

# Amino Acid and Protein Kinetics in Renal Failure: An Integrated Approach

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Even apparently healthy patients on dialysis have significant loss of lean body mass. Patients with chronic renal failure without coexisting metabolic acidosis or inflammation have decreased protein turnover, with balanced reduction in protein synthesis and breakdown. However, regional and whole-body protein kinetic studies indicate that hemodialysis (HD) induces net increase in protein breakdown. Whole-body protein turnover studies show that HD is associated with decreased protein synthesis, but proteolysis is not increased. Muscle protein kinetics studies, however, identify enhanced muscle protein breakdown with inadequate compensatory increases in synthesis as the cause of the catabolism. Transmembrane amino acid-transport kinetics studies show that the outward transport is increased more than the inward transport of amino acids during HD. Altered intracellular amino acid transport kinetics and protein turnover during HD could be caused by the loss of amino acids in the dialysate or cytokine activation. Cytokines may be released from peripheral blood mononuclear cells and skeletal muscle during HD. Preliminary evidence indicates that intradialytic increase in cytokines activates the ubiquitin-proteasome pathway. An intradialytic increase in albumin and fibrinogen synthesis is facilitated by interleukin-6 and the constant supply of amino acids derived from skeletal muscle catabolism. Protein anabolism can be induced in end-stage renal disease patients by repletion of amino acids, and perhaps treatment with recombinant human insulin-like growth factor.

Semin Nephrol 26:158-166 © 2005 Elsevier Inc. All rights reserved.

**KEYWORDS** end-stage renal disease, protein turnover, cytokines, inflammation, nutrition

Malnutrition is common in patients with chronic renal failure (CRF). Decreased lean body mass is a reliable marker of protein energy malnutrition and an important predictor of mortality in end-stage renal disease (ESRD) patients. It is believed that several factors such as the accumulation of uremic toxins, anorexia, metabolic acidosis, loss of metabolizing renal tissue, perturbations in the production or responsiveness to catabolic and anabolic hormones, and activation of cytokines act in concert to contribute to the muscle wasting in uremia. Although renal replacement therapy attenuates some of the earlier-mentioned abnormalities, it also promotes muscle protein catabolism through activation of cytokines and loss of amino acids (AAs) in the dialysate.

A healthy adult synthesizes and degrades at least 200 g/d of

tissue protein; consequently a sustained change in protein balance will have a major impact on lean body mass, even if the change from baseline is small.<sup>1</sup> Only about 20% of the protein synthesis comes from daily AA consumption.<sup>2</sup> The remaining 80% is derived from recycling of AAs from protein breakdown, mostly in skeletal muscle.<sup>2,3</sup> Thus, protein turnover should be studied in the context of AA transport kinetics for better understanding of the process.

## Amino Acid Profile in CRF/ESRD

A number of alterations in muscle AA levels and exchange across peripheral tissues in postabsorptive and protein-fed states have been described in patients with moderate renal insufficiency. Plasma essential AAs (EAAs), notably branched-chain AAs (BCAAs), is decreased in patients with CRF. The normal kidney is involved actively in the metabolism of AAs and therefore the loss of functioning renal mass may be an important factor in the abnormal

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aminogram in CRF.<sup>4</sup> The liver and muscle cooperate in the homeostasis of plasma AA levels. Several observations suggest that CRF alters this cooperative role between liver and muscle. In the postprandial phase, most NEAAs are captured and metabolized by the splanchnic organs, particularly the liver, whereas the EAAs, mainly BCAAs, escape from the splanchnic region and enter the hepatic vein.<sup>5</sup> Accordingly, under normal conditions the postprandial AA profile is characterized by a large increase in EAAs, but only a small increase in NEAAs. However, in CRF patients, protein meal<sup>6</sup> or AA ingestion<sup>7</sup> is followed by an exaggerated increase of NEAAs in arterial blood. This difference possibly is caused by a defect in AA metabolism by splanchnic organs mediated by resistance to the action of insulin or metabolic acidosis. The peripheral uptake of total EAAs in CRF is not different from healthy volunteers, but the uptake of NEAAs is increased in response to increased NEAA concentration in the blood.<sup>8</sup> The nonbalanced profile of AAs taken up by peripheral tissue may affect protein synthesis because an appropriate profile of AAs is necessary to replenish protein in muscle.<sup>9</sup>

Renal replacement therapy does not normalize the AA profile. When compared with patients on hemodialysis (HD), peritoneal dialysis (PD) patients have higher intracellular, but lower plasma, concentrations of AAs.<sup>10</sup> Raj et al<sup>11</sup> reported that during nocturnal HD, the progressive increase in plasma total AA, EAA, NEAA, and BCAA concentrations on change over to nocturnal HD, in which the weekly Kt/V is significantly higher than conventional hemodialysis. However, abnormalities in the ratios of EAAs/NEAAs, valine/glycine, and tyrosine/phenylalanine persisted.

