ELSEVIER

SEMINARS IN
NEPHROLOGY

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.
Semin Nephrol 24:484-487 © 2004 Elsevier Inc. All rights reserved.

KEYWORDS muscle proteolysis, capase-3, ubiquitin, proteasome, acidosis, insulin resistance

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.[1] 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.[2]

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.[3] 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.[4] 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).[3] 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.[3] 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-

Nephrology Division, Department of Internal Medicine, University of Texas Medical Branch, Galveston, TX.
Address reprint requests to: William E. Mitch, MD, University of Texas Medical Branch, 9.138A Medical Research Building, 301 University Blvd., 1064 Galveston, TX 77555-1064.

 0270-9295/04/$-see frontmatter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.semnephrol.2004.06.014

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.[3] 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.[5] For example, the E3α enzyme ubiquitinates only proteins with unusual NH_2-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.[6-8] We found that E3α also is involved in the accelerated loss of muscle protein caused by acute diabetes.[9] 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.[10] 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.[3] 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.[11-13] 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.[9]

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.[11,14-17] 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 HCO_3 increased in these patients after more intensive dialysis, there was a significant decrease in the ubiquitin mRNA level,[18] consistent with down-regulation of the Ub-P'some system.[11]

Diabetes and Activation of the Ub-P'some System

Another common factor in patients with kidney disease or acidosis is insulin resistance.[19,20] 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.[12,13] We also measured a sharp increase in the rate of ubiquitin conjugation to muscle proteins.[9] These findings suggest that defective insulin-mediated cell signaling pathway is a critical mechanism that accelerates muscle protein breakdown in uremia.

Glucocoticoids 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.[13,21-23] 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.[24]

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.[3,25] 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-

Table 1 Evidence for the protein catabolic influence of metabolic acidosis

Subjects and Reference	Measurements of Effectiveness	Outcome of Trial
Normal adults[27]	Induced acidosis, amino acid, and protein metabolism	Acidosis increased amino acid and protein degradation
Normal adults[28]	Induced acidosis, nitrogen balance, and albumin synthesis	Acidosis induced negative nitrogen balance and suppressed albumin synthesis
Chronic renal failure[29]	Nitrogen balance before and after treatment of acidosis	$NaHCO_3$ improved nitrogen balance
Chronic renal failure[30]	Essential amino acid and protein degradation before and after treatment of acidosis	$NaHCO_3$ suppressed amino acid and protein degradation
Chronic renal failure[31]	Muscle protein degradation and acidosis	Proteolysis was proportional to acidosis and blood cortisol levels
Chronic renal failure[32]	Nitrogen balance before and after treatment of acidosis	$NaHCO_3$ reduced urea production and nitrogen balance
Hemodialysis[33]	Protein degradation before and after treatment of acidosis	$NaHCO_3$ decreased protein degradation
Hemodialysis[34]	Serum albumin levels before and after treatment of acidosis	$NaHCO_3$ increased serum albumin
CAPD[35]	Protein degradation before and after treatment of acidosis	$NaHCO_3$ decreased protein degradation
CAPD[36]	Weight and muscle gain before and after treatment of acidosis	Increasing dialysis buffer increased weight and muscle mass

Abbreviation: CAPD, continuous ambulatory peritoneal dialysis.

ciently 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

1. Lowrie EG, Lew NL: Death risk in hemodialysis patients: The predictive value of commonly measured variables and an evaluation of the death rate differences among facilities. Am J Kidney Dis 15:458-482, 1990
2. Mitch WE: Insights into the abnormalities of chronic renal disease attributed to malnutrition. J Am Soc Nephrol 13:S22-S27, 2002
3. Mitch WE, Goldberg AL: Mechanisms of muscle wasting: The role of the ubiquitin-proteasome system. N Engl J Med 335:1897-1905, 1996
4. Schild H, Rammensee H-G: Perfect use of imperfection. Nature 404: 709-710, 2000
5. Ciechanover A, Schwartz AL: How are substrates recognized by the ubiquitin-mediated proteolytic system? Trends Biochem Sci 14:483-488, 1989
6. Varshavsky A: The N-end rule: Functions, mysteries, uses. Proc Natl Acad Sci U S A 93:12142-12149, 1996
7. Solomon V, Lecker SH, Goldberg AL: The N-end rule pathway catalyzes a major fraction of the protein degradation in skeletal muscle. J Biol Chem 273:25216-25222, 1998
8. Solomon V, Baracos V, Sarraf P, et al: Rates of ubiquitin conjugation increase when muscles atrophy, largely through activation of the N-end rule pathway. Proc Natl Acad Sci U S A 95:12602-12607, 1998
9. Lecker SH, Solomon V, Price SR, et al: Ubiquitin conjugation by the N-end rule pathway and mRNAs for its components increase in muscles of diabetic rats. J Clin Invest 104:1411-1420, 1999
10. Gomes MD, Lecker SH, Jagoe RT, et al: Atrogin-1, a muscle-specific F-box protein is highly expressed during muscle atrophy. Proc Natl Acad Sci U S A 98:14440-14445, 2001
11. Bailey JL, Wang X, England BK, et al: The acidosis of chronic renal failure activates muscle proteolysis in rats by augmenting transcription of genes encoding proteins of the ATP-dependent, ubiquitin-proteasome pathway. J Clin Invest 97:1447-1453, 1996
12. Price SR, Bailey JL, Wang X, et al: Muscle wasting in insulinopenic rats results from activation of the ATP-dependent, ubiquitin-proteasome pathway by a mechanism including gene transcription. J Clin Invest 98:1703-1708, 1996
13. Mitch WE, Bailey JL, Wang X, et al: Evaluation of signals activating ubiquitin-proteasome proteolysis in a model of muscle wasting. Am J Physiol 276:C1132-C1138, 1999
14. Hara Y, May RC, Kelly RA, et al: Acidosis, not azotemia, stimulates branched-chain amino acid catabolism in uremic rats. Kidney Int 32: 808-814, 1987
15. May RC, Kelly RA, Mitch WE: Mechanisms for defects in muscle protein metabolism in rats with chronic uremia: The influence of metabolic acidosis. J Clin Invest 79:1099-1103, 1987
16. England BK, Greiber S, Mitch WE, et al: Rat muscle branched-chain ketoacid dehydrogenase activity and mRNAs increase with extracellular acidemia. Am J Physiol 268:C1395-C1400, 1995
17. Mitch WE, Medina R, Greiber S, et al: Metabolic acidosis stimulates muscle protein degradation by activating the ATP-dependent pathway involving ubiquitin and proteasomes. J Clin Invest 93:2127-2133, 1994
18. Pickering WP, Price SR, Bircher G, et al: Nutrition in CAPD: Serum bicarbonate and the ubiquitin-proteasome system in muscle. Kidney Int 61:1286-1292, 2002

