Insights into the Abnormalities of Chronic Renal Disease Attributed to Malnutrition

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Abstract. Low values of serum proteins and loss of lean body mass are commonly found in patients with chronic renal insufficiency (CRI) and especially in dialysis patients. These abnormalities have been attributed to malnutrition (i.e., an inadequate diet), but available evidence indicates that this is not the principal cause. In contrast, there is persuasive evidence that secondary factors associated with the CRI condition cause abnormalities in protein turnover and ultimately result in low serum protein levels and loss of lean body mass. Recent reports have identified some factors that could interfere with the control of protein turnover in CRI patients, including acidosis, inflammation, and/or resistance to anabolic hormones. Each of these stimulates protein breakdown in muscle and activates a common proteolytic pathway, the ubiquitin-proteasome pathway. Moreover, acidosis or inflammation suppress hepatic albumin synthesis. Understanding the biochemical mechanisms that regulate the ubiquitin-proteasome and other catabolic pathways are required to identify new strategies for preventing protein deficits that are associated with CRI.
Table 1. Premises that implicate malnutrition as the cause of abnormal serum proteins and lean body mass in patients with kidney disease

- Dialysis alone will improve metabolic processes and correct these abnormalities
- Dialysis stimulates a patient’s appetite to correct these abnormalities
- A low-protein diet per se (i.e., malnutrition) is responsible for these abnormalities

(compared with changes in the degradation of amino acids and protein (13)). Even after days of starvation, feeding did not significantly increase protein synthesis (14). Similar responses to dietary protein occur in patients with uncomplicated kidney disease (i.e., without other systemic problems) (15,16). However, increasing protein intake increases the accumulation of waste products, and in rats with CRI, excess dietary protein reduces the efficiency of using protein for growth and slows growth (17–19). Even the major anabolic hormone, insulin, stimulates muscle protein synthesis minimally in the intact organism, because in vivo insulin primarily acts to suppress protein degradation (20,21). There is good evidence that uremia induces resistance to the anabolic effects of insulin or insulin-like growth factor–1 (IGF-1) (22,23), making it even less likely that these hormones will stimulate protein synthesis. Excess protein intake by dialysis patients will require more dialysis to control waste product accumulation; unfortunately, increasing dialysis by itself, can stimulate catabolism, causing loss of lean body mass (24,25).

**Cellular Protein Turnover**

Maintaining protein stores means that the rates of synthesis and degradation of cellular protein are equal. This is a complicated process. First, the turnover rate of individual proteins varies widely, from minutes (regulatory factors, etc.) to days (structural proteins in muscle). Second, integrated protein turnover is far greater than the turnover of plasma proteins. Approximately 3.5 to 4.5 g protein/kg body wt per d (equivalent to 245 to 315 g protein/d in a 70-kg adult) are synthesized and degraded each day (26). Muscle is roughly 20% protein; therefore, this is the equivalent of protein contained in 1.2 to 2 kg muscle being turned over each day! Because enzymes, transcription factors, and regulatory proteins are critical for the function and survival of cells and because the turnover of individual cellular proteins varies so widely, it is not surprising that protein synthetic and protein degradative pathways are precisely controlled. Fortunately, loss of lean body mass appears to be the most prominent consequence of impaired protein turnover in CRI (presumably, there are factors controlling the function of metabolic pathways or gene expression besides the concentration or content of proteins being degraded at an accelerated rate and/or synthesized at a slower rate).

**Factors Causing Protein Deficits**

How can CRI change protein turnover and cause loss of lean body mass? Potential factors are listed in Table 2. Experimental studies have demonstrated that the metabolic acidosis of CRI stimulates the catabolism of protein and amino acids; these results have been confirmed in CRI patients (27–31). Another factor in CRI that could cause protein deficits is inflammation: uremia is associated with high circulating levels of acute phase reactant proteins (3,32). Evidence that links protein deficits in hemodialysis patients to inflammation includes high levels of acute phase reactant proteins and low levels of serum albumin (33,34). In addition, tumor necrosis factor and interleukins are high in the blood of hemodialysis or CRI patients (35,36); these cytokines/inflammatory factors could cause excessive protein catabolism and suppression of hepatic synthesis of albumin (34,37–40). The third mechanism in CRI is resistance to the anabolic effects of insulin (low levels of insulin stimulate protein degradation in muscle) (41–44). Similar evidence that the anabolic effects of IGF-1 are impaired by CRI has been reported (23).

