Adaptation To Low-Protein Diets In Renal Failure: Leucine Turnover and Nitrogen Balance

Timothy H. J. Goodship,1 William E. Mitch, Robert A. Hoerr, David A. Wagner, Theodore I. Steinman, and Vernon R. Young

T.H.J. Goodship, Department of Medicine, Brigham and Women's Hospital, Boston, and Laboratory of Human Nutrition and Clinical Research Center, Massachusetts Institute of Technology, Cambridge, MA
W.E. Mitch, The Renal Division, Emory University School of Medicine, Atlanta, GA, and Department of Medicine, Brigham and Women's Hospital, Boston, MA
R.A. Hoerr, V.R. Young, Laboratory of Human Nutrition and Clinical Research Center, Massachusetts Institute of Technology
D.A. Wagner, Shriners Burn Institute, Boston, MA
T.I. Steinman, Department of Medicine, Beth Israel Hospital, Boston, MA

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ABSTRACT

In normal subjects, short to intermediate responses to dietary protein restriction include decreased amino acid oxidation and protein degradation plus increased utilization of amino acids for protein synthesis; these responses are activated to improve amino acid utilization and nitrogen balance. To assess whether chronic renal failure (CRF) impairs the adaptive responses to a low-protein diet, we measured nitrogen balance and the kinetics of infused L-(15N, 1-13C)leucine during fasting and feeding. In six adult CRF and four control (C) subjects, 0.6 (LP) and 1.0 (HP) g protein kg⁻¹ day⁻¹ diets were compared. LP reduced feeding stimulated oxidation of leucine by 26% in CRF and 33% in C (P = NS). During fasting, oxidation was unaffected by diet or CRF. For both groups, feeding suppressed protein degradation to the same extent; leucine incorporation into protein did not change. Nitrogen balance during the two diets was the same with C and CRF, as was protein balance estimated from results of measured leucine kinetics. Thus, patients with CRF can activate appropriate adaptive responses to LP inasmuch as reduced amino acid oxidation occurring with feeding and estimates of protein balance did not differ from control.

Key Words: Chronic renal failure, low-protein diet, nitrogen balance, leucine turnover.

It has been known for many decades that a reduction in the intake of protein can ameliorate the signs and symptoms of advanced uremia (1). Dietary regimens introduced in the 1960s (2, 3), however, were not widely accepted because of the danger of malnutrition (4). More recently, it has been reported that protein-restricted diets may retard the rate of progression of renal failure (5). Although this possibility has re-awakened interest in protein-restricted dietary regimens, concern about their nutritional adequacy persists.

If malnutrition is to be avoided, metabolic adaptation (6) to a low protein diet must occur in order to maintain nitrogen balance and normal organ function. In normal subjects, it has been suggested that metabolic adaptation results primarily from a reduction in amino acid oxidation and, perhaps, by a reduction in protein degradation induced by feeding (7). These responses lead to increased efficiency of amino acid utilization. It is not known what mechanisms are activated by CRF patients fed a low protein diet nor to what extent their adaptive responses are the same as those of normal subjects.

In the present study we have addressed these questions by measuring nitrogen balance in CRF patients given two levels of dietary protein: the mean daily requirement for healthy adults, 0.6 g kg⁻¹ day⁻¹ (8), and a “standard” intake, 1.0 g kg⁻¹ day⁻¹ (8). At the end of each 1-wk dietary period, we also analyzed the kinetics of leucine turnover using a primed constant infusion of L-[15N,1-13C]leucine (9). The amino acid leucine was chosen because its metabolism best represents the adaptive responses that we wished to examine. The 1-wk periods were chosen to compare adaptive mechanisms to a low-protein diet in the two groups in terms of amino acid metabolism and estimated rates of protein synthesis and breakdown because normal subjects activate adaptive responses within 1 wk (6, 7).
TABLE 1. Clinical characteristics of CRF patients and normal subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age</th>
<th>IBW (kg)</th>
<th>% IBW</th>
<th>Primary Disease</th>
<th>Serum Creatinine (mg%)</th>
<th>BUN at start (mg%)</th>
<th>GFR (ml/min)</th>
</tr>
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<tr>
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<td>139</td>
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<tr>
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<td>64</td>
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<tr>
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<td>F</td>
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<td>M</td>
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<td>119</td>
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<td></td>
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<tr>
<td>9</td>
<td>F</td>
<td>61</td>
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<td>128</td>
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<td>10</td>
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<td>106</td>
<td>106</td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

* IBW, ideal body weight; BUN, blood urea nitrogen; GFR, glomerular filtration rate calculated as the average of the urea and creatinine clearance; GN, glomerulonephritis.