## Amino Acid Transport Kinetics and Protein Turnover

Skeletal muscle provides a quantitatively important storage system for AAs. It helps to maintain the concentrations of AAs in the blood within normal limits under changing conditions. Concentrations of most AAs in the intracellular water are considerably higher than their concentrations in the plasma.<sup>12</sup> All AAs, however, are not concentrated in the cell to the same degree. The tissue to plasma ratio may vary from 2 to 58, depending on the tissue and AA studied.<sup>13</sup> This is a result of independently regulated active transport systems for AAs in the cell membrane. Concentrations of AAs in the blood and muscle-free pool are determined by protein turnover, interorgan AA exchange, and intracellular recycling of AAs.<sup>14-16</sup> In catabolic states and starvation, AAs are released from the muscle into the blood to be used in other organs. On the other hand, AAs can be taken up actively by muscle during anabolism. Fig 1 shows the interorgan and intracellular recycling of AAs.

Alanine and glutamine constitute about 60% to 70% of AA released by muscle, although they only account for less than 15% of the total muscle protein.<sup>17</sup> Such data indicate that these 2 AAs are synthesized de novo in skeletal muscle.<sup>18</sup> Conversely, there are more of the BCAAs present in the mus-

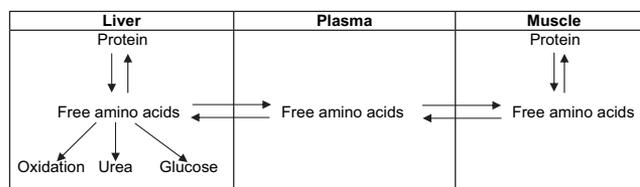


Figure 1 Interorgan and intracellular recycling of AAs.

cle than are released, indicating that a significant component of these AAs is used within the muscle with a lesser amount released into the plasma compartment. Raj et al<sup>19</sup> studied the transport kinetics of essential and nonessential AAs during HD using a 3-pool model (artery, vein, and muscle). The plasma AA concentrations decreased during HD as a result of loss in the dialysate. However, intradialytic decreases in arterial concentrations of total AAs (24.9% versus 15.9%), EAAs (33.4% versus 15.8%), NEAAs (25.4% versus 15.8%), and BCAAs (20.5% versus 9.5%) were higher than that in the vein, reflecting net release from the muscle.<sup>20</sup> Study of intracellular AA transport kinetics showed that the outward transport (from muscle to vein) is significantly higher than the inward transport (artery to muscle). Despite the decrease in the concentrations of AAs in the blood compartment and net efflux of AAs from the muscle, the intracellular concentrations remained relatively stable intradialysis.

AAs can appear in the cell via transport from arterial blood, from protein breakdown, and/or from de novo synthesis in the case of nonessential AAs. Phenylalanine is neither synthesized de novo nor degraded in the muscle. Hence, intracellular appearance of phenylalanine represents protein synthesis and intracellular appearance is an index of protein degradation. For alanine and glutamine, intracellular appearance represents the sum of de novo synthesis and appearance from proteolysis. During HD, the intracellular appearance of alanine and glutamine derived from protein breakdown increased by 74.3% and 60% respectively, but the de novo synthesis of alanine and glutamine did not change significantly.<sup>20</sup> These results indicate that intracellular AA concentration is maintained during HD, predominantly by augmented protein catabolism.

