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The Effect of Hemodialysis on Protein Metabolism
A Leucine Kinetic Study

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Abstract
To assess the effect of hemodialysis on protein metabolism, leucine flux was measured in seven patients before, during, and after high efficiency hemodialysis using cuprophane dialyzers and bicarbonate dialysate during a primed-constant infusion of L-[1-13C]leucine. The kinetics (μmol/kg per h, mean±SD) are as follows: leucine appearance into the plasma leucine pool was 86±28, 80±28, and 85±25, respectively, before, during, and after dialysis. Leucine appearance into the whole body leucine pool, derived from plasma L-[1-13C]α-ketoisocaproate enrichment, was 118±31, 118±31, and 114±28 before, during, and after dialysis, respectively. In the absence of leucine intake, appearance rate reflects protein degradation, which was clearly unaffected by dialysis. Leucine oxidation rate was 17.3±7.8 before, decreased to 13.8±7.8 during, and increased to 18.9±10.3 after dialysis (P = 0.027). Leucine protein incorporation was 101±26 before, was reduced to 89±23 during, and returned to 95±23 after dialysis (P = 0.13). Leucine net balance, the difference between leucine protein incorporation and leucine release from endogenous degradation, was -17.3±7.8 before, decreased to -28.5±11.0 during, and returned to -18.9±10.3 after dialysis (P < 0.0001). This markedly more negative leucine balance during dialysis was accountable by dialysate leucine loss, which was 14.4±6.2 μmol/kg per h. These data suggest that hemodialysis using a cuprophane membrane did not acutely induce protein degradation. It was, nevertheless, a net catabolic event because protein synthesis was reduced and amino acid was lost into the dialysate. (J. Clin. Invest. 1993 91:2429–2436.) Key words: hemodialysis • leucine kinetics • protein flux

Introduction
It is generally believed that hemodialysis is a protein catabolic process (1–3). This notion stems from the observation of that blood urea nitrogen rebounds rapidly after dialysis and the fact that 6–8 g of amino acids are lost to the dialysate per treatment (4–6). Furthermore, Farrell and colleagues (7) and Ward et al. (8) noted that urea generation rate, calculated from urea kine-

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1. Abbreviations used in this paper: KIC, α-ketoisocaprate; NPCR, normalized protein catabolic rate; PCR, protein catabolic rate.

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Table I. Demographic, Nutritional, and Dialysis Status of the Study Subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age/Sex</th>
<th>BMI</th>
<th>Serum albumin</th>
<th>Kt/V*</th>
<th>NPRC</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.A.</td>
<td>56/F</td>
<td>29.6</td>
<td>3.9</td>
<td>1.31</td>
<td>0.98</td>
</tr>
<tr>
<td>J.D.</td>
<td>32/M</td>
<td>20.5</td>
<td>3.2</td>
<td>0.71</td>
<td>0.77</td>
</tr>
<tr>
<td>E.B.</td>
<td>54/F</td>
<td>31.1</td>
<td>4.2</td>
<td>1.36</td>
<td>1.41</td>
</tr>
<tr>
<td>T.D.</td>
<td>23/F</td>
<td>17.2</td>
<td>4.1</td>
<td>1.77</td>
<td>1.14</td>
</tr>
<tr>
<td>R.F.</td>
<td>46/M</td>
<td>26.8</td>
<td>4.2</td>
<td>0.86</td>
<td>0.97</td>
</tr>
<tr>
<td>V.M.</td>
<td>39/F</td>
<td>21.8</td>
<td>4.7</td>
<td>1.11</td>
<td>0.81</td>
</tr>
<tr>
<td>R.S.</td>
<td>20/M</td>
<td>19.3</td>
<td>4.7</td>
<td>1.02</td>
<td>0.94</td>
</tr>
</tbody>
</table>

BMI, body mass index; F, female; M, male. Kt/V and NPRC represent dialysis adequacy index and estimated daily protein intake (see Methods).

