Coordinated increase in albumin, fibrinogen, and muscle protein synthesis during hemodialysis: role of cytokines

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Submitted 2 October 2003; accepted in final form 17 December 2003

Raj, Dominic S. C., Elizabeth A. Dominic, Robert Wolfe, Vallabh O. Shah, Arthur Bankhurst, Philip G. Zager, and Arny Ferrando. Coordinated increase in albumin, fibrinogen, and muscle protein synthesis during hemodialysis: role of cytokines. Am J Physiol Endocrinol Metab 286:E658–E664, 2004. First published January 13, 2004; 10.1152/ajpendo.00444.2003.—Serum albumin, fibrinogen levels, and lean body mass are important predictors of outcome in end-stage renal disease (ESRD). We estimated the fractional synthesis rates of albumin (FSR-A), fibrinogen (FSR-F), and muscle protein (FSR-M) in nine ESRD patients and eight controls, using primed constant infusion of L-[ring-13C6]phenylalanine. Cytokine profile and arteriovenous balance of amino acids were also measured. ESRD patients were studied before (Pre-HD) and during hemodialysis (HD). Plasma IL-6, IL-10, and C-reactive protein increased significantly during HD. Despite a decrease in the delivery of amino acids to the leg, the outflow of the amino acids increased during HD. The net balance of amino acids became more negative during HD, indicating release from the muscle. HD increased leg muscle protein synthesis (45%) and catabolism (108%) but decreased whole body protein catabolism (15%). FSR-A during HD (9.7 ± 0.9%/day) was higher than pre-HD (6.5 ± 0.9%/day) and controls (5.8 ± 0.5%/day, P < 0.01). FSR-F increased during HD (19.7 ± 2.6%/day vs. 11.8 ± 0.6%/day, P < 0.01), but it was not significantly different from that of controls (14.4 ± 1.4%/day). FSR-M intradialysis (1.77 ± 0.19%/day) was higher than pre-HD (1.21 ± 0.25%/day) and controls (1.30 ± 0.32%/day, P < 0.001). Pre-HD FSR-A, FSR-F, and FSR-M values were comparable to those of controls. There was a significant and positive correlation between plasma IL-6 and the FSRs. Thus, in ESRD patients without metabolic acidosis, the fractional synthesis rates of albumin, fibrinogen, and muscle protein are not decreased pre-HD. However, HD increases the synthesis of albumin, fibrinogen, and muscle protein. The coordinated increase in the FSRs is facilitated by constant delivery of amino acids derived from the muscle catabolism and intradialytic increase in IL-6.

protein catabolism; protein turnover; inflammation; amino acid; end-stage renal disease

LOW SERUM ALBUMIN, INCREASED FIBRINOGEN, and decreased lean body mass are important predictors of mortality and morbidity in the general population and in patients with end-stage renal disease (ESRD). In addition to being nutritionally modulated, albumin is also a negative acute-phase protein. Fibrinogen, on the other hand, is an important positive acute-phase protein. Loss of muscle mass in ESRD may reflect decreased muscle protein synthesis, increased catabolism, or both. The relationship between albumin, fibrinogen metabolism, and muscle protein turnover is complex and interrelated. In some physiological and pathological conditions, there is concomitant increase in fractional synthesis rates of albumin (FSR-A), fibrinogen (FSR-F), and muscle protein (FSR-M) (13), but in others the responses may be discordant (14, 36). The response to injury, sepsis, and inflammation is characterized by an increase in total liver protein synthesis, along with depressed muscle protein synthesis.

Uremia is a state of microinflammation, with elevated cytokine and positive acute-phase protein levels (20), which is further augmented by hemodialysis (HD) (19). Acute-phase protein synthesis is transcriptionally regulated by cytokines (4). Increased production of cytokines and muscle protein breakdown have both beneficial and detrimental effects on the associated disease states. In the acute phase of illness, there is increased production of inflammatory cytokines, which accelerates the breakdown of skeletal muscle, providing the substrate for the liver to mount an acute-phase response (15). The acute-phase response is an important pathophysiological phenomenon that modulates the response to inflammation. This includes synthesis of proteins by the liver, which may positively or negatively influence the inflammatory process (15). However, continued loss of lean body mass is deleterious to the host and contributes to morbidity and mortality. Although intradialytic increases in FSR-A and FSR-F have been reported (8), their relationship to FSR-M and cytokine activation have not been rigorously studied. Arteriovenous balance studies have demonstrated net release of amino acids from the muscle during HD (18, 21, 34). We hypothesized that cytokine activation and release of amino acids from the muscle during HD may mediate the observed intradialytic increase in FSR-A and FSR-F.