The AAs released from protein catabolism can have any 1 of 3 fates: they can be re-used for protein synthesis, used for synthesis of other AAs, or may exit the muscle pool into the vein. Augmented intradialytic AA efflux from the muscle could be a compensatory mechanism to maintain the plasma AA concentration in the face of intradialytic loss of AAs or a result of ineffective use of AAs or both. Protein synthesis efficiency, an estimate of the fraction of AAs that appear in the intracellular pool, is directed to protein synthesis. The muscle protein synthesis efficiency decreased from 41% to 30% during HD.<sup>20</sup>

## Methods of Studying Protein Turnover

The protein balance may be studied using nitrogen balance, arteriovenous balance of AAs, or by using tracer methodol-

ogy. The classic technique of nitrogen balance, which involves estimation of the nitrogen intake and output, and the changes in body nitrogen pools, are measured over a period of time. In this method, intake generally is overestimated and output is underestimated, resulting in an error toward positive body balances. Unlike nitrogen balance studies, which reflect net change in body protein stores over a period of time, whole-body protein turnover studies provide rapid analysis of specific dynamic and discrete processes that are important to the understanding of whole-body protein balance. With constant infusion of the tracer, an isotopic equilibrium is achieved, a state in which the tracer/tracee ratio is constant. Any change in enrichment over time occurs as a consequence of dilution from the unlabeled tracee. From this data the rate of appearance and hence the protein turnover may be estimated. The protein turnover can be estimated at the whole-body or regional level. Whole-body protein kinetics are studied generally by using L-(1-<sup>13</sup>C) leucine as a tracer. Rates of protein synthesis and breakdown are measured as the rates of the AA appearance into and disappearance from plasma. Leucine removal from the circulation can occur only by 2 routes: leucine oxidation (LO) and incorporation into protein. Consequently, nonoxidative leucine disposal (NOLD) provides an index of whole-body protein synthesis. Regional protein kinetics across the limb or splanchnic region can be derived from arterial and venous enrichment and AA concentrations and blood flow rate across the region. L-(ring <sup>13</sup>C<sub>6</sub>) phenylalanine typically is used to estimate protein turnover in the muscle because it is neither synthesized nor metabolized by muscle; its appearance rate, therefore, represents protein degradation, and its disappearance represents protein synthesis. The same data can be used to estimate the transmembrane AA transport kinetics (see later).

## Protein Turnover in CRF

Baliga et al<sup>21</sup> measured the incorporation of 3H phenylalanine into the gastrocnemius muscle of rats with varying degrees of CRF and reported that the protein synthesis rate was depressed when renal function was less than 30% of normal. Rats with CRF have an impaired response to the anabolic effect of insulin.<sup>22</sup> Also, hepatic protein synthesis is decreased in CRF rats by 30% to 40% despite an increase in the number of liver polysomes.<sup>23</sup> The results from human studies are more controversial than those from animal studies. In patients with CRF (without metabolic acidosis), there is a balanced reduction in both protein synthesis and catabolism.<sup>24</sup> Adey et al<sup>25</sup> showed that CRF patients have decreased synthesis of mixed muscle proteins. The muscle protein synthetic rate was correlated negatively with the severity of renal failure. A positive linear relationship between renal function and the rate of whole-body protein degradation has been reported.<sup>26</sup> During euglycemic hyperinsulinemia, the decrease in proteolysis, net leucine flux into protein, and LO in CRF patients was not different from that of controls.<sup>27</sup> In response to AA infusion, the leucine turnover and LO increased equally in controls and CRF patients, but the increase

in NOLD was more pronounced in controls compared with CRF patients. These results suggest that there are some subtle defects in protein metabolism in CRF.

The Modification of Diet in Renal Disease study showed that dietary protein intake begins to decrease when the glomerular filtration rate is approximately 25 mL/min, and the calorie intake decreases even earlier.<sup>28</sup> Major adaptation to a reduction in protein intake involves a decrease in protein turnover through marked reduction in protein degradation, and a small decrease in protein synthesis. Tom et al<sup>29</sup> reported that in CRF patients (glomerular filtration rate  $18 \pm 2$  mL/min) placed on a very low protein diet for more than a year maintained a neutral nitrogen balance and did not show any change in whole-body protein synthesis, degradation, or LO during follow-up evaluation. Thus, adaptive responses for protein conservation are operative in stable patients with mild and moderate renal failure. However, these adaptations may not be representative of the response to dietary protein restriction in the presence of more advanced CRF, which frequently is complicated by metabolic acidosis.