METHODS

Subjects

Six CRF patients and four normal, healthy, adult control subjects, approximately matched for age and sex, participated in the study (Table 1). The CRF patients were not being treated by dialysis, and the normal subjects had no abnormalities of hepatic or renal function and were taking no medicines. Several of the CRF patients were taking antihypertensive medicines and/or phosphate binders but no corticosteroids, insulin, or other drugs known to affect protein metabolism. The experimental protocols were approved by the Massachusetts Institute of Technology (MIT) Committee on the use of Humans as Experimental subjects, the Executive Committee of the MIT Clinical Research Centre (CRC) and the Committee for the Protection of Human Subjects from Research Risks of the Brigham and Women’s Hospital. Written informed consent was obtained from each subject.

Experimental Design

The study consisted of two consecutive 1-wk periods while the subjects were hospitalized. The subjects received a diet containing 1 g protein kg⁻¹ day⁻¹ for 1 wk and 0.6 g kg⁻¹ day⁻¹ during the other week; the order of the diet periods was randomized to decrease the possible carryover effects of the previous dietary intake, since a low protein diet had been prescribed for CRF patients 1-4 as part of their long-term therapy. At both levels of protein intake the diets were formulated to contain adequate amounts of the essential amino acids. An energy intake equivalent to the calculated BMR plus 25% and other dietary constituents were kept constant throughout both diet periods. The daily energy intakes of the CRF patients and the normal subjects were 32.5 ± 3.5 and 32.7 ± 4.5 kcal/kg, respectively. These values are similar to those shown to produce nitrogen equilibrium in CRF patients consuming diets supplying 0.6 g protein kg⁻¹ day⁻¹ and were considered adequate for the age and sedentary nature of the subjects (10-12). Urine and feces were collected and analyzed for nitrogen balance; aliquots from a 24 h dietary homogenate also were analyzed for nitrogen content. Nitrogen balance (g N/day), measured over the last 5 days of each week, was calculated from nitrogen intake and urinary and fecal nitrogen excretion, and the daily change in the urea N pool was calculated from serial measurements of body weight and BUN, as described previously (13, 14).

Isotope Preparation and Infusion

L-[¹⁵N,¹³C]leucine (99% ¹³C and ¹⁵N, Tracer Technologies, Inc., Somerville, MA) and NaH¹³CO₃ (90% ¹³C; Kor Isotopes, Cambridge, MA) were tested for sterility and non-pyrogenicity using culture techniques, rabbit body temperatures, and bimubus amebocyte lysate assays (Findlay Research Inc. Fall River, MA). The isotopes were diluted with normal saline and administered in the following dosages: L-[¹⁵N,¹³C]leucine, 7.5 μmol/kg as a priming dose and 7.5 μmol kg⁻¹ hr⁻¹ for the continuous infusion; NaH¹³CO₃, 0.087 mg/kg as a priming dose.

On the seventh day of each week, following a 10-h fast, the subjects underwent a primed constant infusion of L-[¹⁵N,¹³C]leucine to measure leucine kinetics (Figure 1). At 7:15 a.m., following a 10-h fast, a 20-gauge catheter was inserted into a vein of each antecubital fossa, using local anesthesia (1% Lidocaine). One catheter was used for the primed constant infusion of L-[¹⁵N,¹³C]leucine and the other for blood sampling. The patency of both catheters
was maintained by slow infusion of 0.15 M saline. At 7:30 a.m. and 7:45 a.m., samples of blood and expired air were collected to determine basal $^{15}$N, $^{13}$C enrichment of plasma leucine, and basal $^{13}$C enrichment of plasma a-ketoisocaproate (KIC) and expired CO$_2$.