This study was designed to examine the effect of hemodialysis and intradialytic activation of cytokines on albumin, fibrinogen, and muscle protein synthesis. We estimated arteriovenous balance of amino acids across the leg and fractional synthesis rates of albumin, fibrinogen, and muscle protein in nine ESRD patients and eight controls. ESRD patients were studied before (Pre-HD) and during HD.

MATERIALS AND METHODS

Methods

The study population included nine ESRD patients and eight controls. ESRD patients were placed on a 35 kcal/kg and 1.2 g protein/kg diet. Low serum albumin, increased fibrinogen, and decreased lean body mass are important predictors of mortality and morbidity in the general population and in patients with end-stage renal disease (ESRD). In addition to being nutritionally modulated, albumin is also a negative acute-phase protein. Fibrinogen, on the other hand, is an important positive acute-phase protein. Loss of muscle mass in ESRD may reflect decreased muscle protein synthesis, increased catabolism, or both. The relationship between albumin, fibrinogen metabolism, and muscle protein turnover is complex and interrelated. In some physiological and pathological conditions, there is concomitant increase in fractional synthesis rates of albumin (FSR-A), fibrinogen (FSR-F), and muscle protein (FSR-M) (13), but in others the responses may be discordant (14, 36). The response to injury, sepsis, and inflammation is characterized by an increase in total liver protein synthesis, along with depressed muscle protein synthesis.

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MATERIALS AND METHODS

Methods

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patients consumed the recommended diet at home for a minimum of 14 days before the study. Dietary intake was confirmed by a 3-day dietary history. Predialysis plasma bicarbonate (HCO₃⁻) was checked 3 wk before the experiment. If plasma HCO₃⁻ was <22 meq/l, patients were initiated on oral NaHCO₃ supplementation, and the dose was adjusted to achieve a target plasma HCO₃⁻ ≥22 meq/l. The experiments were performed only when the patient’s plasma HCO₃⁻ was >22 meq/l over a period of ≥2 wk.

Subjects were admitted to the General Clinical Research Center (GCRC) at the University of New Mexico 1 day before the experiment. ESRD patients were studied 72 h after dialysis treatment. This timing was selected to estimate the synthesis rates at the peak of metabolic abnormalities. All of the studies were performed in a postabsorptive state after an overnight fast. On the day of the experiment, diabetic subjects were given one-half of the usual dose of insulin or oral hypoglycemic agent. Blood sugar was closely monitored during the experiment and maintained within normal range by additional doses of regular insulin administered subcutaneously. Polyethylene catheters were inserted into the femoral artery and vein on the same side for blood sampling and in the nonaccess forearm veins for arterial lines. Leg volume was estimated using an anthropometric formula as described previously (7).

L-[ring-¹³C₆]phenylalanine (99% atom percent excess) was purchased from Cambridge Isotope Laboratories (Andover, MA). After blood samples were obtained for background amino acid enrichment, a primed (2 µmol/kg) continuous (0.1 µmol·kg⁻¹·min⁻¹) infusion of L-[ring-¹³C₆]phenylalanine was initiated through the forearm vein. Tracer infusion was continued throughout the experiment. Blood samples for enrichment were collected as previously described (7) (Fig. 1). Blood samples were taken at 0 and 300 min to estimate FSR-A and FSR-F before dialysis and at 540 min to calculate FSR during dialysis. Arteriovenous balance studies were performed before and during HD. Blood flow to the lower extremity was measured by aortic flow probe. Leg volume was estimated using an anthropometric formula as described previously (7).

Hemodialysis was initiated at 300 min and continued for 4 h. Patients’ usual blood and dialysate flow rates were used. Dialysate composition was as follows: 139 meq/l sodium (Na⁺), 35 meq/l HCO₃⁻, 2.5 meq/l calcium (Ca²⁺), 1 meq/l magnesium (Mg²⁺), 200 mg/dl dextrose, and potassium (K⁺) per patient’s need. A new polysulfone membrane (F70, Fresenius, Hemoflow) was used. Anticoagulation was not used during dialysis to minimize the risk of bleeding. Representative spent dialysate samples were collected to estimate the amino acid concentrations.

