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Muscle Atrophy in Chronic Kidney Disease Results from Abnormalities in Insulin Signaling

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Abstract

Muscle atrophy is a significant consequence of chronic kidney disease (CKD) that increases a patient’s risk of mortality and decreases their quality of life. The loss of lean body mass results, in part, from an increase in the rate of muscle protein degradation. In this review, the proteolytic systems that are activated during CKD and the key insulin signaling pathways that regulate the protein degradative processes are described.

Skeletal muscle is a dynamic organ that provides a rich source of amino acids and carbon chains that can be mobilized during stress or chronic pathological conditions like chronic renal failure (CKD), sepsis or diabetes. Estimates of protein turnover rates in skeletal muscle of healthy individuals indicate that nearly 1.5 kg muscle mass turns over daily (1). Given this extraordinary level of protein metabolism in skeletal muscle, one can easily envision how even a minor change in the rate of protein degradation without a reciprocal change in synthesis would have profound physiological effects over time in conditions like CKD.

There are numerous reports documenting the cachexia or loss of muscle mass that is frequently seen in patients with chronic kidney disease. Using experimental models of CKD, the causes of muscle atrophy have been extensively studied. May et al. found that CKD attenuated insulin-stimulated protein synthesis and increased protein degradation in skeletal muscle (2). These studies were subsequently extended by identifying the proteolytic pathways in muscle that are augmented during CKD. The major pathway that is up-regulated during CKD, as well as most other chronic diseases, is the ubiquitin-proteasome pathway. This multi-enzyme system involves the targeting of muscle proteins by a series of enzymatic reactions and the subsequent degradation by a large protein complex called the proteasome (Figure 1). Proteins are targeted for proteasomal degradation by the covalent attachment of polymeric chains of a 7-kDa protein, ubiquitin, to the ε-amino group of lysine residues in the substrate proteins. The key substrate recognition component in this process is a group of enzymes called E3 ubiquitin ligases which are the largest known family of functionally-related proteins (>300) in mammals. Once the substrate protein is “polyubiquitinated”, it is degraded by the proteasome, a large (2000 kDa)
multi-subunit complex found in all mammalian cells. The proteasome contains subunits that recognize the polyubiquitin chains while others utilize ATP in a process that uncoils the substrate proteins and inserts them into the core of the proteasome where they are cleaved to small peptides and amino acids by multiple proteolytic subunits. In muscle, when the atrophy process is initiated, genes encoding two muscle-specific E3 ligases, atrogin-1 (also known as MAFb) and muscle-specific ring finger-1 (MuRF1) as well as ubiquitin and a select group of proteasome subunits are upregulated (3). This process occurs uniquely in skeletal muscle cells.

Other proteolytic pathways are also upregulated during muscle atrophy. Lysosomal protein degradation, the other major pathway for protein degradation in most cells, was also found to be increased in some models of muscle atrophy (4). This pathway degrades endocytosed membrane proteins as well as intracellular proteins through a process called autophagy. A third important proteolytic pathway that is activated during muscle atrophy is caspase-3. Caspase-3 is generally thought of as an effector protease that participates in apoptosis. However, given the multinucleated nature of muscle fibers in vivo, it is unlikely that apoptosis occurs in the classical fashion. The cross-sectional area of muscle fibers get smaller during atrophy but the number of fibers does not decrease. So what does caspase-3 do? We found it cleaves actin present in myofibrillar complexes and in doing so, it generates a 14-kDa peptide fragment that serves as a biomarker of muscle atrophy (5;6). Examination of muscle samples from both CKD rats and hemodialysis patients revealed higher levels of this 14-kDa actin fragment, indicating that muscle wasting was actively occurring.

In CKD, two physiological signals have been identified that work in concert to activate protein degradation in skeletal muscle – glucocorticoids and metabolic acidosis (7–9). Studies by Bailey et al. revealed that the metabolic acidosis of CKD stimulates protein degradation indirectly (10). They examined the effects of metabolic acidosis on the intracellular muscle cell pH (pHi) and determined that the pHi is maintained during CKD or metabolic acidosis. It was concluded that an acidosis-related signal must regulate muscle protein metabolism. Subsequently, we utilized cultured muscle cells to delineate the roles of acidosis and glucocorticoids in the induction of protein degradation. As with animals, glucocorticoids and acidification acted in concert to stimulate muscle protein degradation (8). It is, however, important to note that glucocorticoids, when given in pharmacologic doses, suppress protein synthesis as well as augment the degradation of myofibrillar proteins independently of metabolic acidosis (11). This apparent interaction between acidosis and glucocorticoids suggested a common signaling mechanism of action. One possibility that we and others have examined is that CKD/metabolic acidosis/glucocorticoids work by affecting insulin signaling.