19. DeFronzo RA, Alvestrand A, Smith D, et al: Insulin resistance in uremia. J Clin Invest 67:563-568, 1981
20. DeFronzo RA, Beckles AD: Glucose intolerance following chronic metabolic acidosis in man. Am J Physiol 236:E328-E334, 1979
21. Price SR, England BK, Bailey JL, et al: Acidosis and glucocorticoids concomitantly increase ubiquitin and proteasome subunit mRNAs in rat muscle. Am J Physiol 267:C955-C960, 1994
22. May RC, Masud T, Logue B, et al: Chronic metabolic acidosis accelerates whole body proteolysis and leucine oxidation in awake rats. Kidney Int 41:1535-1542, 1992
23. Wing SS, Goldberg AL: Glucocorticoids activate the ATP-ubiquitin-dependent proteolytic system in skeletal muscle during fasting. Am J Physiol 264:E668-E676, 1993
24. Isozaki Y, Mitch WE, England BK, et al: Interaction between glucocorticoids and acidification results in stimulation of proteolysis and mRNAs of proteins encoding the ubiquitin-proteasome pathway in BC3H-1 myocytes. Proc Natl Acad Sci U S A 93:1967-1971, 1996
25. Pereira BJ, Shapiro L, King AJ, et al: Plasma levels of IL-1B, TNF-a and their specific inhibitors in undialyzed chronic renal failure, CAPD, and hemodialysis patients. Kidney Int 45:890-896, 1994
26. Mitch WE, Price SR: Transcription factors and muscle cachexia: Have we defined a therapeutic target? Lancet 357:734-735, 2001
27. Reaich D, Channon SM, Scrimgeour CM, et al: Ammonium chloride-induced acidosis increases protein breakdown and amino acid oxidation in humans. Am J Physiol 263:E735-E739, 1992
28. Ballmer PE, McNurlan MA, Hulter HN, et al: Chronic metabolic acidosis decreases albumin synthesis and induces negative nitrogen balance in humans. J Clin Invest 95:39-45, 1995
29. Papadoyannakis NJ, Stefanides CJ, McGeown M: The effect of the correction of metabolic acidosis on nitrogen and protein balance of patients with chronic renal failure. Am J Clin Nutr 40:623-627, 1984
30. Reaich D, Channon SM, Scrimgeour CM, et al: Correction of acidosis in humans with CRF decreases protein degradation and amino acid oxidation. Am J Physiol 265:E230-E235, 1993
31. Garibotto G, Russo R, Sofia A, et al: Skeletal muscle protein synthesis and degradation in patients with chronic renal failure. Kidney Int 45: 1432-1439, 1994
32. Williams B, Hattersley J, Layward E, et al: Metabolic acidosis and skeletal muscle adaptation to low protein diets in chronic uremia. Kidney Int 40:779-786, 1991
33. Graham KA, Reaich D, Channon SM, et al: Correction of acidosis in hemodialysis decreases whole-body protein degradation. J Am Soc Nephrol 8:632-637, 1997
34. Movilli E, Zani R, Carli O, et al: Correction of metabolic acidosis increases serum albumin concentration and decreases kinetically evaluated protein intake in hemodialysis patients: A prospective study. Nephrol Dial Transplant 13:1719-1722, 1998
35. Graham KA, Reaich D, Channon SM, et al: Correction of acidosis in CAPD decreases whole body protein degradation. Kidney Int 49:1396-1400, 1996
36. Stein A, Moorhouse J, Iles-Smith H, et al: Role of an improvement in acid-base status and nutrition in CAPD patients. Kidney Int 52:1089-1095, 1997