*Kt/V and protein catabolic rate (PCR) as he regularly dialyzed at home for 4.5–5 h three times a week. Both parameters were determined from the 3-h dialysis sessions performed preceding the experiment. Kt/V of urea represents the “dose of dialysis” and is used as a dialysis adequacy index, where \( k \) equals dialyzer urea clearance (ml/min), \( t \) is time on dialysis (min), and \( V \) is volume of urea distribution (ml). Dialyzer clearance was measured from the spent dialysate volume and dialysate and blood urea nitrogen concentration. Dialysis time was recorded. Volume of urea distribution was calculated from the single-pool variable volume kinetics (13, 15). Urea appearance rate or urea generation rate (\( G \)) was also derived from the urea kinetic equations. PCR = \( \left[ (G \times 1.44) + 1.71 \right]/0.154 \) (9). NPRC expresses PCR per body weight, which was normalized for a standard urea volume of distribution of 58%, thus NPRC = PCR/\( V/0.58 \). In steady state, NPRC is an estimate of dietary protein intake. Vascular access recirculation was <7% in all subjects. Study dialses were performed as described with the exception that the time was shortened to 2.5 h to accommodate the experiment.

Experimental design

Materials. L-[1-13C]leucine, (99 atom% 13C), and NaH13CO3, 95 atom% 13C, were purchased from Merck Sharp & Dohme/Isotopes, Dorval, Quebec, Canada. Infusates were prepared aseptically the afternoon before the experiment.

Procedures. Subjects were admitted to the Clinical Research Center the day before the study. After a 12-h overnight fast, leucine infusion was started at 0700 the next morning. Gauge 20 needles were inserted into the arterial and the venous ends of the vascular access, the former for blood sampling and the latter for infusion. Isotopic [1-13C]leucine (see below) was infused for 4 h from 0700 to 1100 hours before dialysis, continued for 2.5 h during high efficiency hemodialysis, and for 4 h after dialysis (total infusion time was 10.5 h). The first 2 h of the infusion was used to achieve isotopic equilibrium; data from the third and fourth hours served as the predialysis baseline to which dialysis and postdialysis values were compared. CO2 production rate (FCO2), atom percent excess of [1-13C]leucine and [1-13C]KIC in the plasma, and atom percent excess of 13CO2 in the expired gas were quantitated before infusion and every 30 min for 2 h before, during, and after dialysis. Additionally, total plasma leucine concentration was measured before, during, and after dialysis. Spent dialysate was also collected during the study and, after appropriate mixing, aliquots were taken for measurement of leucine concentration.

The protocol was approved by the Committee on Human Research of the University of Iowa College of Medicine.

Leucine turnover kinetics. Leucine kinetics were measured by a primed-constant infusion technique during substrate and isotopic steady state (16–18). The priming solution consisted of 4.0 μmol/kg of L-[1-13C]leucine and 0.11 mg/kg of NaH13CO3. In anticipation of leucine loss into the dialysate, the constant infusion was given at the rate of 6.0 μmol/kg per h.

Quantitation of amino acid metabolism was on the basis of the relationship stating that \( Q = S + C = B + I \), where \( Q \) is flux or total turnover rate, \( S \) is the rate of amino acid incorporation into protein or protein synthesis, \( C \) is the rate of amino acid oxidation, \( B \) represents the rate of amino acid release from endogenous protein breakdown, and \( I \) is the rate of exogenous intake. In the postabsorptive state, \( I \) equals 0 and \( B \), therefore, equals \( Q \). In general, routes of nonoxidative leucine disposal other than \( S \) are assumed to be negligible (12, 19). This statement is true in the pre- and postdialysis period when \( S = Q - C \). During dialysis, when nonoxidative disposal of leucine must take into account dialysate loss, then \( S = Q - (C + D) \) where \( D \) equals dialysate leucine loss. Leucine loss was measured in the spent dialysate and the loss was assumed to be linear during the entire 2.5 h of treatment. Net \( B \) or net leucine balance was derived from the difference between leucine protein incorporation (\( S \)) and leucine degradation (\( D \)); the latter being equal to \( Q \) or flux.

Leucine flux [\( Q \)] denotes movement of leucine into (rate of appearance [\( Ra \)]) and out of (rate of disappearance [\( Rd \)]) the metabolic pool. In steady state, \( Ra = Rd \).

\[ Q = \frac{[E_i - E_f]}{1 - i} \]

where \( E_i \) is [1-13C] leucine enrichment in the infusate (100%), \( E_f \) is [1-13C] leucine or [1-13C] α-keto 150 caproate (KIC) enrichment in the plasma at isotopic plateau, and \( i \) is 1-13C leucine infusion rate [μmol/kg per h]. Leucine appearance rate into the plasma leucine pool was calculated from plasma [1-13C] leucine enrichment (16), whereas leucine appearance rate into the whole body leucine pool was derived from plasma [1-13C] KIC enrichment (20, 21).

