Muscle wasting in chronic kidney disease: the role of the ubiquitin proteasome system and its clinical impact

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Abstract Muscle wasting in chronic kidney disease (CKD) and other catabolic diseases (e.g. sepsis, diabetes, cancer) can occur despite adequate nutritional intake. It is now known that complications of these various disorders, including acidosis, insulin resistance, inflammation, and increased glucocorticoid and angiotensin II production, all activate the ubiquitin–proteasome system (UPS) to degrade muscle proteins. The initial step in this process is activation of caspase-3 to cleave the myofibril into its components (actin, myosin, troponin, and tropomyosin). Caspase-3 is required because the UPS minimally degrades the myofibril but rapidly degrades its component proteins. Caspase-3 activity is easily detected because it leaves a characteristic 14kD actin fragment in muscle samples. Preliminary evidence from several experimental models of catabolic diseases, as well as from studies in patients, indicates that this fragment could be a useful biomarker because it correlates well with the degree of muscle degradation in dialysis patients and in other catabolic conditions.

Keywords Ubiquitin–proteasome system (UPS) • Caspase-3 • 14kD actin fragment • Muscle wasting • Protein degradation • Muscle atrophy • Uremia • Chronic kidney disease (CKD)

Maintenance of protein stores in chronic kidney disease

In uremic patients, protein stores are frequently depressed when assessed by a low serum prealbumin and weight loss, which includes loss of muscle mass [1, 2]. In pediatric patients with chronic kidney disease (CKD), linear growth is impaired and muscle mass is reduced. Although these findings have been attributed to “malnutrition”, many uremic patients with muscle wasting have not developed the problems because of inadequate diet; instead, they have complications that induce a complex series of physiological and biochemical adaptations, resulting in protein catabolism [3, 4]. In children and adults with CKD, these complications include metabolic acidosis, insulin resistance, increased glucocorticoid production, high levels of angiotensin II (Ang II), and inflammation [5–8]. Many observational studies and mechanistic investigations have attempted to explain this loss of protein stores, and especially the loss of muscle mass. There are at least three conclusions from these studies. First, rates of protein synthesis in muscle are generally unchanged, whereas rates of protein degradation tend to increase [5, 9]. Second, the daily rates of protein turnover in cells are so high (3.5–4.5 g protein/kg per day) that even a small increase in protein degradation (and/or a decrease in protein synthesis) will cause marked protein depletion over time [10]. Third, the increase in muscle protein degradation in uremia and most other catabolic disease states is mostly due to programmed activation of the ubiquitin–proteasome system (UPS) [5, 11]. Therefore, to understand muscle wasting, one must understand the UPS.
The ATP-dependent, ubiquitin–proteasome system (UPS)

Over the past two decades, progress in understanding the action and regulation of the UPS has been at the center of attempts to understand the control of protein turnover. The UPS includes concerted actions of enzymes that link ubiquitin (Ub), a member of the heat-shock protein family, to protein substrates that are destined for degradation (Fig. 1). When a chain of at least four to five ubiquitins are linked to a protein, it is marked for degradation in a second step mediated by the proteasome [12, 13]. Specifically, the tagged protein will be recognized by the 26S proteasome, a very large multicatalytic protease complex that not only recognizes Ub-conjugated proteins but also removes Ub, unwinds the protein, and injects it into the central core of the 26S proteasome. Once inside this central, tube-like structure, the protein substrate is degraded into small peptides [14].

Three enzymatic components are required to link Ub to proteins that are destined for degradation. There appears to be only one E1 (Ub-activating) enzyme and around 40 E2 (Ub-carrier or conjugating) proteins. The key enzyme, however, is the E3 enzyme, which accounts for the exquisite specificity of proteins to be degraded. There are at least a thousand E3 enzymes (Ub ligase), and each can recognize a specific protein substrate and catalyze the transfer of an activated Ub from the E2 carrier protein to the substrate protein [10, 15].

Since the initial reports that the UPS recognizes specific proteins and tags them for destruction, knowledge about proteolytic processes in the proteasome has exploded. Thousands of proteins have been recognized as being degraded by the UPS, and novel cellular functions are now known to be regulated by Ub conjugation. In terms of protein breakdown, the major functions of the pathway are:

**Rapid removal of proteins** Protein degradation is irreversible, and hence, destruction of a protein generally leads to a complete termination of cellular process mediated by the protein. Consequently, protein degradation is critical for the regulation of metabolism and cell turnover. The rapid degradation of specific proteins also permits cells (as well as the organism) to rapidly adapt to a change in physiological conditions (e.g. requiring a switch to glucose as an energy source involves converting protein stores into amino acids that can be used in gluconeogenesis).