Plasma samples were obtained pre- and post-HD for blood urea nitrogen (BUN), creatinine, electrolytes, albumin, and fibrinogen. Albumin was measured by the bromcresol green method, prealbumin by nephelometry, insulin-like growth factor (IGF), catecholamines, and glucagon by radioimmunoassay (RIA), thyroid-stimulating hormone (TSH), insulin, cortisol, and C-reactive protein (CRP) by immulite chemiluminescence. Cytokines (TNF-α, IL-1, IL-6, and IL-10) were measured using commercially available ELISA kits (R&D Systems, Minneapolis, MN) according to the manufacturer’s directions. Assays were performed in duplicate, and the mean of the two measurements was used. The inter- and intra-assay coefficients of variation for each of the cytokines were <6%.

Muscle biopsies were performed at the second hour to measure isotopic carbon enrichment of bound and free phenylalanine in the muscle. The second biopsy was obtained at the fifth hour to estimate the fractional synthesis rates before HD. The third biopsy was obtained at the fourth hour of dialysis. Biopsies were taken from the lateral portion of the vastus lateralis muscle 20 cm above the knee by use of a Bergstrom biopsy needle. Fat and connective tissue were removed and the samples frozen in liquid nitrogen and stored at −80°C for future analysis.

**Analytical Methods**

**Blood.** Plasma samples were stored at −80°C for future analysis of tracer enrichment and amino acid concentrations. Amino acids were separated using cation exchange chromatography and dried under vacuum using a Speed-Vac (38). The enrichment of free amino acids in the plasma sample was determined by gas chromatography-mass spectrometry (GC-MS) (GC HP 5890 and MSD HP 5989; Hewlett-Packard, Palo Alto, CA) by selected ion [mass-to-charge ratio (m/z)] monitoring. Chemical ionization was used for nitrogen-acetyl-α-propyl ester (NAP) derivatives of phenylalanine (m/z 336, 342, and 346). Data are expressed as tracer-to-tracee ratios.

For determination of arteriovenous amino acid balance, 3 ml of blood from femoral artery and vein were collected into lithium-heparin tubes, centrifuged, and frozen at −80°C until analyzed. Free amino acid concentrations were determined by HPLC (Waters 2960 system; Milford, MA), precolumn derivatization, and o-phthalaldehyde-3-mercaptopropionic acid.
Albumin and fibrinogen. Albumin was isolated by ethanol extraction from trichloroacetic acid (TCA)-precipitated plasma proteins, as described previously (39). In short, citrated plasma was precipitated with 10% TCA and centrifuged, and the supernatant was discarded. The protein pellet was dissolved in absolute alcohol. The resulting supernatant was decanted to obtain isolated plasma albumin. The pellet was dried under vacuum. Albumin was hydrolyzed at 110°C for 24 h with 6 N constant-boiling HCl. The protein hydrolysates were washed through a cation exchange column to isolate purified amino acids. NAP derivatives of the purified albumin amino acids were then separated and analyzed by GC-MS. L-[ring-13C6]phenylalanine enrichment of albumin was determined from an M + 6/M + 3 ratio and an isotopic dilution curve.

To determine isotopic enrichment in fibrinogen, blood samples were transferred into heparin sodium-treated tubes and centrifuged at 4°C. Fibrinogen was precipitated from 2 ml of plasma by adding 40 μl of calcium chloride (1 M) and 4 U of thrombin and incubating at 22°C for 2 h (10). The clot was washed and centrifuged. The purity of the isolate was confirmed by SDS-gel electrophoresis. The resulting pellet was analyzed for phenylalanine enrichment as described before.

Muscle. Muscle samples were weighed, and protein was precipitated with 500 μl of 14% perchloric acid. The supernatant was collected after homogenization of the issue and centrifugation. The amino acids in the pooled supernatant were separated using cation exchange chromatography. The isotopic enrichment of the intracellular amino acid was determined on their NAP derivatives in the electron impact mode. Intracellular enrichment was corrected on the basis of the chloride method (5). The tissue pellet was further washed with saline and absolute alcohol and dried at 50°C overnight. The precipitated protein was hydrolyzed at 110°C for 24 h with 6 N constant-boiling HCl. The protein hydrolysate was then processed as blood samples, and phenylalanine enrichment was measured by GC-MS (GC 8000 series, MD 800; Fisons Instruments, Manchester, UK) using chemical ionization and the standard curve approach (9).