## Metabolic Acidosis and Protein Turnover

May et al<sup>30</sup> first reported that metabolic acidosis increased protein catabolism in skeletal muscle. These investigators showed that when metabolic acidosis was induced by NH<sub>4</sub>Cl, growth was stunted and urinary nitrogen excretion increased compared with paired controls. Net protein degradation was increased in muscles from acidotic rats, both in the absence and presence of insulin. The catabolic response to acidosis is at least in part caused by a coordinated increase in branched-chain ketoacid dehydrogenase activity<sup>30</sup> and the adenosine triphosphate-dependent ubiquitin-proteasome (Ub-PC) proteolytic pathway in skeletal muscle.<sup>31</sup> Plasma insulin-like growth factor-1 (IGF-1) concentration decreases in response to chronic metabolic acidosis.<sup>32</sup> Garibotto et al<sup>33</sup> showed that net proteolysis correlated positively with plasma bicarbonate and cortisol levels in CRF patients. Correction of metabolic acidosis in continuous ambulatory peritoneal dialysis (CAPD) and HD patients decreased both protein degradation and synthesis, but it had no effect on leucine oxidation.<sup>34,35</sup>

## Hormonal Regulation of Protein Metabolism

Protein turnover and AA transport kinetics are regulated by hormones. Insulin has been shown to influence the activity of at least 4 distinct transport systems of AAs, namely systems A, ASC, Nm, and Xc.<sup>36</sup> Uremia is associated with insulin and catecholamine resistance, and glucocorticoid excess.<sup>37,38</sup> Quantitative or qualitative insulin deficiency may result in muscle wasting through depressed protein synthesis and increased proteolysis.<sup>39,40</sup> Lim et al<sup>41</sup> studied the effect of insulin and insulin plus AA infusion in ESRD patients before and after initiation of HD. They showed that insulin alone reduced proteolysis and LO, and insulin plus AAs increased net

protein synthesis. These data indicate that response to the protein anabolic actions of insulin is preserved in ESRD patients. Glucocorticoids have a permissive effect on muscle proteolysis.<sup>42</sup> Glucagon, epinephrine, and norepinephrine augment cortisol-induced nitrogen loss.<sup>43</sup> Growth hormone (GH) administration has been shown to stimulate AA transport and protein synthesis, which probably is mediated by IGF-1. GH treatment increased muscle IGF-1 messenger RNA (mRNA) levels in control but not in CRF rats.<sup>44</sup> Skeletal muscle resistance to GH in CRF is caused, at least in part, by impaired Janus-associated kinase 2–signal transducers and activators of transcription 5 phosphorylation and translocation.<sup>44</sup> Furthermore, CRF rats also are resistant to the IGF-1–induced suppression of protein degradation and stimulation of protein synthesis.<sup>45</sup>

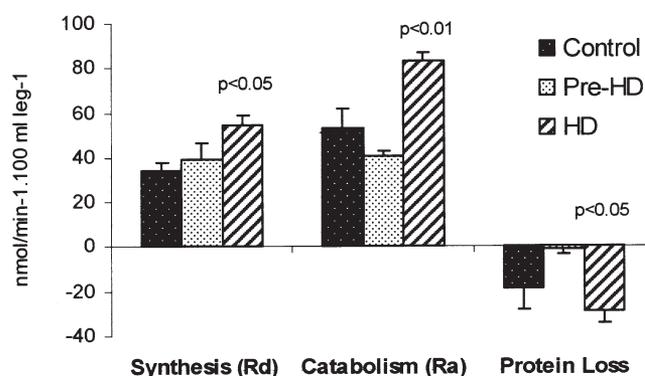
## Protein Turnover During Hemodialysis

The dietary requirement of protein is higher in dialysis patients than in normal subjects and in nondialyzed patients with CRF.<sup>46</sup> Healthy subjects have a daily minimum protein requirement of  $0.6 \text{ g} \cdot \text{kg}^{-1}/\text{day}^{-1}$ .<sup>47</sup> Although non-HD patients with CRF may be in nitrogen balance with  $0.6 \text{ g} \cdot \text{kg}^{-1}/\text{day}^{-1}$  of high-quality protein,<sup>48</sup> signs of malnutrition are present in ESRD patients (on HD) consuming approximately  $1.0 \text{ g} \cdot \text{kg}^{-1}/\text{day}^{-1}$  of protein.<sup>49</sup> Borah et al<sup>50</sup> showed that nitrogen balance is always more negative or less positive, depending on the intake, on dialysis days compared with nondialysis days.