**Regulation of gene transcription** Ub conjugation affects gene transcription because many transcription factors become conjugated to Ub, and transcription activators are degraded by the proteasome [16]. This process regulates transcriptional activity by removing “spent” activators and resetting a promoter for additional rounds of transcription [17]. Second, the ability of transcription factors to function varies with their location within the cell. For example, nuclear factor (NF)-κB, a proinflammatory transcriptional factor, is kept outside the nucleus because movement into the nucleus is blocked by its association with an inhibitory protein, IκB. Destruction of IκB, initiated by the IKK kinase and carried out by the UPS, frees NF-κB, which then translocates to the nucleus to stimulate gene transcription [18]. A clinical application of this function of the UPS has developed in oncology. Bortezomib (Velcade, PS-341), a proteasome inhibitor, has proven to be beneficial in patients with multiple myeloma and is currently in clinical trials for the treatment of other cancers [19, 20]. The proposed mechanism of action involves the ability of bortezomib to prevent the UPS-induced destruction of IκB, thereby blocking the activation of NF-κB (an antiapoptosis transcription factor), leading to an increase in apoptosis [21]. Inhibition of the proteasome, therefore, will induce apoptosis of the neoplastic cells [22]. In addition, myeloma cells are also particularly dependent upon NF-κB to produce essential growth factors [especially interleukin 6 (II-6)]; when NF-κB is inactive, the growth of myeloma cells is depressed.

**Quality-control mechanism** The UPS selectively eliminates abnormally folded or damaged proteins that have arisen because of missense or nonsense mutations, biosynthetic errors, proteins damaged by oxygen radicals, or by
denaturation. For example, in cystic fibrosis, the mutant form of the transmembrane conductance regulator protein (CFTR) is selectively degraded before it reaches the cell surface [23]. The UPS catalyzes destruction of this mutant CFTR because its tertiary structure is abnormal. Another example is the degradation of misfolded proteins within the endoplasmic reticulum. Endoplasmic-reticulum-associated degradation (ERAD) of proteins removes misfolded proteins by targeting them for destruction by proteasomes in the cytoplasm [24].

**Influencing the function of the immune system** The UPS is responsible for creating antigens from the degradation of foreign proteins (e.g. viral particles). The antigens are presented on the major histocompatibility complex as class I molecules. In this way, the 26S proteasome exerts dual roles of removing foreign proteins and creating a stimulus of the immune system [15, 25].

**As a source of amino acids** When carbohydrate calories are rapidly needed or when cells must respond to catabolic diseases/conditions, there is breakdown of cell proteins, especially skeletal muscle proteins. The UPS degrades muscle proteins to provide amino acids that can be converted to glucose (i.e. gluconeogenesis). An undesired consequence of this activity could be an inappropriate loss of muscle protein.

**Functions of Ub not associated with proteolysis** Ub can also be conjugated to proteins as a monomer (rather than as the typical Ub chain). When this occurs on cell-surface proteins, the protein is internalized into the endocytic pathway to be degraded in lysosomes [26, 27].

**Uremia-activated mechanisms that accelerate loss of muscle protein**

Results from rodent models of CKD have established that accelerated muscle protein catabolism involves many of the same cellular mechanisms that cause muscle wasting in other catabolic conditions, such as cancer, starvation, insulin deficiency/resistance, or sepsis [10, 28]. The principal mechanism causing muscle atrophy in CKD involves activation of the UPS. Evidence for this includes the presence of higher levels of mRNAs encoding certain components of the UPS, as well as a similar pattern of increases and decreases in the expression of about 100 atrophy-related genes (also called atrogenes) [5, 28]. Changes in atrogenes include decreased expression of various growth-related genes and increased expression of components of the UPS. Patients with different clinical conditions associated with muscle atrophy exhibit similar increases in mRNAs encoding components of the UPS (e.g. an increase in mRNAs encoding Ub and proteasome subunits) [11, 29–31]. In these cases, changes in gene expression are most likely due to transcriptional regulation because we have shown that uremia or abnormal insulin responses increase the transcription of Ub and subunits of the proteasome [5, 6, 32]. Additional evidence linking the UPS to protein degradation in catabolism is the finding that the increase in protein degradation in the muscle of rats with CKD (and other muscle-wasting conditions) can be blocked by inhibitors of the proteasome [5, 6, 33]. Considered together, these results indicate that muscle wasting is a specific and carefully orchestrated program.