Calculations

Amino acids entering the leg through the femoral artery (F in) and leaving the leg via femoral vein (F out) were estimated as

\[ F_a = C_a \cdot BF \]
\[ F_{out} = C_v \cdot BF \]

where \( C_a \) and \( C_v \) are concentration of amino acid in the artery and vein, respectively, and BF is plasma flow rate.

Net amino acid balance (NB) in the leg was calculated as

\[ NB = (C_a - C_v) \cdot BF \]

The rate of disposal of arterial phenylalanine (R a) is an index of the absolute amount of protein synthesis. R a is calculated as

\[ R_a = [(E_a - E_v)/E_a] \cdot BF \]

where E a and E v are enrichment of amino acid in the artery and leaving the leg via femoral vein (F out) were estimated as

\[ E_a \]
\[ E_v \]

The whole body phenylalanine flux was calculated from the rate of tracer infusion (μmol·kg\(^{-1}\)·min\(^{-1}\)) divided by the arterial enrichment of phenylalanine. FSR-M was calculated by dividing the increment in enrichment in the product (ΔEP) by the average in the intracellular enrichment (precursor) from the first and second muscle biopsies. ΔEP is the difference in the enrichment of the muscle protein between the first and second muscle biopsies. The use of intracellular amino acid enrichment as a precursor for protein synthesis has been validated in vivo studies (37).

\[ \text{FSR} = \frac{\Delta EP}{T} \cdot \frac{1}{E_{m(1)} + E_{m(2)} - 1440 \cdot 100} \]

where \( E_{m(1)} \) and \( E_{m(2)} \) represent the L-[ring-13C6]phenylalanine enrichment in the free muscle pool in the consecutive biopsies. T is the time interval between first and second biopsies.

ΔIncrement in protein-bound L-[ring-13C6]phenylalanine enrichment (ΔEP) between first and second biopsies (ΔEP) is calculated as follows:

\[ \Delta EP = \frac{(13C)^2C_{(2)} - (13C)^2C_{(1)}}{1.5} \]

where (1) and (2) represent the isotope ratio from first and second biopsies.

FSR-A and FSR-F (%/day) between times t1 and t2 can be expressed by the following equation

\[ \text{FSR} = \frac{E_{b(t_2)} - E_{b(t_1)}}{\int_{t_1}^{t_2} E_{f(t)} dt} \]

where \( E_{b(t)} \) is defined as the enrichment of phenylalanine bound in albumin at time \( t \) and \( E_{f(t)} \) the enrichment of phenylalanine free in plasma at time \( t \). The equations for \( E_{f(t)} \) will be determined by fitting the following nonlinear regression curves to the values for \( E_{f(t_1)} \) and \( E_{f(t_2)} \)

\[ E_{f(t)} = a(1 - e^{-bt}) \]

where \( b > 0 \), \( a > 0 \) are regression parameters.

These regression curves were chosen to account for the behavior of plasma phenylalanine enrichment during the primed constant infusion (2).

Subjects

Healthy volunteers were recruited from the GCRC database for healthy volunteers. ESRD patients were recruited from the outpatient dialysis unit. There were two diabetic patients in the control and ESRD arms each. Only patients on maintenance HD for ≥90 days and without residual renal function were included. Patients with infective or inflammatory condition, pregnancy, liver dysfunction, bleeding tendency, catabolic illness, or unexplained weight loss were excluded from the study. The study was approved by the Human Research Review Committee at the University of New Mexico Health Sciences Center.

Statistical Analysis

Data are given as means ± SE. α Value was set at 0.05. Paired and unpaired t-tests were used when applicable. Repeated measures of analysis of variance (ANOVA) were used, with pre- and post-HD as the repeating factor and ESRD vs. control as the grouping factor, with a post hoc Tukey test. Linear regression analysis was used to identify relations between variables.