The rate of 13CO2 release from tracer leucine oxidation was calculated as follows: \( F_{13CO_2} = (FCO_2 \times ECO_2/BW) \times \left( 60 \times 41.6/100 \times 0.81 \right) \).

\( F_{13CO_2} \) is CO2 production rate, \( E_{CO_2} \), 13CO2 enrichment in the expired gas at isotopic steady state, \( BW \) is body weight (kg). The constants 60 [min/h] and 41.6 [μmol/ml at standard temperature and pressure] convert \( F_{13CO_2} \), from ml/min to μmol/h. The factor 100 changes atom percent excess from a percent to a fraction, and the factor 0.81 represents the fraction of 13CO2 produced by [1-13C]leucine oxidation released from the body bicarbonate pool into the expired gas. (We have not independently measured CO2 recovery from the bicarbonate pool in the uremic subjects). The rate of leucine oxidation [\( C \)] is then (16)

\[ C = F_{13CO_2}/[E_{CO_2} - 1]/E_f \times 100 \times F_{13CO_2}/[E_{CO_2} - 1]/E_f \times 100 \]

where \( D_{13CO_2} \) is the amount of labeled CO2 lost in the dialysate. \( D_{13CO_2} \) was not measured but was calculated as follows: we first estimated plasma CO2 concentration from arterial PCO2. Plasma CO2 concentration [mmol/liter] = arterial PCO2 × 0.0301, where 0.0301 is the solubility coefficient of CO2. Then, \( D_{13CO_2} \) (μmol/kg/h) = [plasma CO2 (mmol/liter) × QBG (blood flow rate, liters/min) × 1,000 × 60] × [expired CO2 (atom % excess)/100] divided by body weight (kg). Arterial PCO2, QBG, and expired 13CO2 enrichment were the measured parameters. In isotopic steady state, expired 13CO2 enrichment reflects the circulating 13CO2 enrichment (which is in equilibrium with body CO2 and bicarbonate pool). Since dialysate bicarbonate represents loss from the same circulating pool, we assumed that the 13CO2 enrichment of dialysate CO2 at isotopic steady state was the same as that of the expired CO2. Since product isotopic enrichment (in this case breath and dialysate 13CO2) cannot exceed precursor isotopic enrichment, the above calculation, therefore, represents maximal potential \( D_{13CO_2} \) (see Tables III and IV). In other words, we are assuming complete plasma clearance of 13CO2 during its passage through the dialyzer. In using dissolved CO2 to calculate dialysate CO2 loss, we reasoned that in the dialysate where carbonic anhydrase is absent and where contact between blood and dialysate is of transient duration (single-pass system where blood and dialysate flow rates were, respectively, 0.4 and 0.5
To account for the changes in the natural abundance of $^{13}$CO$_2$ in expired gas during dialysis, additional studies were performed in four subjects in whom expired gases were collected as described below before, during, and after identical hemodialysis but in the absence of L-[1-13C]leucine and NaH$^{13}$CO$_3$ administration. The average change in expired $^{13}$CO$_2$ enrichment of these four subjects at each dialysis and postdialysis period was subtracted from individual measured values of all study subjects during the same experimental period to yield corrected values for $^{13}$CO$_2$ enrichment that were then used in above equations to generate data on leucine oxidation and leucine protein incorporation.

**Leucine and CO$_2$ measurement.** [1-13C] -enrichment of plasma leucine and plasma KIC was measured by gas chromatography mass spectrometry (22, 23) and $^{13}$CO$_2$ enrichment in the expired gas was measured by isotope ratio mass spectrometry as described previously (16). Leucine content in the plasma and the spent dialysate was measured by an amino acid analyzer (model 6300; Beckman Instruments, Inc., Fullerton, CA) using norleucine as the internal standard (24). The data were analyzed using Waters Expert Ver. 6.2 1A software (Waters Instrs. Inc., Rochester, MN).