Insulin resistance and CKD are intimately associated. CKD increases the risks of developing diabetes and vice versa. Many studies have examined the effects of CKD on insulin/IGF-1 action and it has been well-established that its growth-promoting effects are impaired in skeletal muscle. Importantly, Ikizler and colleagues reported that insulin resistance is linked to accelerated muscle protein degradation in non-diabetic, hemodialysis patients, thus underscoring the role that insulin has in regulating protein turnover (12). To understand the causes of muscle atrophy in CKD, it is important to first review the relevant insulin signaling pathways.

Insulin (and IGF-1) modulate both protein synthesis and degradation in skeletal muscle but this review will focus on the regulation of protein degradation. The main pathway for insulin’s metabolic effects, including the suppression of protein degradation, is the Class I phosphatidylinositol 3-kinase (PI3K)/Akt pathway (Figure 2). Insulin binds to its receptor (IR) and activates an internal receptor tyrosine kinase activity. As a consequence, the receptor undergoes auto-phosphorylation which, in turn, provides a binding site for the insulin receptor substrate (IRS) proteins. In skeletal muscle cells, two major isoforms of the IRS proteins, IRS-1

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and IRS-2, are expressed and each independently recognizes and binds to phosphorylated tyrosine residues in the IR. Upon interaction with the receptor, they become substrates for the IR kinase activity and undergo phosphorylation on tyrosine residues. Class I PI3K is composed of a p85 regulatory and p110 catalytic subunits which form an active enzyme complex when the p85 subunit docks to the phosphorylated tyrosine residues in the IRS proteins. PI3K catalyzes the production of the lipid second messenger phosphatidyinositol (3,4,5)-triphosphate or PIP3 which then activates the serine kinase Akt. Akt serves as is a nexus or branch point for a variety of downstream signaling pathways. One target of Akt is the FOXO (e.g., FOXO1, FOXO3a) family of transcription factors which are important regulators of metabolic processes including protein degradation in skeletal muscle. Phosphorylation of the FOXOs by Akt prevents them from translocating to the nucleus. When activated (i.e., dephosphorylated), nuclear FOXOs increase the expression of a variety of genes including the atrogin-1 and MuRF1 E3 ligases which are tightly linked to the muscle atrophy process (13; 14).

A second major signaling system that is activated by the insulin receptor is the MEK/ERK mitogen-activated protein (MAP) kinase pathway. Generally speaking, this pathway regulates cell growth and survival functions rather than essential metabolic processes like glucose transport. Like the PI3K/Akt system, this pathway ultimately accomplishes many of its functions by modulating specific gene targets.

To characterize which insulin pathways are affected by CKD, we first examined whether acidosis affects PI3K/Akt signaling in the controlled environment of cultured L6 muscle cells (15). Reducing the pH of the culture media to pH 7.1 suppressed PI3K activity associated with IRS-1 and subsequent Akt phosphorylation. To confirm the role of the PI3K/Akt pathway in the regulation of protein degradation, we measured the rate of protein degradation in the presence and absence of insulin after adding the pharmacologic inhibitor of PI3K, LY294002 or infecting cells with an adenovirus encoding a dominant negative p85α PI3K subunit. Both treatments attenuated the suppression of protein degradation by insulin. Other studies using inhibitors of the proteasome confirmed that the proteolytic pathway that was suppressed by insulin was the ubiquitin-proteasome pathway.

We next examined PI3K/Akt signaling in the muscles of CKD rats to validate our findings with cultured muscle cells. Previous studies had shown that CRF had minimal effects on the binding properties of the insulin and IGF-1 receptors, leading to the conclusion that any CKD-associated defects in signaling must be post-receptor in nature (16;17). Therefore, we studied CKD rats (induced by 7/8th nephrectomy) that were rendered acidotic by feeding them a high protein diet (18). Insulin-stimulated tyrosine phosphorylation of IRS-1 tended to be lower in CKD rats but the reduction did not reach significance. Basal PI3K activity associated with IRS-1 was significantly decreased but the complex remained sensitive to a pharmacologic dose of insulin. In contrast to IRS-1, tyrosine phosphorylation of IRS-2 (the other major IRS protein in muscle) was increased as was IRS-2-associated PI3K activity. This increase in the functional status of IRS-2 was likely due to an increase in the amount of IRS-2 protein that was detected in muscle lysates by Western blot analysis. Importantly, the phosphorylation of Akt activity was suppressed in CRF rat muscle, suggesting that the reduction in IRS-1/PI3K/Akt signaling was a factor contributing to the muscle atrophy of CRF. To determine whether the acidosis of CRF was responsible for the impaired insulin signaling, a subset of CRF rats were given oral bicarbonate supplements to prevent them from becoming acidotic and the IRS-1-associated PI3K activity in muscle was compared to the activity in untreated CRF rats. Correction of acidosis improved the IRS-1/PI3K function just as it attenuated muscle protein degradation in earlier studies by Mitch and colleagues (2). Rabkin et al. subsequently reported a similar depression of basal IRS-1 signaling and Akt activity in CRF rat muscles. Moreover, they found that mechanically overloading the muscle to induce growth signals restored many aspects of
the defective insulin signaling and attenuated muscle loss. The conclusion from the cell and animal experiments combined is that CKD alters IRS-1 function, leading to reduced PI3K/Akt signaling. This in turn, results in FOXO-mediated transcription of atrogin-1 and MuRF1.

