Review of muscle wasting associated with chronic kidney disease\textsuperscript{1–3}

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ABSTRACT

Muscle wasting increases the morbidity and mortality associated with chronic kidney disease (CKD) and has been attributed to malnutrition. In most patients, this is an incorrect diagnosis because simply feeding more protein aggravates uremia. Instead, there are complex mechanisms that stimulate loss of skeletal muscle, involving activation of mediators that stimulate the ATP-dependent ubiquitin-proteasome system (UPS). Identified mediators of muscle protein breakdown include inflammation, metabolic acidosis, angiotensin II, and neural and hormonal factors that cause defects in insulin/insulin-like growth factor I (IGF-I) intracellular signaling processes. Abnormalities in insulin/IGF-I signaling activate muscle protein degradation in the UPS and caspase-3, a protease that disrupts the complex structure of muscle proteins to provide substrates for the UPS. During the cleavage of muscle proteins, caspase-3 leaves behind a characteristic 14-kD actin fragment in the insoluble fraction of muscle, and characterization of this fragment identifies the presence of muscle catabolism. Thus, it could become a marker of excessive muscle wasting, providing a method for early detection of muscle wasting. Another consequence of activation of caspase-3 in muscle is stimulation of the activity of the proteasome, which increases the degradation of muscle proteins. Treatment strategies for blocking muscle wasting include correction of metabolic acidosis, which can suppress muscle protein losses in patients with CKD who are or are not being treated by dialysis. Correcting acidosis also improves bone metabolism in CKD and hence should be a goal of therapy. Exercise training is a potentially beneficial approach, but side effects in women have not been adequately tested. Although insulin resistance occurs early in the course of CKD, there are no effective means of correcting it. Consequently, new therapies that can safely suppress muscle wasting are needed. Am J Clin Nutr 2010;91(suppl):1128S–32S.

MUSCLE WASTING IN CHRONIC KIDNEY DISEASE

Muscle wasting complicates common clinical syndromes such as diabetes, chronic kidney disease (CKD), trauma, burns, and immobilization due to spinal cord injury or stroke (1–4). This is a devastating complication because it not only promotes a sedentary lifestyle, leading to decreased quality of life, but also jeopardizes cardiovascular health by increasing morbidity and mortality (5, 6). The presence of muscle wasting in patients with CKD is especially relevant because CKD is a significant public health problem, with >20,000,000 Americans afflicted with CKD and a rising prevalence expected in ensuing decades (7). Observational reports suggest that muscle wasting in patients with CKD is progressive (8, 9), and hence it contributes to spiraling health care costs. Fortunately, a considerable amount has been learned about muscle protein homeostasis since Schoenheimer's discovery in the 1930s that skeletal muscle is in a state of continual turnover (10). Recently, certain mechanisms that regulate muscle turnover have been characterized and methods for its early detection and treatment are being studied. Still, there is much to be learned to enable us to design therapies that will safely suppress muscle wasting.

Skeletal muscle turnover varies depending on age, sex, and specific factors that affect protein synthesis and breakdown. In a normal adult, \( 3.5–4.5 \) g protein/kg body weight are synthesized and degraded each day; the majority of these proteins are intracellular proteins (11). Although there is no storage form of protein per se, when catabolic conditions are present, skeletal muscle is degraded at an accelerated rate that leads to sarcopenia. In adults, protein degradation is the major mechanism for providing amino acids to be converted into glucose and used in normal activities. However, in catabolic conditions, the balance is shifted toward excessive protein degradation that results in muscle wasting.

THE UBIQUITIN-PROTEASOME SYSTEM

Studies in humans and rodents have identified the ubiquitin-proteasome system (UPS) as the major pathway degrading protein in skeletal muscle. Although lysosomal cathepsins and calcium-dependent calpains can be up-regulated in catabolic conditions, their role in the degradation of myofibrillar proteins in catabolic conditions, including CKD, is substantially below that of the UPS. For example, inhibition of lysosomal and calcium-dependent proteolytic pathways in isolated muscles showed that blocking these pathways did not eliminate the acceleration of protein degradation, but inhibition of proteasome activity did block the excessive loss of muscle proteins (12). In fact, the UPS is responsible for the degradation of the bulk of proteins in all cells (13).