CO$_2$ production rate and collection of expired gas. CO$_2$ production rate ($F_{CO2}$) was determined by a portable metabolic gas monitor [model M21; Utah Medical Products, Midvale, UT]. Subjects were given sufficient time to become familiarized with the equipment and the procedures. During measurement, a mouthpiece attached to a two-way valve was used. Expired gas was sampled by a small gas line leading from the mouthpiece to an infrared CO$_2$ sensor. Simultaneously, tidal volume and frequency of respiration were recorded by a pneumotachograph and expired ventilation by an ultrasonic flow transducer (25). Partial pressure of the inspired CO$_2$ was taken from that of the atmosphere. $F_{CO2}$ was calculated by standard equations and corrected for standard pressure and temperature, dry. Before each experiment, the equipment was calibrated with a standard reference gas consisting of 10% CO$_2$ and the flow transducer was checked by a calibrated syringe. Immediately after each measurement, the expired gas was collected into a Douglas bag and a sample was transferred anaerobically into sealed vacuum tubes (Venoven; Terumo Medical, Elkton, MO) for quantitation of $^{13}$CO$_2$ enrichment by isotope ratio mass spectrometry.

**Arterial blood gas and serum bicarbonate.** Arterial pH, P$_{CO2}$, and serum bicarbonate concentration were measured before, at 60 and 90 min during dialysis, and immediately after dialysis. These measurements were determined to estimate CO$_2$ and bicarbonate fluxes during the dialysis procedure (see Table IV). Bicarbonate gain ($G_{bic}$) was calculated as (post-HCO$_3$ - pre-HCO$_3$) / (BW x 0.4), where HCO$_3$ represents serum bicarbonate levels post- and predialysis, BW is post dialysis body weight, and 0.4 represents bicarbonate distribution space or 40% of body weight. This 40% distribution appears to be appropriate for the pH ranges of our patients, 7.33-7.45 (26). Bicarbonate loss ($L_{bic}$) was calculated as [(pre-HCO$_3$ - post-HCO$_3$) / 2] x UF, where UF is ultrafiltration or fluid removal during dialysis. Net HCO$_3$ gain is the difference between HCO$_3$ gain and loss. If all the bicarbonate gained from the dialysate were to be titrated to CO$_2$, the predicted maximal amount of CO$_2$ release = (Net HCO$_3$ gain / 150) x 24, where 150 is the number of minutes in 2.5 h of dialysis and 24 is Avogadro's number, stating that 1 mol of CO$_2$ occupies a volume of 24 liters, and converts CO$_2$ from mmol to ml.

Statistical analysis. Data were stored in the Clinfo System of the Clinical Research Center of the University of Iowa College of Medicine; statistical analyses were completed using analysis of variance and Duncan's multiple comparison test.

**Results**

Fig. 1 depicts the mean plasma [1-13C]leucine and [1-13C]KIC enrichment before, during, and for 4 h after completion of dialysis. Each graph consists of 18 data points, 5 before initia-
Table III summarizes expired $^{13}$CO$_2$ enrichment and appearance rates; the latter represents the amount of CO$_2$ derived from metabolism of L-$[1-^{13}$C]leucine. Despite an increased production of total CO$_2$ from 7.083 to 7.964 $\mu$mol/kg per h, the appearance rate of $^{13}$CO$_2$ decreased from 0.87 before to a nadir of 0.61 $\mu$mol/kg per h during dialysis. After completion of dialysis, total CO$_2$ production continued to be elevated, but expired gas $^{13}$CO$_2$ enrichment and $^{13}$CO$_2$ appearance rate rose concomitantly so that $^{13}$CO$_2$ appearance rates rose above predialysis values to 1.14 $\mu$mol/kg per h ($P < 0.001$).