How do glucocorticoids contribute to the muscle wasting? It is well accepted that glucocorticoids antagonize insulin’s actions. One responsible mechanism seems to be that glucocorticoids alter PI3K activity by altering the stoichiometry of the enzymes subunits (19). These findings are relevant to the muscle atrophy due to CKD because the amount of p85 subunit in CKD rat muscle was nearly double that of the controls whereas the p110 catalytic subunit was unaffected (18). We found that this response could be mimicked in cultured muscle cells by treatment with dexamethasone for 24 h. Moreover, overexpression of the p85 PI3K subunit in the absence of exogenous was sufficient to increase the degradation actin by caspase-3, a finding consistent with a reduction in PI3K/Akt signaling. Recently, Mitch and colleagues reported that activated glucocorticoid receptors can directly interact with the PI3K p85 subunit, thereby preventing PI3K from docking to IRS-1 and becoming fully activated (20). Thus, the mechanism by which glucocorticoids antagonize insulin/PI3K/Akt signaling appears to be multifactorial. The net result is activation of the FOXOs and induction of atrogin-1 and MuRF1.

Glucocorticoids also support protein degradation by inducing expression of the ubiquitin gene (UbC). In this case, the mechanism involves activation of the MEK/ERK signaling pathway and the transcription factor, Sp1 (21). At first thought, this finding seems paradoxical because insulin simultaneously stimulates both PI3K/Akt and MEK/ERK signaling acutely. However, we found that either chronic dexamethsone treatment (>24 h) or overexpression of a p85 subunit resulted in activation of the MEK/ERK pathway. These data suggested that inhibition of PI3K signaling produces a reciprocal activation of the parallel MEK/ERK pathway by a mechanism that is currently under investigation. However, it is notable that glucocorticoids induce ubiquitin and atrogin-1/MuRF1 expression and both responses occur coordinately and specifically in skeletal muscle cells (22). This is consistent with the selective manner in which protein degradation is increased in skeletal muscle but not other cell types.

In conclusion, metabolic acidosis and increased glucocorticoid production are two CKD-associated signals that act in concert to cause insulin resistance in muscle. The diminished ability of insulin to repress protein degradation is a major contributing factor causing muscle atrophy. Alterations in multiple insulin/IGF-1 signaling pathways lead to the activation of transcription factors that regulate the expression of key atrogenes which encode components of the ubiquitin-proteasome and other proteolytic systems. The result is a higher rate of protein degradation and loss of muscle mass in patients with CKD and other chronic illnesses linked to insulin resistance.

References


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Proteins destined for degradation by the proteasome are first tagged by the addition of a polymeric chain of ubiquitin protein molecules. This process requires energy (i.e., ATP) and involves an E1 ubiquitin-activating enzyme, a member of the E2 ubiquitin-conjugating enzyme family, and a member of the E3 ubiquitin protein ligase family. The substrate protein-polyubiquitin chain conjugate is recognized by subunits of the proteasome. Other subunits of the proteasome exhibit chaperone activity which utilize ATP to unfold the substrate protein and insert the peptide chain into the core of the proteasome where proteolytic subunits cleave the substrate to small peptide and amino acids (AA).
Figure 2. Insulin Signaling Pathways
Shown are the two major insulin signaling pathways. In muscle, the phosphatidylinositol 3-kinase (PI3K)/Akt pathway controls many metabolic responses including glucose transport and protein degradation. Activated insulin receptors phosphorylate the insulin receptor substrate (IRS) proteins. The PI3K p85 regulatory subunit binds to the phosphorylated IRS protein and recruits the PI3K p110 catalytic subunit. The PI3K complex forms phosphatidylinositol (3,4,5) triphosphate which then activates Akt. Akt serves as a nexus enzyme that phosphorylates a variety of downstream effectors. The activated insulin receptor also stimulates a parallel signaling cascade of kinases called the MEK/ERK pathway. Pathway signaling is initiated by the IRS proteins. It ultimately controls many cellular processes involved in cell growth and survival.