Cellular Mechanisms Causing Loss of Muscle Mass in Kidney Disease

William E. Mitch and J. Du

In stable adults or patients with kidney disease, the daily turnover of cellular proteins is very large, amounting to the quantity of protein in 1 to 1.5 kg of muscle. Consequently, even a small but persistent increase in protein degradation or decrease in protein synthesis leads to a substantial loss of muscle mass. In chronic kidney disease, the pathway that degrades muscle protein is the ubiquitin-proteasome system. We tested whether either of two complications of chronic kidney disease, metabolic acidosis or insulin resistance accelerates the loss of muscle protein. Metabolic acidosis activates the ubiquitin-proteasome system and this can explain an large number of clinical conditions in which metabolic acidosis also causes loss of muscle protein. Insulin deficiency as a model of insulin resistance also activates the ubiquitin-proteasome system. Both complications also activate caspase-3 and we found that this protease performs a critical initial step in breaking down the complex structure of muscle to provide actin, myosin and fragments of these proteins as substrates for the ubiquitin-proteasome system. Defects in insulin signalling processes can activate both caspase-3 and the ubiquitin-proteasome system to degrade muscle protein. Understanding mechanisms that activate protein breakdown will lead to therapies that successfully prevent the loss of muscle mass in patients with kidney disease.

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Patients with kidney disease commonly exhibit a low serum albumin level, a low body weight, and anthropometric abnormalities, pointing to a loss of muscle mass. These responses are clinically important because there is a close relationship between a low serum albumin level and mortality in hemodialysis patients. Assigning these abnormalities to malnutrition, however, suggests that they are the result of an inadequate intake of protein and calories. Although an inadequate diet can contribute to this problem, it is only one component of the problems that result in loss of body weight and muscle protein stores.

Notably, even a small and persistent suppression of protein synthesis or stimulation of protein degradation causes a substantial loss of muscle protein. This occurs because the rates of protein synthesis and degradation in normal adults are very large, amounting to 3.5 to 4.5 g protein per kg each day, equivalent to the loss of protein in 1 to 1.5 kg of muscle each day. A major fraction of proteins that are synthesized and degraded each day is needed to replace proteins with transcriptional or translational errors arising during protein synthesis or to remove proteins that are damaged or with a biochemical modification so they no longer function normally. Enzymes or transcription factors that regulate cellular growth and homeostasis also must be removed. But, in catabolic illnesses, there is a further stimulation of the breakdown of proteins in muscle by the ubiquitin-proteasome system (Ub-P’some). It requires 3 components, adenosine triphosphate hydrolysis, the protein cofactor ubiquitin, and the 26S proteasome. Recent reports have provided clues to the signals that activate the Ub-P’some system in muscle.

Biochemical Mechanisms of the Ub-P’some System

The initial step in protein degradation by this system links a substrate protein to a chain of ubiquitin molecules. Ubiquitin is a small protein that is present in all cells and is a member of the heat-shock complex of proteins. Ubiquitin conjugation is initiated by a ubiquitin-activating enzyme, E1, using adenosine triphosphate to create a high-
energy thiolester form of ubiquitin. Activated ubiquitin is transferred to a ubiquitin-carrier, an E2 enzyme. Different E2 enzymes could confer some specificity to the ubiquitin conjugating process, but this is not clear and considering the large number of proteins in cells, there are other means of conferring specificity. The third step transfers activated ubiquitin to the substrate protein using a ubiquitin-protein ligase, an E3 enzyme. This enzyme binds both the protein substrate and the E2 enzyme and transfers activated ubiquitin to the substrate protein. These processes are repeated until a chain of at least 4 ubiquitin molecules are attached. The ubiquitin chain can be recognized by the 26S proteasome, a very large (2,000 kd) complex made up of at least 50 protein subunits. The subunits are arranged in 4 stacked cylinders to form a structure known as the 20S proteasome. On the inner face of the 20S proteasome cylinder, there are 3 proteolytic activities that degrade substrate proteins to small peptides. The 26S proteasome also has protein caps on each end of the 20S proteasome to recognize the ubiquitin-conjugated substrate and inject the protein into the core of the proteasome. Both the ubiquitin conjugation process and the activities of the 26S proteasome require adenosine triphosphate.