Table IV summarizes bicarbonate and CO$_2$ fluxes during hemodialysis. The left half of the table lists ultrafiltration rate and changes in serum HCO$_3^-$ and VCO$_2$ before and after dialysis whereas the right half documents HCO$_3^-$ gain and loss as well as potential CO$_2$ and $^{13}$CO$_2$ losses during treatment. Mean HCO$_3^-$ gain of 187 mmol is due to diffusion of bicarbonate from dialysate to blood and mean HCO$_3^-$ loss of 21 mmol is a result of convective movement of bicarbonate from blood to dialysate consequent to ultrafiltration. The difference between these two processes resulted in a net HCO$_3^-$ gain of 166 mmol during the 2.5 h of treatment. This bicarbonate gain would lead to a predicted increment in VCO$_2$ of 27 ml/min if all the bicarbonates were to titrate acid metabolites. Since only $\sim 80\%$ of the CO$_2$ produced is released, the corrected predicted rise in VCO$_2$ would be 21.6 ml/min. This predicted value is surprisingly close to the measured change in VCO$_2$ before and after dialysis, 20 ml/min. Mean potential CO$_2$ loss was 0.47 mmol/min or 457 $\mu$mol/kg per h whereas mean $^{13}$CO$_2$ loss was only 0.033 $\mu$mol/kg per h. Tolchin et al. (27) using acetate dialysate reported a CO$_2$ loss of 0.3 mmol/min. Since we calculated $^{13}$CO$_2$ loss in the dialysate (Table IV) as complete plasma clearance of $^{13}$CO$_2$ into the dialysate on each pass, D$^{13}$CO$_2$ was overestimated to some extend but was, nonetheless, only $\sim 5\%$ of the $^{13}$CO$_2$ appearance rate (Table III). The net result, then, was little overall effect on oxidation compared with labeled leucine carbon lost via the breath alone.

Fig. 2 illustrates changes in leucine flux, oxidation, protein incorporation, and net leucine balance during and after hemodialysis plotted as percentages of values obtained predialysis.

Table III. Expired Gas $^{13}$CO$_2$ Enrichment and $^{13}$CO$_2$ Appearance Rate

<table>
<thead>
<tr>
<th>Dialysis period</th>
<th>Time</th>
<th>FCO$_3$</th>
<th>$^{13}$CO$_2$ enrichment</th>
<th>$^{13}$CO$_2$ appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
<td>ml/min</td>
<td>$\mu$mol/kg per h</td>
<td>atom % excess</td>
</tr>
<tr>
<td>Before</td>
<td>0</td>
<td>193±46</td>
<td>7,083±1,688</td>
<td>0.0101±0.0042</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>217±48</td>
<td>7,964±1,761</td>
<td>0.0075±0.0026</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>210±44</td>
<td>7,707±1,615</td>
<td>0.0063±0.0024*</td>
</tr>
<tr>
<td>After</td>
<td>60</td>
<td>216±54</td>
<td>7,927±1,982</td>
<td>0.0084±0.0046</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>212±47</td>
<td>7,781±1,725</td>
<td>0.0096±0.0035</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>215±51</td>
<td>7,891±1,872</td>
<td>0.0110±0.0046</td>
</tr>
<tr>
<td></td>
<td>240</td>
<td>219±56</td>
<td>8,027±2,055</td>
<td>0.0118±0.0053</td>
</tr>
<tr>
<td>ANOVA d.f.</td>
<td></td>
<td>6/124</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>0.843</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.539</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean±SD. FCO$_3$ and $^{13}$CO$_2$ appearance represent, respectively, CO$_2$ production rate and CO$_2$ derived from metabolism of [1-13C]leucine. For ease of comparison FCO$_3$ is listed as ml/min as well as $\mu$mol/kg per h. The latter was derived using Avogadro’s number stating that 1 mol of CO$_2$ occupies a volume of 24 liters. Statistical analysis was done using one-way ANOVA and Duncan’s multiple comparison test; the degree of freedom (d.f.), $F$, and $P$ values are listed. * Values that are significantly different from those obtained in the predialysis period.
sis and returned to 18.9±10.3 after dialysis (P = 0.027). The rate of leucine incorporation into protein (S) decreased from 101±26 before dialysis to 89±23 during dialysis, and 95±23 after dialysis (P = 0.132). Dialysate leucine loss (D), measured in the spent dialysate, was 14.4±6.2. Net leucine balance (Net B), derived from the difference between leucine protein incorporation rate and leucine derived from proteolysis declined from –17.3±7.8 before dialysis to –28.5±10.3 during dialysis, and reverted to –18.9±10.3 after dialysis (P < 0.0001).