Berkelhammer et al<sup>51</sup> found that whole-body protein breakdown was not increased in ESRD, but LO was higher compared with controls. In children with ESRD protein flux increased from baseline after initiation of dialysis.<sup>52</sup> Lim et al<sup>53</sup> measured longitudinally whole-body leucine flux in patients with CRF before and after initiation of dialysis. By using mass balance calculations, they found that the protein synthesis is increased more than degradation after initiation of HD. In another study, they showed that HD treatment is associated with normal basal leucine flux, a transient decrease in protein synthesis, and a negative protein balance.<sup>54</sup> Ikizler et al<sup>55</sup> found that HD results in a net whole-body protein loss, which improved postdialysis. Raj et al<sup>19,20,56</sup> estimated the whole-body, regional, and fractional protein kinetics in ESRD patients using phenylalanine, leucine, and lysine as tracers and showed that protein balance is maintained in ESRD patients during the interdialytic period. Although both protein synthesis and catabolism increased during HD, the intradialytic increase in catabolism exceeded that of synthesis, resulting in a net protein loss (Fig 2).

## PD and Protein Kinetics

PD treatment provides 300 to 600 kcal/day<sup>-1</sup>, resulting in sustained hyperinsulinemia in these patients. Goodship et al<sup>24</sup> performed leucine turnover studies in CRF patients before and after 3 months of CAPD treatment. They observed

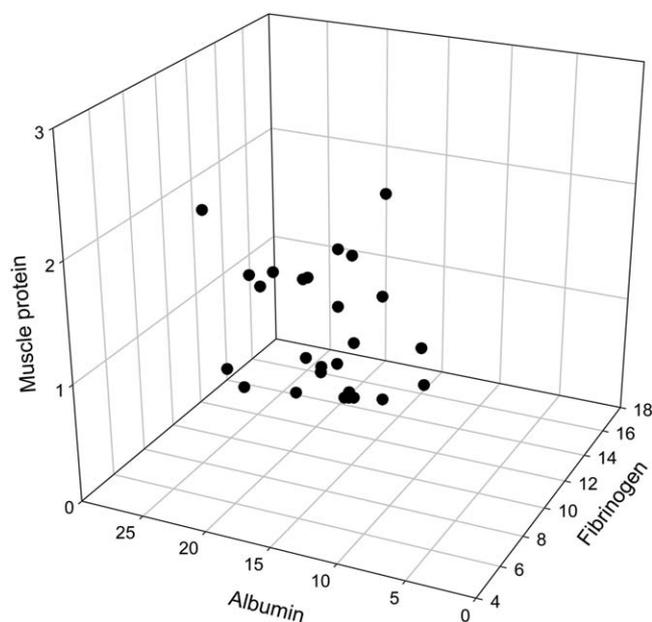


**Figure 2** Muscle protein synthesis and catabolism during HD. ■, Control; ▨, pre-HD; ▩, HD.

that protein turnover is decreased at baseline, but the balance between synthesis and breakdown is higher and remained unchanged after 3 months on CAPD. Long-term use of AA-based PD fluid has been shown to induce a positive protein balance.<sup>57</sup> Approximately 80% of leucine contained in the dialysate solution is absorbed through the peritoneum and approximately 43% of leucine absorbed is used for protein synthesis.<sup>58</sup> PD with dextrose-based dialysate induced an insulin-related decrease in plasma BCAA concentration along with decreased forearm protein breakdown, muscle protein synthesis, and negative protein balance.<sup>59</sup> However, dialysis with PD fluid containing dextrose plus AA increased the arterial concentration of AAs and plasma insulin levels, resulting in increased protein synthesis with no change in protein breakdown. The net effect was a positive nitrogen balance. Castellino et al<sup>60</sup> investigated the effect of a euglycemic insulin clamp and combined insulin plus AA on whole-body protein kinetics in CAPD patients. Basal leucine flux, LO, and NOLD were reduced in CAPD compared with control subjects. In response to insulin, endogenous leucine flux, LO, and NOLD decreased. During insulin plus AA administration, NOLD increased and net AA flux into protein became positive in both controls and CAPD patients to the same degree.