RESULTS

Patient characteristics are described in Table 1. Etiology of renal failure was glomerulonephritis (n = 2), hypertension (n = 2), tubulointerstitial nephropathy (n = 1), diabetes (n = 2), and unknown (n = 2). Protein (1.48 ± 0.09 g·kg\(^{-1}\)·day\(^{-1}\)) and calorie (33.2 ± 3.5 kcal·kg\(^{-1}\)·day\(^{-1}\)) intake in ESRD patients were not different from controls (protein 1.34 ± 0.06 g·kg\(^{-1}\)·day\(^{-1}\), calories 35.0 ± 121 kcal·kg\(^{-1}\)·day\(^{-1}\)). Gly-
Table 1. Patient characteristics and biochemistry

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Pre-HD</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, yr</strong></td>
<td>44.6±4.7</td>
<td>43.5±4.9</td>
<td></td>
</tr>
<tr>
<td><strong>Females, %</strong></td>
<td>1 (12.5)</td>
<td>1 (16.7)</td>
<td></td>
</tr>
<tr>
<td><strong>Diabetics, %</strong></td>
<td>2 (25)</td>
<td>2 (22.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Weight, kg</strong></td>
<td>79.3±17.2</td>
<td>74.8±3.4</td>
<td></td>
</tr>
<tr>
<td><strong>Hemoglobin, g/dl</strong></td>
<td>14.4±0.3a</td>
<td>12.2±0.3</td>
<td>12.3±0.5</td>
</tr>
<tr>
<td><strong>BUN, mg/dl</strong></td>
<td>14.4±1.0</td>
<td>66.3±6.2b</td>
<td>18.4±3.2</td>
</tr>
<tr>
<td><strong>Creatinine, mg/dl</strong></td>
<td>1.1±0.3</td>
<td>9.3±1.06b</td>
<td>4.9±0.8</td>
</tr>
<tr>
<td><strong>Glucose, mg/dl</strong></td>
<td>114±12.6</td>
<td>109.8±14.1</td>
<td>110.2±11.7</td>
</tr>
<tr>
<td><strong>Bicarbonate, mEq/l</strong></td>
<td>23.5±0.5</td>
<td>23.9±0.8</td>
<td>25.5±2.5</td>
</tr>
<tr>
<td><strong>Albumin, g/dl</strong></td>
<td>3.9±1.0c</td>
<td>3.2±0.1</td>
<td>3.4±0.2</td>
</tr>
<tr>
<td><strong>Fibrinogen, g/dl</strong></td>
<td>275.3±12.5</td>
<td>328.7±28.6</td>
<td>402.7±32.1</td>
</tr>
<tr>
<td><strong>Transferrin, μg/dl</strong></td>
<td>220.3±8.6d</td>
<td>168.9±9.9</td>
<td></td>
</tr>
<tr>
<td><strong>Prealbumin, mg/dl</strong></td>
<td>30.2±3.1</td>
<td>29.2±1.8</td>
<td></td>
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<tr>
<td><strong>Hb A1C</strong></td>
<td>7.2±1.1</td>
<td>6.9±1.8</td>
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</tr>
</tbody>
</table>

Values are means ± SE; HD, hemodialysis; BUN, blood urea nitrogen.

*Only in diabetics. \( P < 0.01\), HD vs. Control; \( P < 0.001\), Pre-HD vs. control and HD; \( P < 0.01\), Control vs. Pre-HD and HD; \( P < 0.01\), Control vs. Pre-HD and HD; \( P < 0.01\), Pre-HD vs. Pre HD.