**Metabolic Acidosis Stimulates Amino Acid and Protein Catabolism**

Metabolic acidosis stimulates amino acid and protein catabolism in infants, normal adults, and CRI patients (15,16,45–47). For example, subnormal values of branched-chain amino acids (BCAAs) are common in CRI patients (2), and correction of the acidosis raises the levels of these essential amino acids (48,49). The first documentation of this catabolic response to acidosis was the report that plasma levels of valine, leucine, and isoleucine and the valine concentration in muscle water of acidic rats with CRI (50). These abnormalities were corrected by feeding sodium bicarbonate. The mechanism underlying the accelerated degradation of BCAAs is increased activity of branched-chain ketoacid dehydrogenase (BCKAD), the rate-limiting enzyme in the irreversible oxidation of BCAAs. May et al. (51) reported that the V_max of BCKAD is increased in the muscle of rats with experimentally induced metabolic acidosis; the increase in BCKAD activity in muscle of acidic rats is caused by an increase in the fraction of the enzyme in the dephosphorylated, activated form plus an increase in the mRNAs for E1a, E1b, and E2 subunits of the enzyme (52). These biochemical mechanisms depend on the presence of both acidification and glucocorticoids (53). In humans as well, metabolic acidosis stimulates the degradation of BCAAs; when normal adults were fed NH4Cl to induce acidosis, leucine oxidation increased 25% (46). If CRI patients are treated to raise serum bicarbonate levels from 16 to 21 mM, leucine degradation is suppressed by 29% (29). Lofberg et al.
metabolism was Papadoyannakis also causes protein wasting in CRI patients, including dialysis compared with that of sham-operated, pair-fed rats. The mechanism underlying nitrogen losses was linked to a 90% increase in the rate of muscle protein degradation but was eliminated by correcting acidosis with dietary sodium bicarbonate. Acidosis also causes protein wasting in CRI patients, including dialysis patients. Among the first to show that acidosis impairs protein metabolism was Papadoyannakis et al. (56), who showed that treating CRI patients with sodium bicarbonate improved their nitrogen balance. Williams and colleagues (57) examined acidic CRI patients who were eating a high-protein diet and found that protein degradation did not decrease when patients were switched to a low-protein diet. But, normal adaptive responses were restored when the same CRI patients were treated to raise serum bicarbonate from 18 to 24 mM. Reaich et al. (29) found that acidosis accelerated protein degradation in CRI patients but when acidosis was corrected, protein degradation decreased by 28%. When they gave patients an equimolar amount of sodium chloride, excessive protein breakdown returned. This group subsequently reported that accelerated protein degradation stimulated by acidosis in CRI patients cannot be inhibited by insulin (58). Garibotto et al. (59) measured protein degradation in the forearm of CRI patients and found it to be inversely correlated with serum bicarbonate but directly correlated with the serum cortisol level. Acidosis can also explain a low serum albumin, because it impairs albumin synthesis (60). In fact, Movilli et al. (61) showed that simply correcting metabolic acidosis of hemodialysis patients led to an increase in their serum albumin concentrations.

The cellular pathways by which metabolic acidosis stimulates protein degradation have not been identified; there could be a primary effect of acidification and/or secondary effects due to changes in hormonal responses induced by acidification. For example, metabolic acidosis causes resistance to insulin (62) and impaired function of growth hormone, thyroid hormone, and the conversion of vitamin D to its most active form, 1,25(OH)_{2}cholecalciferol (63–65).

Despite all these reports that document the catabolic effects of metabolic acidosis, there are cross-sectional studies that report little or no relationship between the degree of acidosis and signs of malnutrition in dialysis patients (66). Examination of these reports reveals at least two major problems. First, a single value of serum bicarbonate is not sufficient to define acidosis; and second, there can be a major technical problem in the measurement of serum bicarbonate. A delay in measuring serum bicarbonate creates unpredictable changes in its level, which is relevant because blood chemistries of patients in many dialysis units are performed in laboratories distant from the unit (67).