## Cytokines: Agent Provocateurs of Protein Catabolism

The role of cytokines in the catabolic response to sepsis, injury, and inflammatory states has been well established. Experimental studies in which normal rats were treated with recombinant cytokines<sup>61</sup> and septic rats were treated with cytokine antibodies<sup>62</sup> provide strong evidence that cytokines, especially tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1 (IL-1), facilitate muscle protein breakdown. TNF- $\alpha$  and IL-1 decrease the AA transport, which is facilitated by glucocorticoids.<sup>63</sup> IL-6 transgenic mice have augmented muscle atrophy.<sup>64</sup> Administration of antimurine IL-6–receptor antibody to colon-26 adenocarcinoma mice reduced the muscle wasting, which was associated with reduction in the enzymatic activity of cathepsin B plus L and mRNA levels of



**Figure 3** Coordinated increase in albumin, fibrinogen, and muscle protein synthesis during HD.

cathepsin L and poly-ubiquitin.<sup>65</sup> Nuclear factor  $\kappa$  B (NF- $\kappa$ B) plays a pivotal role in the catabolic response to TNF- $\alpha$ .<sup>66</sup> In skeletal muscle, TNF- $\alpha$  activates NF- $\kappa$ B via a rapid dose-dependent process that requires degradation of the inhibitory protein I-kappa B-alpha. Once I- $\kappa$ B $\alpha$  is degraded, NF- $\kappa$ B is translocated to the myocyte nucleus where it alters gene expression, stimulating protein loss in differentiated muscle cells.

In vitro and in vivo data suggest that cytokine transcription and or production are increased during HD. Although peripheral blood mononuclear cells are an established source of inflammatory cytokines during HD, skeletal muscle also is capable of generating these biomolecules. Raj et al<sup>67</sup> showed increased IL-6 mRNA and protein in the skeletal muscle and a net increase in the efflux of cytokines from the muscle into the vein during HD. They also showed that HD increased the gene expression of caspase-3, a marker of apoptosis; ubiquitin, a marker of proteolysis; and branched-chain keto acid dehydrogenase-E2, a marker of BCAA catabolism in the skeletal muscle.<sup>68</sup> The mRNA levels correlated positively with plasma IL-6.<sup>68</sup> Furthermore, they showed that intradialytic activation of IL-6 is associated with an (1) efflux of AAs from the muscle, (2) induction of negative AA balance, (3) increased protein turnover with net increase in protein catabolism, and (4) increased synthesis hepatic acute-phase proteins, albumin, and fibrinogen.<sup>56</sup> The intradialytic fractional synthesis rates of albumin, fibrinogen, and muscle protein were correlated with each other (Fig 3). In vitro studies indicate that IL-6 increases fibrinogen synthesis, but decreases albumin synthesis.<sup>69</sup> Intradialytic increases in muscle protein, albumin, and fibrinogen correlated positively with plasma IL-6 levels.<sup>56</sup> HD is associated with back transport of endotoxin fragments from the dialysate into the blood compartment. Exposure to endotoxin induces release of IL-6, and

increases synthesis of total liver proteins, including albumin, suggesting that there may be a nonspecific activation of all mRNAs involved in hepatic protein synthesis.<sup>70</sup> Thus, the coordinated increase in the fractional synthesis rates of albumin and fibrinogen probably is facilitated by constant delivery of AAs derived from the muscle catabolism and intradialytic increase in IL-6 level.<sup>56</sup>

## Dissociation Between Regional and Whole-Body Kinetics During HD

Whole-body protein turnover studies show that HD is associated with reduced protein synthesis without a substantial increase in proteolysis. Regional kinetics, on the other hand, document enhanced muscle protein breakdown with an inadequate compensatory increase in synthesis as the cause of the catabolism. It should be emphasized that organ-specific kinetics vary and whole-body turnover is a composite of all the visceral and somatic compartments taken together. During hemodialysis, as compared with that before or without dialysis, muscle kinetics invariably show an increase in protein breakdown.<sup>19</sup> By contrast, whole-body turnover shows only a minimal increase in proteolysis or no evidence of enhanced protein breakdown.<sup>54</sup> The net negative protein balance is more severe in magnitude in the muscle as compared with that of the whole body.<sup>56</sup> The discordance between whole-body and regional kinetics is best explained by the contrasting muscle and hepatic physiology. Raj et al<sup>56</sup> estimated the fractional synthesis rates of albumin, fibrinogen, and muscle protein by the precursor product approach using L(ring <sup>13</sup>C<sub>6</sub>) phenylalanine as a tracer in ESRD patients. They showed that the fractional synthetic rates of albumin, fibrinogen, and muscle protein during HD are increased by 38.6%, 53.5%, and 52.6%, respectively, compared with pre-HD estimates. They hypothesized that the intracellular increase in AAs derived from muscle catabolism stimulates muscle protein synthesis.<sup>71</sup> The use of AAs, however, is less efficient during HD, resulting in an increase in net outward transport of AAs into the vein.<sup>19</sup> The AAs released from the muscle are taken up by splanchnic bed to be used for acute-phase proteins synthesis, including albumin and fibrinogen.