Other questions are why are proteins degraded, and how is the complex program triggered in widely varied pathological conditions (e.g. acidosis in renal failure, low insulin levels in fasting and diabetes, inactivity, or glucocorticoids and cytokines in sepsis and other inflammatory conditions)? In fasting and in other disease states, acceleration of muscle-protein breakdown mobilizes amino acids, which are used for protein synthesis in tissues and for conversion to glucose in the liver [10]. However, if excessive protein degradation persists, the protein loss will have deleterious effects. In CKD, the breakdown of tissue protein produces nitrogenous waste products, which must be excreted to prevent the accumulation of uremic toxins [3, 10]. Finally, in muscle wasting conditions, contractile proteins are lost differentially, whereas in conditions causing atrophy (e.g. aging), all components of muscle cells seem to be affected.

What accounts for muscle-specific response? The answer to this question lies in the involvement of the UPS. The UPS degrades a specific protein depending on which E3 ubiquitin ligase is activated. For example, two Ub ligases, atrogin-1 (also known as MAIBx) and MuRF-1, are found specifically in muscle, and their expression increases dramatically (8- to 20-fold) in catabolic states, causing loss of muscle protein [28, 34, 35]. In mice lacking the genes for either atrogin-1 or MuRF-1, muscles grow normally, but in response to muscle denervation, the ensuing atrophy is 30–50% slower [34]. In addition, the muscle’s content of atrogin-1 mRNA can be considered a biomarker for the rate of proteolysis in muscles responding to a catabolic condition [36–38].

**In uremia, the initial cleavage of myofibrillar proteins is mediated by caspase-3**

Myofibrillar proteins (including actomyosin) comprise about two thirds of the protein in muscle, the major store of amino acids for new protein synthesis and for gluconeogenesis. The UPS readily degrades the main proteins in
myofibrils (i.e. actin, myosin, troponin, or tropomyosin), but it does not readily break up the myofibril into its main component proteins [39]. This means that another proteolytic system must initially digest myofibrils to create substrates that can be degraded by the UPS [40].

Many catabolic conditions are associated with inflammation and/or cell injury, and these conditions activate a cysteine protease called caspase-3. We tested caspase-3 in an in vitro system using purified actomyosin and found that caspase-3 cleaves actomyosin and leaves a characteristic 14kD actin fragment [40]. When we activated caspase-3 in cultured muscle cells, we found that UPS rapidly degraded myofibrillar component proteins, and again, the 14kD C-terminal fragment of actin was left in the muscle cells [40]. The protein-cleaving action of caspase-3 is important because blocking caspase-3 will reduce overall protein degradation in muscle [40]. In addition, we have found the same cleavage processes are present in muscles of rodent models of uremia, type-1 or type-2 diabetes, or in Ang-II-induced hypertension [7, 35, 40, 41]. Moreover, we found that the 14kD actin fragment also accumulates in muscles of patients with loss of muscle mass due to painful osteodystrophy, uremia, or burn injury [42].

**Signals triggering muscle atrophy in kidney disease and other catabolic states**

Complications of CKD, as well as the complex syndrome of uremia, can trigger muscle protein breakdown. The triggering complications include metabolic acidosis, decreased insulin action (including insulin resistance), increased glucocorticoid production, high levels of Ang II, and inflammation [5–8]. Metabolic acidosis is known to cause accelerated protein degradation in infants, children, adults, the elderly, and patients with CKD (Table 1). The mechanism by which metabolic acidosis causes muscle wasting involves activation of the UPS and caspase-3 [40, 43]. In addition, acidosis changes hormone actions, such as insulin resistance and increased glucocorticoid production, which are involved in activating protein degradation [32, 42].

**Table 1** Evidence that metabolic acidosis induces catabolism of protein and amino acids in normal infants, children, and adults, as well as in patients with chronic kidney disease (CKD)

| Subjects investigated | Outcome measurements | Trial outcome |
|-----------------------|----------------------|--------------|
| Infants [68]          | Low-birth-weight, acidotic infants were given NaHCO3 or NaCl | NaHCO3 supplement improved growth |
| Children with CKD [69]| Measured rates of protein degradation in children with CKD | Protein loss was ~ 2-fold higher when HCO3 was < 16 mM compared with > 22.6 mM |
| Normal adults [70]    | Acidosis induced and then measured amino acid and protein metabolism | Acidosis increased amino acid and protein degradation |
| Normal adults [71]    | Induced acidosis and then measured nitrogen balance and albumin synthesis | Acidosis induced negative nitrogen balance and suppressed albumin synthesis |
| Chronic renal failure [72] | Nitrogen balance before and after treatment of acidosis | NaHCO3 improved nitrogen balance |
| Chronic renal failure [73] | Essential amino acid and protein degradation before and after treatment of acidosis | NaHCO3 suppressed amino acid and protein degradation |
| Chronic renal failure [74] | Muscle protein degradation and degree of acidosis | Proteolysis was proportional to acidosis and blood cortisol |
| Chronic renal failure [75] | Nitrogen balance before and after treatment of acidosis | NaHCO3 reduced urea production and improved nitrogen balance |
| Hemodialysis [76]     | Protein degradation before and after treatment of acidosis | NaHCO3 decreased protein degradation |
| Hemodialysis [77]     | Serum albumin before and after treatment of acidosis | NaHCO3 increased serum albumin |
| CAPD [78]             | Protein degradation before and after treatment of acidosis | NaHCO3 decreased protein degradation |
| CAPD [79]             | Weight and muscle gain before and after treatment of acidosis | Raising dialysis buffer increased weight and muscle mass |