At 8:00 a.m. priming doses of NaH$^{13}$CO$_3$ and L-$[^{15}$N, 1-$^{13}$C$]_{	ext{leucine}}$ were given and the constant infusion of L-$[^{15}$N, 1-$^{13}$C$]_{	ext{leucine}}$ began. During the fourth and tenth hour of the infusion, heparinized blood samples were obtained at 15-min intervals, and plasma aliquots were separated and stored at $-80^\circ$C. Insulin levels were measured at each time point. Expired air samples were obtained at the same time using an anesthesia bag equipped with a Rudolf valve. Air samples were injected into 20-ml evacuated tubes without silicone coating (Venoject T-208U, Terumo Medical Corporation, Elkton, MD) and stored at room temperature until analyzed for $^{13}$C enrichment.

Rates of CO$_2$ production were determined on three occasions over 6 min each in the third and ninth hour of the infusion. Volumes were measured with a LB2 CO$_2$ gas analyzer (Beckman Instruments, Inc., Fullerton, CA). Values were corrected for standard temperature and pressure.

During the last 6 h of the infusion the subjects were given small, isocaloric, isonitrogenous meals of a liquid, egg-based, formula diet every 30 min to achieve a metabolic steady-state for the fed condition (7). During the 6 h of feeding, 50% of the total, daily dietary protein, and energy intake was provided.

**Sham Infusion**

Since protein and carbohydrate ingestion can increase $^{13}$CO$_2$ enrichment in expired air (15), a sham infusion without tracers was performed on the fifth day of each week. The subjects were fasted until noon and then fed in a manner identical to the infusion day. Samples of expired air were taken at 15-min intervals between 5 and 6 p.m., and analyzed for $^{13}$C enrichment of CO$_2$. The results were used to correct values obtained at the same time points during the L-$[^{15}$N, 1-$^{13}$C$]_{	ext{leucine}}$ infusion.

**Measurement of Stable Isotope Enrichment**

The $N$-acetyl $n$-propyl (NAP) ester of plasma leucine was created as described previously (16, 17). A selected ion monitoring CI-GCMS (Hewlett Packard 5985 quadrupole instrument) was used to measure the [MH]$^+$, [MH + 1]$^+$, and [MH + 2]$^+$ ions of NAP-leucine ($m/z = 216, 217, and 218$), corresponding to the unlabelled, monolabelled, and dilabelled species. Selected ion monitoring EI-GCMS (Hewlett Packard 5970 quadrupole instrument) was used to measure total $^{15}$N enrichment from the fragment and fragment plus one ions ($m/z = 128 and 129$) (18). Total $^{13}$C enrichment was then calculated by subtraction (9).

A trimethylsilyl-quinoxalinol derivative of plasma KIC (19, 20) was used with EI-GCMS to measure the ions, $m/z = 259 and 260$. The $^{13}$C enrichment of CO$_2$ in expired air was determined by isotope ratio mass spectrometry (Model 3-69-RMS, Nuclide Corp. State College, PA), as previously described (17).

**Calculations**

The model and calculations of leucine metabolism have been described previously (9, 17). Leucine carbon flux ($\varphi_c$) is equal to the sum of leucine disappearance into body proteins (PS) plus leucine oxidation (O), and to the sum of leucine appearance from protein degradation (PD) plus dietary intake of leucine ($I$). Therefore leucine carbon flux equals:

$$\varphi_c = PS + O = PD + I$$

Briefly, leucine carbon flux can be calculated either from the leucine total $^{13}$C enrichment (the sum of plasma $[^{15}$N, 1-$^{13}$C$]_{\text{leucine}}$ enrichments) or from $[1-$13C$]_{\text{KIC}}$ enrichment. We chose to use the $[1-$13C$]_{\text{KIC}}$ enrichment since it has been reported to give a more accurate estimate of the intracellular enrichment of leucine (21). Thus leucine carbon flux in $\mu$mol kg$^{-1}$ h$^{-1}$ ($\varphi_c$) is:

$$\varphi_c = I(E_{\text{KIC}}/E_{\text{sc}} - 1)$$

where $I$ is the L-$[^{15}$N, 1-$^{13}$C$]_{\text{leucine}}$ infusion rate, $E_{\text{KIC}}$, the $^{13}$C enrichment of the L-$[^{15}$N, 1-$^{13}$C$]_{\text{leucine}}$ infused, and $E_{\text{sc}}$, the $^{13}$C enrichment of plasma KIC measured at isotopic equilibrium.