## Molecular Mechanism of Protein Turnover

### Translation Initiation Factors

Translation initiation is regulated by a large number of protein factors termed *eukaryotic initiation factors* (eIF).<sup>72</sup> Translation of mRNA on the ribosome consists of the following 3 phases: initiation, whereby the initiator methionyl-transfer RNA (tRNA) associates with mRNA bound to the 40S ribosomal subunit and the subsequent binding of the 40S ribosome to the 60S subunit to form a complex capable of translation; elongation, during which tRNA-bound AAs are incorporated into growing polypeptide chains; and termina-

tion, during which the completed protein is released from the ribosome. The amount of eIF2 present in the ternary complex is dependent on the activity of eIF2B.<sup>72</sup> Translation of mRNAs occurs through the cap-dependent process involving eIF4E. The eIF4E can be sequestered into an inactive complex through binding of the translational repressor molecules 4E-BP1. Changes in the distribution of eIF4E between active eIF4E-eIF4G and the inactive eIF4E-BP1 complexes have been suggested as the mechanism through which AAs, insulin, IGF-1, and cytokines regulate global protein synthesis.<sup>73-75</sup> Kobayashi et al<sup>76</sup> showed that AA depletion by HD in nonuremic pigs reduced the activity of eIF2B and repletion increased its activity in the skeletal muscle. However, in ESRD patients, even with A repletion, the mRNA levels of eIF2B did not increase (unpublished data).

### Molecular Regulation of Protein Catabolism

Intracellular protein degradation is mediated by 3 well-characterized intracellular proteolytic systems: the lysosomal pathway, calcium-dependent proteinases (calpains), and the Ub-PC pathway, which accounts for about 80% of total proteolytic activity. Busquets et al<sup>77</sup> showed that starvation induced a 125% increase in calcium-dependent proteolysis, a 60% increase in adenosine triphosphate-dependent proteolysis, and no change in lysosomal proteolysis. Calpains play a significant role in myofibrillar protein degradation, especially in the disassembly of the myofibril during early stages of turnover. The lysosomal pathway degrades soluble and extracellular proteins and is not involved in the degradation of myofibrillar proteins. Calcium-dependent proteinases (calpains) degrade cytoskeletal, but not myofibrillar, proteins.

The Ub-PC system is involved in the degradation of the abnormal short-lived proteins. Increased mRNA levels for ubiquitin and proteasome subunits are reported in several muscle-wasting conditions, including CRF.<sup>78</sup> AA loss and cytokine activation can stimulate the Ub-PC pathway in the muscle.<sup>79</sup> The Ub-PC system does not break down the complexes of protein contained in actomyosin or myofibrils. Thus, 1 or more proteases must release constituent proteins of actomyosin before the Ub-PC system can degrade the muscle protein. Du et al<sup>80</sup> showed that activation of caspase-3 is the initial step in the process of accelerated muscle protein loss in catabolic conditions. Phosphatidylinositol 3 kinase activity is the key to activation of muscle proteolysis through regulation of caspase-3 and expression of atrogin-1/MAFbx.<sup>81</sup> Although this observation has not been studied rigorously in patients with renal failure, microarray studies show that a common set of transcriptional adaptations underlie the loss of muscle mass in different pathologic states,<sup>82</sup> including uremia.<sup>83</sup> Patients with renal failure have increased levels of angiotensin II. Infusion of angiotensin II in rats produces cachexia secondary to increased muscle proteolysis by downregulation of phospho-Akt and activation of caspase-3 in skeletal muscle.<sup>84</sup> mRNA levels of polyubiquitin and caspase-3 are increased in the muscle during HD,<sup>68</sup> suggesting that this pathway may be the mediator of protein breakdown intradialysis.