The biochemical processes degrading proteins in the UPS are tightly regulated to avoid uncontrolled degradation of cellular proteins (14, 15). Proteins destined for degradation in the UPS are

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\textsuperscript{2} Presented at the symposium “Cachexia and Wasting: Recent Breakthroughs in Understanding and Opportunities,” held at Experimental Biology 2009, New Orleans, LA, 18 April 2009.

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First published online February 24, 2010; doi: 10.3945/ajcn.2010.28608B.
first conjugated to ubiquitin, a highly conserved protein member of the heat shock protein family. Ubiquitin is joined to lysine residues by the E1 enzyme, an ATP-mediated process. Activated ubiquitin is then transferred to an E2 carrier protein (>20 forms exist) before being joined to the substrate protein in a reaction catalyzed by an E3 enzyme (>500 forms exist). Each of the large number of E3 enzymes recognizes specific proteins, thereby providing specificity to the process. This process is repeated until 5 ubiquitin molecules form a ubiquitin chain that marks the protein substrate for degradation in the 26S proteasome (depicted in Figure 1). This large complex of subunit proteins is composed of a 20S core proteasome and 19S complexes, forming a barrel-shaped structure with a 19S complex at each end. These 19S caps cleave ubiquitin molecules from the protein by processes that include ATPases and recognition sites (16). The 19S complex also catalyzes the unfolding of substrate proteins and facilitates transport of the unfolded protein into the 20S core of the proteasome, which contains protease activities. Inside the 26S proteasome, proteins are cleaved into small peptides. The peptides are released and rapidly hydrolyzed to amino acids by cytoplasmic peptidases; the amino acids are then transported out of the cell.

ROLE OF INSULIN RESISTANCE IN MUSCLE WASTING

There are a number of factors causing muscle wasting in rodent models of CKD, metabolic acidosis, excess angiotensin II, or inflammation (17–20). These conditions share a common pathway that depends on activation of insulin/insulin-like growth factor I (IGF-I) signaling, as do many diseases or disorders. Endogenous glucocorticoids are necessary but not sufficient to cause muscle protein degradation in several catabolic conditions and only fairly recently was the mechanism uncovered (21, 22). The role of endogenous glucocorticoids is to activate the glucocorticoid receptor, which binds to phosphatidylinositol 3-kinase, leading to suppression of the phosphorylation of Akt (23). The decrease in p-Akt up-regulates proteolytic pathways in muscle to cause muscle wasting. This nongenomic mechanism, which impairs intracellular insulin/IGF-I signaling, emphasizes the complex regulation of muscle protein metabolism.

This link between impaired insulin/IGF-1 signaling in muscle leading to a decrease in p-Akt and muscle wasting occurs in several conditions, including excess angiotensin II, inflammation, and CKD with acidosis (17, 19, 20), and results in activation 2 pathways causing muscle protein wasting. First, there is activation of caspase-3 that breaks down the complex protein structure of muscle (see below). Second, a low p-Akt decreases phosphorylation of the forkhead transcription factor, which permits its translocation into the nucleus, where it stimulates the expression of atrogin-1/muscle atrophy F-box (MAFbx) and muscle ring finger 1 (MuRF1) (24). These enzymes are specific, E3 ubiquitin–conjugating enzymes that recognize specific muscle proteins and increase their degradation in the UPS by promoting ubiquitin conjugation to the proteins. The increase in

FIGURE 1. Proteins targeted for degradation are first ubiquinated: the E1 enzyme activates ubiquitin (Ub), which is transferred to 1 of 20–40 E2 carrier proteins. One of ~1000 E3 enzymes catalyzes ubiquitin transfer to the substrate protein in an ATP-dependent reaction. This process is repeated to form a chain of ubiquitin molecules. The ubiquitin chain is recognized by the 19S proteasome, which catalyzes entry of the protein substrate into the 20S core proteasome where it is cleaved to peptides in the 26S proteasome. The peptides are degraded into amino acids to build cell proteins or are released by cells. ADP, adenosine diphosphate. Adapted with permission from reference 11.
these E3 enzymes leads to increased loss of muscle proteins—ie, muscle wasting.