Table 2. Hormone profile and cytokine activation during hemodialysis

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Pre-HD</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL-1, pg/dl</strong></td>
<td>0.99±0.32</td>
<td>1.20±0.34</td>
<td>1.40±0.42</td>
</tr>
<tr>
<td><strong>IL-6, pg/dl</strong></td>
<td>4.23±3.03</td>
<td>11.53±6.73a</td>
<td>27.86±14.83b</td>
</tr>
<tr>
<td><strong>IL-10, pg/dl</strong></td>
<td>1.29±0.85</td>
<td>0.63±0.73</td>
<td>2.22±1.07</td>
</tr>
<tr>
<td><strong>TNF-α, pg/dl</strong></td>
<td>0.46±0.17</td>
<td>1.01±0.21</td>
<td>1.19±0.47</td>
</tr>
<tr>
<td><strong>CRP, mg/dl</strong></td>
<td>1.01±0.19d</td>
<td>3.72±0.89</td>
<td>4.12±0.99e</td>
</tr>
<tr>
<td><strong>Insulin, μU/ml</strong></td>
<td>11.6±4.1</td>
<td>30.9±6.4a</td>
<td>20.5±3.1</td>
</tr>
<tr>
<td><strong>Glucagon, pg/dl</strong></td>
<td>66.2±8.2</td>
<td>147.4±14.8</td>
<td>90.2±8.5</td>
</tr>
<tr>
<td><strong>IGF, μg/l</strong></td>
<td>185.8±10.8</td>
<td>2005.28±7.7</td>
<td></td>
</tr>
<tr>
<td><strong>Cortisol, μg/dl</strong></td>
<td>14.4±3.1</td>
<td>10.3±1.2</td>
<td>17.7±2.4</td>
</tr>
<tr>
<td><strong>Epinephrine, pg/ml</strong></td>
<td>83.5±8.5</td>
<td>40.7±13.1</td>
<td>25.2±5.7</td>
</tr>
<tr>
<td><strong>Norepinephrine, pg/ml</strong></td>
<td>230.1±48.8</td>
<td>319.3±110.9</td>
<td>348.4±78.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. CRP, C-reactive protein. \( P < 0.05\), Pre-HD vs. Control; \( P < 0.001\), HD vs. Pre-HD and Control; \( P < 0.01\), Pre-HD vs. HD; \( P < 0.01\), Control vs. Pre-HD and HD; \( P < 0.05\), Pre-HD vs. Control; \( P < 0.01\), Pre-HD vs. Controls.

Table 3. Amino acid flow across the leg

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Pre-HD</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood flow rate, ml/min</strong></td>
<td>3.8±0.2</td>
<td>3.3±0.1</td>
<td>3.4±0.2</td>
</tr>
</tbody>
</table>

Phenylalanine

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Pre-HD</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F&lt;sub&gt;in&lt;/sub&gt;</td>
<td>345.0±29.7a</td>
<td>280.6±30.4a</td>
<td>216.7±18.3</td>
</tr>
<tr>
<td>F&lt;sub&gt;out&lt;/sub&gt;</td>
<td>364.1±31.4</td>
<td>284.8±31.4</td>
<td>245.7±20.5b</td>
</tr>
</tbody>
</table>

**Values are means ± SE; amino acid data are expressed as nmol-min<sup>-1</sup>-100 ml leg<sup>-1</sup>. Amino acid inflow into the leg (F<sub>in</sub>) decreased during HD, but outflow (F<sub>out</sub>) did not change significantly.**

Table 4. Enrichment of free amino acid in different compartments

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Pre-HD</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Artery</strong></td>
<td>0.195±0.0089</td>
<td>0.133±0.0213</td>
<td>0.157±0.0183</td>
</tr>
<tr>
<td><strong>Vein</strong></td>
<td>0.166±0.0063a</td>
<td>0.113±0.0096</td>
<td>0.105±0.0059</td>
</tr>
<tr>
<td><strong>Albumin</strong></td>
<td>0.0118±0.0041</td>
<td>0.011±0.002</td>
<td>0.025±0.003b</td>
</tr>
<tr>
<td><strong>Fibrinogen</strong></td>
<td>0.031±0.005</td>
<td>0.011±0.005</td>
<td>0.034±0.005</td>
</tr>
<tr>
<td><strong>Muscle free pool</strong></td>
<td>0.055±0.012</td>
<td>0.034±0.009</td>
<td>0.026±0.007d</td>
</tr>
<tr>
<td><strong>Muscle</strong></td>
<td>0.00008±0.00002</td>
<td>0.00005±0.00002</td>
<td>0.00012±0.00004</td>
</tr>
</tbody>
</table>

Values are means ± SE. \( P < 0.001\), Control vs. Pre-HD and HD; \( P < 0.01\), HD vs. Pre HD; \( P < 0.001\), HD vs. Pre-HD; \( P < 0.05\), Pre-HD vs. HD.
stabilized, FSR-A increased during HD and were higher than that of controls ($P < 0.01$; Table 5). FSR-F was higher during HD compared with pre-HD ($P < 0.01$) but not different from controls. The increment $\Delta E_p$ of the enrichment of the muscle protein-bound phenylalanine increased during HD, and the intracellular phenylalanine enrichment decreased. FSR-M increased during HD compared with pre-HD and controls ($P < 0.001$). FSR-M in controls and ESRD patients pre-HD were comparable. There was no significant difference in albumin, fibrinogen, or muscle protein synthesis between diabetic and nondiabetic subjects.

