to degrade muscle protein and stimulate atrophy. Cytokines dramatically reduce mTOR signaling via a NOS-dependent mechanism whereas myostatin signals through FoxO transcription factors. Reduced Akt signaling appears to be a hallmark of both of these responses.
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