When a catabolic condition stimulates muscle cell proteolysis, specific E3 enzymes recognize only certain proteins and catalyze their conjugation to ubiquitin. For example, the E3α enzyme ubiquitinates only proteins with unusual NH2-terminal amino acid residues and participates in a protein-recognition process that is named the N-end rule pathway, which is activated by conditions such as sepsis or starvation. We found that E3α also is involved in the accelerated loss of muscle protein caused by acute diabetes. Another E3 that participates in accelerated conjugation of ubiquitin to muscle proteins was discovered recently when marked up-regulation of the messenger RNA (mRNA) levels of this gene was found in muscle of rat models of fasting diabetes, cancer, or renal failure. This gene is named atrogin because it is activated specifically when muscle protein degradation is accelerated.

Conditions causing muscle proteolysis share another feature, an increase in the mRNAs encoding components of the Ub-P'some system. In muscle of rats with chronic renal failure or diabetes, the higher levels of ubiquitin and proteasome subunit mRNAs are caused by increased transcription of these genes. The finding that mRNA levels encoding components of the Ub-P'some system are high in a number of conditions causing muscle atrophy indicates that this response must represent a common transcriptional program for muscle atrophy.

### Acidosis and Activation of the Ub-P’some System

What are the stimuli activated by kidney disease to cause loss of muscle protein? We identified metabolic acidosis as one important factor that stimulates the degradation of essential amino acids and protein in the muscles of rats. Others reported that acidosis stimulates the loss of protein and essential amino acids in normal adults and patients with renal insufficiency or those treated by hemodialysis or continuous ambulatory peritoneal dialysis (Table 1). Recently, we obtained evidence for activation of the Ub-P’some system in muscle biopsy specimens obtained from continuous ambulatory peritoneal dialysis patients with mild metabolic acidosis. After the serum HCO₃ increased in these patients after more intensive dialysis, there was a significant decrease in the ubiquitin mRNA level, consistent with down-regulation of the Ub-P’some system.

### Diabetes and Activation of the Ub-P’some System

Another common factor in patients with kidney disease or acidosis is insulin resistance. This is relevant because we found that acute diabetes accelerates protein degradation in muscles of rats and sharply increases the mRNAs encoding ubiquitin and proteasome subunits. We also measured a sharp increase in the rate of ubiquitin conjugation to muscle proteins. These findings suggest that defective insulin-mediated cell signaling pathway is a critical mechanism that accelerates muscle protein breakdown in uremia.

### Glucocorticoids and Activation of the Ub-P’some System

In acidosis, diabetes, or starvation, glucocorticoids are essential for activation of the Ub-P’some system in muscle of rats. In these experiments, the adrenal glands were removed but muscle protein degradation was not induced by acidosis, acute diabetes, or starvation. Moreover, a physiologic dose of glucocorticoids did not activate muscle proteolysis in adrenalectomized rats, but the combination of the same dose of glucocorticoids plus the catabolic illness stimulated muscle protein breakdown. Thus, glucocorticoids have a permissive effect in stimulating the Ub-P’some system to degrade protein and increase transcription of genes encoding components of the system.

### Inflammation, Protein Degradation, and the Ub-P’some System

Experimental evidence indicates that inflammation can suppress protein synthesis or stimulate protein degradation and patients with kidney failure often have high serum levels of cytokines and acute phase reactant proteins. Clinical associations, however, cannot identify how inflammation causes a change in protein synthesis or degradation and loss of lean body mass. Moreover, the associations are not suffi-
ciency definitive to recommend therapies that would block inflammatory responses.26

**New Directions**

A review of the characteristics of conditions that accelerate muscle protein loss led us to examine apoptotic pathways as an initial step in the proteolytic process. We found evidence that caspase activation can be the initial enzyme stimulating muscle protein loss. One method that could link activation of proteolytic and apoptotic systems would be defects in insulin cellular signaling pathways. If confirmed, these findings could lead to new therapies for patients with kidney failure.

**References**


**Table 1 Evidence for the protein catabolic influence of metabolic acidosis**

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Abbreviation: CAPD, continuous ambulatory peritoneal dialysis.