Skeletal muscle protein synthesis was correlated with albumin ($r^2 = 0.23$, $P = 0.05$) and fibrinogen fractional synthetic rates ($r^2 = 0.27$, $P < 0.02$). There was a modest but significant correlation between albumin and fibrinogen synthesis ($r^2 = 0.28$, $P = 0.05$). Plasma IL-6 levels correlated positively with albumin ($r^2 = 0.36$, $P < 0.01$), fibrinogen ($r^2 = 0.26$, $P < 0.05$), and muscle protein ($r^2 = 0.32$, $P < 0.01$) synthesis rates.

**DISCUSSION**

This study demonstrates 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. The FSR-A, FSR-F, and FSR-M in controls were comparable to pre-HD estimates. The FSR-A, FSR-F, and FSR-M in controls and ESRD patients pre-HD were comparable. There was no significant difference in albumin, fibrinogen, or muscle protein synthesis between diabetic and nondiabetic subjects.

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Albumin and fibrinogen are both synthesized in the liver. Although albumin is catabolized in a mass-dependent manner, the catabolic rate of fibrinogen remains constant, despite considerable fluctuation in circulating mass of fibrinogen. Experimental models of renal failure in rats, as well as studies in dialysis patients, have shown that renal failure per se does not suppress albumin synthesis (25). However, Kaysen and Schoenfeld (24) observed that albumin synthesis is lower in hypoalbuminemic ESRD patients compared with those with normal plasma albumin levels. Giordano et al. (17) reported that FSR-A and FSR-F are increased in ESRD patients with normal nutritional status. Although plasma albumin was significantly lower in ESRD patients than in controls, FSR-A and FSR-F were comparable to pre-HD estimates. We also observed a correlation between albumin and fibrinogen synthesis during dialysis. Although the intradialytic increase in albumin synthesis rate (64%) was higher than that of fibrinogen (34%), we found that the increase in FSR-F (53.5%) tended to be larger than that of FSR-A (38.6%). Similar to our study, Prinsen et al. (32) recently reported that the absolute synthesis rates of albumin and fibrinogen in ESRD patients on peritoneal dialysis are higher than those of controls. They also observed a correlation between albumin and fibrinogen synthesis rates. The specific enrichment of tracer in serum may not be reflective of intraperitoneal tracer level. Intradialytic loss of tracer may lead to underestimation of intraperitoneal enrichment. However, it is reasonable to assume that both tracer and trace are lost in the dialysate at the same rate; hence, the error in estimation of intraperitoneal tracer level, if it exists, is minimal.

Animal studies have indicated that muscle protein synthesis is decreased and catabolism is increased in uremia (1). On the other hand, despite clinical evidence of malnutrition, protein turnover studies in stable nonacidotic CRF patients have failed to demonstrate any consistent abnormality (12, 29). Garibotto et al. (16) observed that in CRF there is a parallel increase in protein synthesis and catabolism. In our study, the FSR-M pre-HD value was not different from that of controls. This may be due to the fact that the patients did not have metabolic acidosis and that they were consuming an adequate protein diet. The effect of hemodialysis on protein turnover was studied by Lim et al. (27). They reported normal basal leucine flux, a transient decrease in protein synthesis, and a negative protein balance during HD. Raj et al. (34) estimated protein turnover and amino acid transport kinetics in ESRD patients before and during HD by use of stable isotopes of phenylalanine, leucine, lysine, alanine, and glutamine (34). Both protein synthesis and catabolism increased during HD. However, the increase in protein catabolism was higher than in synthesis, resulting in net protein loss. The present study confirms the previous observation from our laboratory that HD promotes skeletal muscle protein catabolism. Despite an increase in muscle breakdown, whole body protein synthesis was not increased during HD. Dissociation between muscle protein turnover and whole body protein kinetics has been observed by other investigators also (21, 27). More studies specifically designed to address this disparity are warranted.