CAPD continuous ambulatory peritoneal dialysis
It is important to emphasize that the correction of metabolic acidaemia has been shown to decrease protein breakdown in various clinical trials, indicating why maintaining normal serum bicarbonate levels should be part of standard clinical care (Table 1).

The finding that various diseases with muscle wasting are caused by activation of the UPS, plus the fact that coordinated changes in the expression of genes in muscle occur in different catabolic states, suggest that catabolic states activate a common cellular signaling pathway [28]. One signaling pathway is a decrease in phosphatidylinositol 3-kinase (PI3K) activity (Fig. 2). The involvement of this signaling pathway follows from the finding that several catabolic illnesses, including sepsis, acidosis, uremia, and diabetes, are characterized by insulin resistance [41, 44, 46, 47]. In normal muscle, binding of insulin or insulin-like growth factor (IGF)-1 to their receptors increases the activities of PI3K and its downstream target, Akt. In insulin-resistant conditions or with deficiency of IGF-1, the activity of this signaling pathway is depressed [35, 48, 49]. When PI3K activity falls, there is decreased production of phosphatidylinositol-3,4,5 phosphate (PIP3), leading to decreased phosphorylation and activity of the downstream serine/threonine kinase, Akt. This is a key step, because activated Akt is a major stimulator of growth-related processes via phosphorylation of the downstream kinases, GSK1 and mTOR/S6 kinase, stimulating protein synthesis. On the other hand, reduced PI3K-Akt signaling (as occurs in insulin resistance) enhances protein breakdown in muscle [41]. The rise in muscle protein losses is associated with two catalytic processes: first, caspase-3 is activated to break down the complex structure of muscle; second, there is increased expression of the E3 ubiquitin ligases, atrogin-1 and MuRF-1, to degrade the proteins made available by caspase-3 [35–37] (Fig. 2). The result is muscle wasting.

How are the two mechanisms activated? In studies of muscles from insulin-deficient rats or db/db mice (a model of insulin resistance), we found that accelerated muscle protein degradation increases the level of the proapoptotic factor, Bax [35, 41]. This is relevant because activated Bax causes the release of cytochrome C from mitochondria. Cytochrome C release, in turn, activates caspase-3 to trigger actomyosin/myofibril cleavage, leaving behind the 14kD actin fragment marker [35]. The activation of atrogin-1, and hence the UPS, involves another mechanism: changes in activity of the fork-head transcription factors (FoxO 1, 3 and 4). When these transcription factors are phosphorylated by Akt, they cannot enter the nucleus to stimulate transcription of atrogin-1. However, when PI3K/Akt activities are low, the FoxOs are not phosphorylated, so they can enter the nucleus to increase the transcription of atrogin-1, resulting in an increase in muscle protein degradation [35–37].

### Table 2

| Hormone                  | Acidosis-induced response                                      |
|--------------------------|----------------------------------------------------------------|
| Growth hormone (GH) [80–84] | Suppressed GH secretion                                      |
| Insulin [44, 85, 86]     | Lower IGF-1 response                                          |
| Insulin-like growth factor (IGF)-1 [81, 84, 87] | Suppressed insulin-stimulated glucose metabolism             |
| Thyroid hormone [82, 88] | Decreased IGF-1 in plasma, and kidney and liver (but not in muscle) |
| Glucocorticoids [89]     | Increased glucocorticoid production                           |
| Parathyroid hormone (PTH) [90, 91] | Decreased sensitivity of PTH secretion to changes in plasma calcium |
| Vitamin D [91]           | Suppressed activation to 1,25 (OH)2-cholecalciferol             |

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![Fig. 2](image_url)
The influence of Akt on the other E3 ubiquitin ligase involved in muscle proteolysis, MuRF-1, is not as clearly established, and it may be linked to inflammation, as activation of NF-κB will cause overproduction of MuRF1 and muscle atrophy [37].