Myostatin is a negative growth regulator for skeletal muscle. Myostatin decreases protein synthesis by inhibiting cell proliferation and DNA synthesis by blocking satellite cell activity.<sup>85</sup> Preliminary evidence from our laboratory indicates that this pathway is involved in sarcopenia in patients with ESRD.<sup>83</sup>

## Interventions to Attenuate Protein Catabolism in ESRD

### Replacement of AAs

The total loss of AAs is approximately 9 to 13 g/HD.<sup>86,87</sup> Exogenous AA infusion improves inward transport of AAs<sup>88,89</sup> and increases protein synthesis,<sup>88,89</sup> but does not decrease protein catabolism<sup>88,89</sup> or improve synthesis efficiency.<sup>89</sup> Replacement of AAs by an intravenous route and through dialysate prevents a decrease in the plasma concentrations of AAs. AA-based dialysate improves protein synthesis in PD patients.<sup>59</sup> Pupim et al<sup>90</sup> showed that intradialytic parenteral nutrition promotes an increase in protein synthesis, a decrease in proteolysis at the whole-body level, and an increase in forearm muscle protein synthesis. They also showed that exercise may complement the anabolic response of intradialytic parenteral nutrition.<sup>90</sup> Saad et al<sup>91</sup> also found that with AA repletion during HD, both protein synthesis and breakdown in the muscle are increased. The net protein balance became less negative with AA infusion. Investigators have observed that a close link exists between muscle protein synthesis and breakdown and the link may supersede any direct influence of AA concentration on muscle catabolism. In catabolic or anabolic states, the extent of changes in muscle protein synthesis and breakdown differs with respect to each other, but the 2 factors generally change in the same direction.<sup>76</sup>

### IGF and Protein Turnover

IGF-1 promotes cellular uptake of glucose and AA, enhances protein synthesis, and suppresses protein degradation. IGF-1<sup>92</sup> and GH<sup>93</sup> administration stimulates skeletal muscle protein synthesis and a positive AA balance. The stimulation of protein synthesis by these hormones is related to an increased rate of inward transport of AAs.<sup>94</sup> Dose-response studies have shown impaired stimulation of protein synthesis and suppressed inhibition of protein degradation by IGF-1 in epitrochlearis muscle of CRF rats.<sup>95</sup> However, IGF-1 has been shown to induce net protein accretion in the muscle by increasing protein synthesis in the face of unchanged protein breakdown in malnourished ESRD patients.<sup>96</sup> In CAPD patients, IGF-1 induced a positive nitrogen balance, resulting in a sustained reduction in dialysate and urine nitrogen and serum urea nitrogen output.<sup>97</sup>

### Attenuation of Inflammation and Protein Turnover

Attenuation of inflammatory response and treatment with anticytokine antibodies have been shown to decrease protein

catabolism. Pentoxifylline is a xanthine-derived phosphodiesterase inhibitor, which can downregulate TNF gene expression and decrease circulating levels of TNF- $\alpha$  and TNF- $\alpha$ -soluble receptor. Biolo et al<sup>98</sup> showed that pentoxifylline decreased whole-body proteolysis in CRF through down-regulation of the TNF- $\alpha$  system.

## Conclusion and Future Directions

Sarcopenia is a strong predictor of outcome in a variety of acute and chronic illnesses. Even in apparently healthy patients on dialysis there is significant loss of lean body mass. Despite variations in protein turnover, a balance between protein synthesis and catabolism is maintained in uncomplicated CRF. The adaptive responses fail and the equilibrium is disturbed in the presence of catabolic stress or metabolic acidosis. However, HD induces an unbalanced increase in synthesis and catabolism, with a net increase in protein catabolism. Protein breakdown during HD could be caused by intradialytic loss of AAs, activation of cytokines, and defective use of AAs. Interventions aimed at these specific causes may attenuate protein breakdown in ESRD.

Our understanding of AA and protein metabolism has reached new dimensions with the use of tracer methodology. However, there are a number of unanswered questions that need to be explored. Re-use of amino acids released from protein breakdown is impaired during HD.<sup>56</sup> Although it is reasonable to assume that it is a result of the intradialytic loss of AAs and cytokine activation, their precise role in mediating this abnormal physiology is unknown. This is an important and potential area of research. AAs and cytokines modulate protein synthesis through translation-initiating factors. The current state of knowledge does not allow us to draw any concrete conclusion regarding whether this regulatory pathway is altered by uremia. Leucine is the principal AA that modulates the availability of eIF4E.<sup>99</sup> It will be interesting to explore whether supplementation of leucine alone will augment protein synthesis during HD. If effective, this will be a less labor-intensive intervention. The seminal work by Mitch and Goldberg<sup>79</sup> has defined the role of Ub-PC in muscle protein breakdown. However, the contribution of lysosomal- and calcium-dependent (calpains) pathways needs further investigation. Understanding the physiology of protein and AA metabolism and its derangement is vital to design targeted interventions.

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