Table 5. Regional and whole body protein metabolism in end-stage renal disease

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Pre-HD</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractional albumin synthesis rate, %/day</td>
<td>5.8 ± 0.5</td>
<td>6.5 ± 0.9</td>
<td>9.7 ± 0.9</td>
</tr>
<tr>
<td>Fractional fibrinogen synthesis rate, %/day</td>
<td>14.1 ± 1.4</td>
<td>11.8 ± 0.6</td>
<td>19.7 ± 2.6</td>
</tr>
<tr>
<td>Fractional muscle protein synthesis rate, %/day</td>
<td>1.30 ± 0.32</td>
<td>1.21 ± 0.25</td>
<td>1.77 ± 0.19</td>
</tr>
<tr>
<td>Leg muscle protein synthesis, nmol·100 ml⁻¹·min⁻¹ (Rd)</td>
<td>33.7 ± 4.1</td>
<td>39.1 ± 7.3</td>
<td>54.7 ± 4.0</td>
</tr>
<tr>
<td>Leg muscle proteinolysis, nmol·100 ml⁻¹·min⁻¹ (Rn)</td>
<td>52.8 ± 9.2</td>
<td>40.7 ± 2.4</td>
<td>83.1 ± 3.6</td>
</tr>
<tr>
<td>Net protein loss, nmol·100 ml⁻¹·min⁻¹</td>
<td>19.1 ± 9.3</td>
<td>1.5 ± 1.9</td>
<td>29.1 ± 5.3</td>
</tr>
<tr>
<td>Whole body proteolysis, nmol·kg⁻¹·min⁻¹</td>
<td>0.55 ± 0.03</td>
<td>0.76 ± 0.08</td>
<td>0.65 ± 0.07</td>
</tr>
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Fractional synthesis rates of albumin, fibrinogen, and muscle protein increased during HD. Net muscle proteolysis was increased during HD, but whole body proteolysis did not change significantly. *$P < 0.01$ HD vs. Controls and Pre-HD; **$P < 0.01$, HD vs. Pre-HD; ***$P < 0.001$, HD vs. Control and Pre-HD; ****$P < 0.05$, HD vs. Control and Pre-HD; *****$P < 0.001$, HD vs. Control and pre HD; ******$P < 0.05$, pre-HD vs. HD, *******$P < 0.01$, Pre-HD vs. Control.
Hepatic and muscle protein synthesis rates seem to be interrelated. In healthy humans, albumin accounts for 50% and fibrinogen for 10% of total liver protein synthesis (30). However, in disease states, fibrinogen synthesis may briefly increase 10- to 20-fold and dominate liver synthesis. In vitro studies have shown that IL-6 increases fibrinogen synthesis but decreases albumin synthesis (11). However, 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 (3). Jahoor et al. (22) demonstrated that inflammatory stress decreases FSR-M but increases FSR-A and FSR-F. Similarly, in head injury patients, muscle protein synthesis is lower, but rates of albumin and fibrinogen synthesis were higher than in controls (28). Despite the intradialytic loss of amino acids, there was a coordinated increase in albumin, fibrinogen, and muscle protein synthesis. We found a significant positive correlation between IL-6 and FSR-A, FSR-F, and FSR-M. We previously reported a correlation between genes promoting protein catabolism and IL-6 (33). These results are consistent with the hypothesis that an intradialytic increase in IL-6 induces hepatic and muscle protein turnover. A positive correlation between protein synthesis and catabolism has been reported from our laboratory (6, 34). Availability of precursor amino acids is a potent modulator of protein synthesis (26). It is possible that the intracelluar increase in amino acids derived from muscle catabolism stimulates muscle protein synthesis (6). The utilization of amino acids, however, is less efficient during HD, resulting in an increase in net outward transport of amino acids into the vein (34). This is supported by the observation that amino acid delivery to the leg (Flna) decreased but efflux (Fena) increased during HD. Previous studies have demonstrated the flow of amino acids from the muscle to visceral organs (31, 35). The amino acids released from the muscle are taken up by the splanchnic bed to be utilized for visceral organs (31, 35). The amino acids released from the muscle catabolism stimulates muscle protein synthesis. The determination of low d5-phenylalanine enrichment (0.002–0.009 atom percent excess), after conversion to phenylalanine, in relation to protein turnover studies by gas chromatography/electron ionization mass spectrometry. Rapid Commun Mass Spectrom 6: 421–424, 1992.