Impaired function of satellite cells is another link between impaired insulin/IGF-I signaling and muscle protein loss (20). Satellite cells are under the basal lamina of myofibers, and in response to muscle injury, they proliferate and differentiate to repair the injury. The second function of satellite cells is to maintain muscle mass, a function that is impaired by CKD. The mechanisms by which impairment in satellite cell function cause loss of muscle mass are similar to those caused by defects in IGF-I signaling. Thus, insulin/IGF-I signaling plays a central role in satellite cell functioning, just as it does for regulating muscle protein turnover.

**THE 14-kD ACTIN FRAGMENT**

Activation of caspase-3 in muscle stimulates protein degradation by disrupting the complex structure of muscle proteins to provide substrates for the UPS (22). The action of caspase-3 can be detected by the presence of a characteristic 14-kD actin fragment in the insoluble fraction of muscle. The density of this actin fragment can serve as a marker to detect muscle wasting at early stages (25). The ability to detect muscle wasting is emphasized because other methods are mainly useful when a significant amount of muscle mass has been lost, making it difficult to judge the effectiveness of therapeutic interventions. For example, muscle size can be estimated by nuclear magnetic resonance imaging, by dual-energy X-ray absorptiometry (DXA), by repeated measures of amino acid/protein turnover using isotopically labeled amino acids, or by measuring nitrogen balance. These methods can be cumbersome, in part because they are based on steady state conditions that are difficult to obtain in ill patients. In patients with CKD, DXA scanning has the added difficulty that it does not distinguish between lean muscle and extracellular fluid (26). We examined the potential of another method of detecting accelerated muscle protein degradation; we studied muscle biopsies and used Western blotting to evaluate the density of the 14-kD actin fragment and compared it with values obtained in muscle biopsies from normal adults. These studies were undertaken because we found increased density of the 14-kD actin fragment in muscle biopsies of animal models of CKD, diabetes, or inflammation (compared with values present in normal muscle) (20, 22, 27).

In patients with severe osteoarthritis of the hip joint that leads to inactivity and a loss of muscle mass, we found an increase in the 14-kD actin fragment; similarly, the 14-kD actin fragment was found in muscle of patients who were losing muscle mass after a serious burn injury or in muscle of hemodialysis patients (25). In patients with severe osteoarthritis and documented loss of muscle mass, muscle biopsies were obtained during hip replacement surgery and muscle protein degradation was measured simultaneously based on the turnover of infused, labeled amino acids. Even though the number of patients was small, we found a significant ($r = 0.787$) correlation between the density of the 14-kD actin fragment and the measured rate of protein breakdown. In addition to correlating the rate of protein degradation in muscle, we found that the 14-kD actin fragment can predict a benefit of a therapeutic intervention based on results from hemodialysis patients who were undergoing exercise training. These results, although preliminary, indicate the following: 1) the 14 kD actin fragment in muscle biopsies is increased in catabolic states, 2) the level is correlated with the rate of protein degradation measured independently, and 3) the effectiveness of anticitabolic therapy could be assessed serially by measuring the 14-kD actin fragment.