Biochemical Mechanisms Stimulating Protein Loss

Recent reports have given insights into biochemical mechanisms linking acidosis to the stimulation of protein degradation in muscle cells. England et al. (68) studied protein turnover in BC_{3}H-1 myocytes and found that acidification of the incubation media increased cellular protein degradation; this was not corrected by adding insulin. These studies were extended to show that acidification-induced protein catabolism in BC_{3}H-1 myocytes requires glucocorticoids. There was no increase in protein degradation upon acidification of the media unless glucocorticoids were present and accelerated proteolysis was blocked by adding RU 486, the steroid receptor antagonist (69). Similar findings were obtained in rats with NH_{4}Cl-induced metabolic acidosis (28). These results imply that acidification activates one or more pathways of protein breakdown in muscle cells.

The pathway that degrades muscle protein has been identified as the ATP-ubiquitin-proteasome pathway (26). This pathway uses energy for reactions that conjugate ubiquitin to substrate proteins that are destined for degradation. Ubiquitin is present in all cells and a member of the heat-shock protein family. Its conjugation to a substrate protein leads to recognition of the protein by the 26S proteasome. The proteasome removes ubiquitin, unravels the protein, and inserts it into the central core of the proteasome, where it is rapidly degraded to peptides. Functions of the proteasome also require energy. Bailey et al. (27) proved that the acidosis of CRI stimulates the ubiquitin-proteasome pathway in muscle to degrade protein when they showed that inhibiting the pathway prevents excessive muscle protein catabolism. Responses to metabolic acidosis stimulate more than flux through a proteolytic pathway; they also activate a program of protein breakdown. Specifically, signals that increase muscle proteolysis via the ATP-ubiquitin-proteasome–dependent pathway also increase the levels of mRNAs encoding components of the pathway. The latter response is due to increased transcription of genes that encode ubiquitin and proteasome subunits (27,42,44). This presumably functions to increase the capacity of the pathway to degrade protein. Results that suggest activation of a catabolic program are reminiscent of the increase in mRNAs encoding subunits of BCKAD in muscle of rats with metabolic acidosis (52).

Besides acidification, factors that could stimulate loss of lean body mass in CRI include the responses to inflammation and resistance to anabolic hormones (Table 2). Interestingly, there is considerable evidence that both of these factors cause muscle protein degradation by stimulating the ubiquitin-proteasome pathway. Kaysen (34) was the first to associate high levels of acute-phase reactant proteins in dialysis patients with
abnormal protein turnover: specifically, low values of serum albumin, a major marker of accelerated mortality in dialysis patients (70). There is evidence that dialysis patients have high circulating levels of inflammatory cytokines (e.g., interleukin-1 and -8 and tumor necrosis factor) (35,36). In fact, dialysis has been characterized as a chronic inflammatory state that increases the risk of cardiovascular disease (3,32). The relevance to loss of lean body mass is that injection of inflammatory cytokines causes excessive protein catabolism (37–40). Moreover, it is well established that animal models of diseases and disorders associated with inflammation (e.g., sepsis, cancer, burns, etc.) activate protein degradation through the ubiquitin-proteasome system (26). However, interactions between inflammatory mediators and activation of the ATP-ubiquitin-proteasome pathway are complex: Du et al. (71) examined the role of inflammation by studying the ability of NF-κB, the inflammatory transcription factor, to activate the ubiquitin-proteasome pathway in skeletal muscle cells. Contrary to expectations, they found high levels of NF-κB in quiescent muscle cells and showed that NF-κB acts to suppress transcription of proteasome subunit genes; incubation of cells with cytokines also reduces the rate of protein degradation. Clues to the physiologic significance of these results were provided when they found that glucocorticoids block the ability of NF-κB to suppress transcription of proteasome subunit genes and activate protein degradation in muscle cells (71). Thus, minor inflammatory illnesses (e.g., upper respiratory infection, etc.) that release cytokines would actually suppress muscle inflammation and glucocorticoid production, the suppressive effect of proteasome subunit genes; incubation of cells with cytokines also reduces the rate of protein degradation. Clues to the physiologic significance of these results were provided when they found that glucocorticoids block the ability of NF-κB to suppress transcription of proteasome subunit genes and activate protein degradation in muscle cells (71). Thus, minor inflammatory illnesses (e.g., upper respiratory infection, etc.) that release cytokines would actually suppress muscle protein loss by mechanisms that involve activation of NF-κB. However, if the disease/disorder stimulates more intense inflammation and/or resistance to anabolic hormones activate catabolic pathways to degrade muscle protein. In CRI, certain factors (e.g., metabolic acidosis) that interfere with the control of protein turnover can be easily addressed. Progress in this area to create treatment strategies will require insights into the biochemical mechanisms that regulate the proteolytic pathways.

References