Besides acidosis and depressed insulin/IGF-1 action, another complication of CKD that participates in muscle wasting is increased glucocorticoid production. The complexity of these interactions is great because increased glucocorticoids can cause insulin insensitivity [32, 41, 50], and both insulin deficiency and insulin resistance increase glucocorticoid production. Glucocorticoids exert a permissive effect on protein degradation in muscle caused by several catabolic conditions. For example, activation of muscle protein breakdown does not occur in adrenalectomized animals with metabolic acidosis or with acute diabetes unless the animals are also given a physiological dose of glucocorticoids [32, 51–53]. Similarly, the increase in muscle proteolysis induced by Ang II or sepsis is largely eliminated by inhibiting the glucocorticoid receptor [7, 54]. This response to glucocorticoids is “permissive” because the same physiological level of glucocorticoids does not stimulate muscle protein degradation unless the animals are either acidic or insulin deficient. These complex interactions actually make “physiological sense” because glucocorticoids evolved to integrate stress responses in different tissues. When glucose is needed, glucocorticoids mobilize amino acids from muscle protein. At the same time, glucocorticoids induce gluconeogenic enzymes in liver to catalyze the conversion of the amino acids to glucose and urea.

In children, accelerated loss of protein stores associated with glucocorticoid therapy frequently results in impaired linear growth. One glucocorticoid-dependent mechanism causing these defects is impaired response to insulin (insulin insensitivity). An allied mechanism is impaired response to IGF-1 arising from decreased or impaired action of growth hormone. Indeed, administration of growth hormone has been shown to improve linear growth of children being treated chronically with glucocorticoids [55]. Moreover, growth hormone has been shown to improve growth of children with chronic catabolic diseases such as CKD; whereas growth hormone improves growth, it does not improve growth in children to (or close to) a normal height for age [56–58]. The impaired response is likely due to the multiple complications of CKD, resulting in retarded growth. Interestingly, there is a small uncontrolled study of five children who were being treated with chronic intermittent hemodialysis and growth hormone. The children were then placed on an intensified daily hemodialysis regimen (3 h/day, five to six times a week) for a median time of 1.5 years [59]. The new regimen led to significant catchup growth into the range of a normal height for age. Intensified dialysis plus growth hormone could correct acidosis, improve insulin and IGF-1 signaling and responses to other hormones, and remove unidentified uremic toxins. These responses emphasize the complexity of sorting out mechanisms for muscle wasting in CKD.

Another catabolic factor associated with CKD is Ang II. Infusion of Ang II into rodents causes both anorexia and muscle protein loss by mechanisms that depend on glucocorticoids [7]. Also, there is the knotty problem of understanding the influence of inflammation. In this case, the mechanism(s) causing muscle wasting in inflammation is not clear. Suggested responses include the ability of certain inflammatory mediators to cause insulin resistance, as well as a more direct influence of inflammatory mediators on muscle protein metabolism (Fig. 2). The problem is difficult because the link between an increase in inflammatory markers in uremic patients (e.g. C-reactive protein) to loss of muscle mass has not been established [60, 61].

Finally, there is an intriguing protein—myostatin—a member of the transforming growth factor (TGF)-β family of cytokines. It is produced in skeletal and cardiac muscle and regulates muscle growth by limiting it [62]. Overexpression of myostatin in muscle leads to loss of protein mass via inhibition of Akt phosphorylation with an increase in active FoxO1; this increases the expression of atrophy-related genes [63, 64]. Underexpression of myostatin results in skeletal muscle hypertrophy [65]. An increase in myostatin expression is found in several cachexia-associated disease states. However, there is limited information about the influence of kidney disease on myostatin expression and function beyond changes in myostatin mRNA [66, 67].

**Conclusion**

In this brief review of mechanisms causing muscle protein losses, we discussed how a complex series of biochemical reactions are coordinated to create a genetic program that degrades muscle proteins. We also identified an initial step in muscle proteolysis that leaves behind a biomarker in muscle, the 14kD actin fragment, resulting from caspase-3. We emphasized how the UPS causes muscle wasting in uremia, as well as the role UPS plays in the regulation of cellular functions, ranging from the control of the cell cycle to activities that promote cancer. Indeed, inhibitors of proteasome activity have emerged as novel chemotherapeutic agents. Involvement of the UPS in such a wide range of functions explains why the 2004 Nobel Prize in Chemistry was awarded to Avram Hershko, Aaron Ciechanover, and Irwin Rose for their discovery of Ub
and its role in orchestrating cellular protein turnover (http://nobelprize.org/chemistry/laureates/2004/).

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