**PREVENTION AND POTENTIAL THERAPY FOR MUSCLE WASTING**

**Endurance and resistive training**

There are reports that exercise could suppress or prevent muscle wasting (28). Wang et al (29) reported that resistance exercise in CKD mice exhibited an increase in protein synthesis and a reduction in protein degradation. In patients with CKD, Storer et al (30) reported that endurance exercise (ie, bicycle pedaling) undertaken just before a dialysis improves strength, power, fatigability, and physical performance in maintenance hemodialysis patients. In a controlled, randomized study of 26 predialysis subjects, the concentrations of inflammatory mediators (ie, C-reactive protein, interleukin-6) were reduced after 12 wk resistance training (31). These findings suggest a beneficial effect of aerobic and resistance training on muscle mass in both predialysis and dialysis patients.

**Correction of metabolic acidosis**

Metabolic acidosis frequently occurs in CKD and impairs growth of children with CKD and stimulates muscle wasting in patients with CKD and normal adults. Experimentally, CKD-induced growth impairment persists despite supplementation of amino acids, insulin treatment, and suppression of lysosomal pathways (18). Clinically, it has been known for many decades that correcting metabolic acidosis in children with renal tubular acidosis improves their growth. In patients with CKD, their nitrogen balance significantly improved when the plasma bicarbonate concentration was corrected by providing alkali supplements (36%; $P = 0.014$) (32). Perhaps the most persuasive evidence is the report of Stein et al (33), who carried out a year-long, randomized trial designed to examine the effects of correcting metabolic acidosis in continuous ambulatory peritoneal dialysis patients. Correction of acidosis led to a 2-kg weight gain and evidence of an increase in muscle mass based on anthropometric estimates. Likewise, in hemodialysis patients, correction of metabolic acidosis blocked the increase in muscle protein breakdown. The consensus is that alkali therapy should be given to achieve a plasma HCO$_3^-$ $> 22$ mmol/L for patients with metabolic acidosis of any cause (34).

**Testosterone**

More than 60% of men with advanced CKD have low plasma concentrations of testosterone that might contribute to muscle wasting (35, 36). Potential mechanisms by which a low testosterone concentration might cause muscle catabolism include altered IGF-I signaling and an increase in myostatin (a protein that suppresses muscle growth) (37). Regardless, 100 mg nandrolone/wk given for 24 wk increased appendicular lean mass $\sim 2$-fold based on DXA scanning ($P < 0.001$) (38). Additional information is needed before testosterone replacement therapy
Correction of insulin resistance

The db/db mouse was studied as a model of insulin resistance and activation of caspase-3. The mechanism of the UPS was revealed, providing an explanation for the loss of muscle mass (27). Interestingly, administration of a thiazolidinedione improved insulin resistance, and increased insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity and p-Akt in muscle along with suppression of protein breakdown in muscle. We were unable to find investigations of the influence of insulin sensitizers on changes in muscle protein metabolism in patients with CKD, but there is evidence that diabetes mellitus is the strongest predictor of lost lean body mass (independently of variables such as age, sex, serum albumin, inflammation markers, and dialysis modality) (39). In nondiabetic hemodialysis patients, insulin resistance has been associated with increased muscle protein breakdown—indicating that, in patients with CKD, as in results from rodent models of CKD, there is a close relation between abnormal insulin/IGF-I signaling and muscle wasting. Consequently, mechanisms that impair insulin/IGF-I signaling should be identified to maximize the likelihood of developing treatment strategies.

CONCLUSIONS

The loss of muscle mass complicates clinical syndromes and erodes patient quality of life. In patients with CKD, muscle wasting is common and studies of animal models of CKD and other catabolic conditions indicate that impaired insulin/IGF-I intracellular signaling stimulates protein degradation in muscle. These defects activate caspase-3 and the UPS to stimulate muscle protein catabolism. If these defects can be overcome by exercise or other methods, accelerated protein loss could be blocked. There is evidence that muscle biopsies could prove useful in identifying patients with accelerated muscle protein catabolism, and therefore potentially to evaluate the success of interventions such as correction of metabolic acidosis. However, it is clear that new strategies are needed to combat muscle protein losses.

The authors’ responsibilities were as follows—BTW and WEM: wrote and edited the manuscript. The authors had no conflict of interests to declare.

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