Despite significant dialysate leucine loss, 14.4 μmol/kg per h, plasma leucine concentration (nmol/ml), however, was not reduced; 130±30 before dialysis, 131±26 during dialysis, and 164±28 after dialysis (P = 0.014).

**Discussion**

During primed-constant infusion of isotopic leucine, we failed to detect any evidence to implicate that hemodialysis stimulates protein degradation directly because plasma enrichment of [1-13C]leucine and [1-13C]KIC was not reduced (Fig. 1). Furthermore, leucine appearance rates into the plasma leucine pool, derived from plasma [1-13C]leucine enrichment, was essentially stable during and for 4 h after dialysis (Table II). To circumvent the possibility that [1-13C]leucine enrichment may underestimate leucine appearance into the whole body system (12), we used plasma [1-13C]KIC enrichment or the "reciprocal pool" (21) method to estimate leucine appearance rate into the total body leucine pool. Again, plasma [1-13C]KIC enrichment was remarkably constant during the entire period of observation. Since intracellular leucine pool in dialysis patients with adequate protein intake is normal (28, 29) and because the observed plasma [1-13C]leucine to [1-13C]KIC ratio of 0.73 is similar to that found in normal subjects (20), it is reasonable to assume that the measured values in our subjects reflected accurately body protein breakdown rates. If protein degradation was accelerated, we should have observed a reduction in plasma [1-13C]leucine and [1-13C]KIC enrichment and a rise in their respective flux rates. In our study, the labeled leucine was infused into the free amino acid pool, plasma in this instance. Dilution of the labeled leucine could occur either by leucine intake or degradation of body protein with release of unlabeled leucine. Since our experiment was done in the postabsorptive state, the magnitude of the isotopic dilution is proportional to proteolysis because intake = 0, and leucine, and essential amino acid, could be derived only from protein breakdown. On the basis of these leucine appearance rates, we conclude that hemodialysis, as performed in our unit, using a cuprophane membrane, bicarbonate dialysate, and treated water with zero bacteria colony counts, did not directly stimulate protein degradation acutely. The postdialysis blood urea nitrogen rebound must, therefore, be explained on the basis of urea dysequilibrium and redistribution (11).

In contrast to the marked stability of the leucine appearance rates, leucine oxidation and leucine protein incorporation rates fluctuated significantly throughout the study (Table V, Figs. 2 and 3). During dialysis, leucine oxidation was reduced. Such reduction, accompanied by increased CO2 production, is perhaps best explained as a counter-regulatory response to augmented leucine loss in the dialysate. Alternatively, it may reflect a change in body fuel oxidation because of metabolism of glucose supplied in the dialysate. We did not measure plasma
Figure 2. Changes in leucine kinetics during and after hemodialysis. Changes in leucine flux, oxidation, and protein incorporation were expressed as percent of predialysis values whereas changes in leucine net balance were plotted as percent change from predialysis. Results are listed as mean±SEM. *Values that are statistically different from those obtained during predialysis.

Table V. Oxidative and Nonoxidative Leucine Disposal

<table>
<thead>
<tr>
<th>Dialysis period</th>
<th>Time (min)</th>
<th>Oxidation (C)</th>
<th>Dialysate loss (S)</th>
<th>Protein incorporation (S)</th>
<th>Net leucine balance</th>
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</thead>
<tbody>
<tr>
<td>Before</td>
<td></td>
<td>17.3±7.8</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>During</td>
<td>60</td>
<td>14.9±6.0</td>
<td>14.4±6.2</td>
<td>84±20*</td>
<td>−17.3±7.8</td>
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<tr>
<td></td>
<td>150</td>
<td>13.0±7.0*</td>
<td>14.4±6.2</td>
<td>92±24</td>
<td>−27.7±11.9*</td>
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<tr>
<td>After</td>
<td>60</td>
<td>16.3±9.8</td>
<td></td>
<td>104±27</td>
<td>−16.3±9.8</td>
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<tr>
<td></td>
<td>120</td>
<td>18.2±9.4</td>
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<td>97±22</td>
<td>−18.2±9.4</td>
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<td></td>
<td>240</td>
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<td></td>
<td>89±21</td>
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<td>ANOVA d.f.</td>
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<td></td>
<td>6/124</td>
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<td>1.661</td>
<td></td>
<td>1.461</td>
<td></td>
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<tr>
<td></td>
<td>P</td>
<td>0.137</td>
<td></td>
<td>0.197</td>
<td></td>
</tr>
</tbody>
</table>

Although leucine oxidation rate and leucine loss into the dialysate were directly measured, leucine protein incorporation rate and net leucine balance were calculated (see Methods). All values are listed as mean±SD. Statistical analysis was performed with ANOVA and Duncan's multiple comparison test; the degrees of freedom (d.f.), F, and P values are listed. * Values that are significantly different from those obtained during the predialysis period.
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...tion and Amgen Center), Research Grant and actually are via the

...istance, Sometimes: VCO2 in this instance. The same table also provides information on potential maximal amounts of dialysate 13CO2 loss, which is 0.033 μmol/kg per h. We have taken this small amount of dialysate 13CO2 loss into consideration in the calculation of leucine oxidation rates (see Methods). Since D13CO2 loss is only a small fraction, ~ 5% of total expired 13CO2 loss, the inclusion of such item did not alter the results of leucine oxidation and protein incorporation rates.

We did not measure labeled bicarbonate dialysate loss but have calculated its potential loss on the basis of the worse scenario that plasma H13CO3 is totally cleared during each pass through the dialyzer and that the direction of flux is entirely from the blood to the dialysate. Such calculations increased the estimated leucine oxidation rate and yielded a further reduction in leucine protein incorporation rate and a greater negative net leucine balance during dialysis. Because the calculations did not alter the overall conclusions of this work and are fraught with assumptions, the derived data are not included in this paper. It should be emphasized that during bicarbonate dialysis, the major route of bicarbonate/CO2 loss is via the lungs, 533 mmol/h, and not the dialyzer, which lost only 31 mmol/h (Tables III and IV).

These findings are consistent with the report of Borah et al. (9), documenting that nitrogen balance is always less on the day of dialysis, and with our own data showing that lengthening the interdialytic interval tends to improve nitrogen balance (10). By measuring arterio-venous amino acid gradient and total blood flow across the leg, Gutierrez and colleagues (14) found that 150 min of sham hemodialysis in normal control subjects led to increased amino acid release, suggesting accelerated protein breakdown. If our patients had a twofold increase in amino acid release, we should have observed a marked reduction in plasma [1-13C]leucine and [1-13C]KIC enrichment and an increase in the calculated leucine flux, and we did not. Moreover, we found changes during dialysis whereas Gutierrez and associates (14) detected changes after dialysis. These discrepancies are difficult to reconcile and may be related to differences in the techniques used.

Our inability to find a change in plasma isotopic dilution could not be due to technical difficulty as the method of primed-constant infusion of a stable isotope is sufficiently sensitive to detect as little as a 2% change in flux rate (12, 16). Recycling of labeled leucine may mask an increase in leucine flux, although label recycling is usually not a significant problem for the first 6–8 h of isotope infusion. Our study was 10.5-h long; thus it is possible that we might have missed a small increase in flux in the latter part of the experimental period.

The amount of leucine lost into the dialysate, 2.26 mmol or 296 mg, was similar to that reported by Wolfson et al. (6). Despite this removal, serum leucine level was not reduced during dialysis and actually rose modestly after dialysis. The maintenance of a constant leucine level must be regulated by a balance between catabolism and anabolism. In our study, leucine protein incorporation rate, on the basis of plasma [1-13C]KIC enrichment, was reduced from a predialysis value of 101 to a nadir of 84 μmol/kg per h during dialysis (Table V). This magnitude of reduction would actually provide 2.86 mmol of free leucine on the basis of a mean body weight of 67.3 kg in our subjects. This amount is very close to the measured dialysate loss, 2.26 mmol. Thus, maintenance of a steady serum leucine level in our study was the result of reduced leucine incorporation into protein.

Finally, it should be emphasized that our data pertain only to acute changes during and immediately after dialysis and do not necessarily exclude the possibility that repeated cytokine release with hemodialysis might enhance protein catabolism over a long period of time (30, 31).

In summary, we found that hemodialysis using a cuprophan membrane and bicarbonate dialysate did not increase protein degradation because leucine appearance rates were stable. The procedure, nevertheless, was a net catabolic event because protein synthesis was reduced and there was a net loss of amino acids into the spent dialysate.

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References


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