Leucine nitrogen flux ($\varphi_n$) calculated from the enrichment of L-$[^{15}$N, 1-$^{13}$C$]_{\text{leucine}}$, is:

$$\varphi_n = I(E_{\text{KIC}}/E_{\text{sc}} - 1)$$

where $E_{\text{sc}}$ is the $^{15}$N enrichment of the L-$[^{15}$N, 1-$^{13}$C$]_{\text{leucine}}$ infused and $E_{\text{KIC}}$ is the $^{15}$N enrichment of plasma leucine measured at isotopic equilibrium. The method for measuring $^{15}$N does not permit measuring $^{13}$C simultaneously, but it is likely that L-$[^{15}$N$]_{\text{leucine}}$ is only a fraction of all $^{15}$N-labelled leucine species.

Leucine oxidation (O) calculated from the $^{13}$CO$_2$ expiration rate and the $^{13}$C enrichment of plasma KIC, is:

$$O = F_{\text{CO}_2}[1/E_{\text{sc}} - 1/E_{\text{KIC}}] \times 100$$
where \( F_{13CO_2} \) is the rate of expired \(^{13}\)CO\(_2\) (\(\mu\)mol\(^{13}\)Ckg\(^{-1}\) h\(^{-1}\)) calculated from the \(^{13}\)C enrichment of CO\(_2\) in expired air and CO\(_2\) production rate assuming the recovery of \(^{13}\)CO\(_2\) to be 81\% for the fed state and 75\% for the fasted state, based on direct determination of \(^{13}\)C-bicarbonate recovery in normal subjects eating similar diets to those given in the present study (22). \(^{13}\)C enrichment of CO\(_2\) in the fed state was corrected for the increment in background \(^{13}\)C caused by feeding, which was measured during the sham infusion. If the background rate of \(^{13}\)C0\(_2\) expired had not been included, rates of leucine oxidation would have been overestimated by 9.4 ± 1.9\%. However, the background was measured for each patient on each diet and included in the calculation.

The rate of disappearance of leucine into body proteins (PS) was calculated from leucine carbon flux.

\[
\text{PS} = Q - \text{PD} + Q_c - I
\]

where PS is the rate of leucine disappearance into body proteins, \(Q\) is the rate of leucine appearance from protein degradation, \(PD\) is the rate of leucine appearance from protein degradation, \(Q_c\) is the rate of leucine appearance from protein degradation, and \(I\) is the rate of dietary leucine intake.

**Calculation of Isotopic Nitrogen Balance**

The balance between leucine disappearance into body protein and leucine appearance from protein breakdown was calculated in the fasting and fed state at both dietary levels. The leucine balance can be converted to a nitrogen equivalent, assuming that body protein contains (by weight) 8\% leucine and 16\% nitrogen (23). This value was calculated for fasting and fed states at each dietary level and then corrected to 12 h each to yield an estimate of daily nitrogen balance.

**Statistical Analysis**

Values are reported as mean ± SEM, and analyzed using paired and unpaired t tests, or analysis of variance; \(P < 0.05\) was considered statistically significant. Rates are expressed as \(\mu\)mol kg\(^{-1}\) h\(^{-1}\).

**RESULTS**

**Plasma Insulin**

Means of the two values of plasma insulin obtained before the infusion (basal) and of the five values obtained during the fasting and fed states are shown in Figure 2. Plasma insulin levels before and during the isotope infusions.

![Figure 2. Plasma insulin levels before and during the isotope infusions.](image)

**TABLE 2. Individual values for components of nitrogen balance in patients with CRF (gN/day ± SEM)**

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>(\lambda_0)</th>
<th>(\lambda)</th>
<th>(\mu)</th>
<th>(\nu)</th>
<th>(\delta)</th>
<th>(\epsilon)</th>
<th>(\Delta)</th>
<th>(\Delta)</th>
<th>(B_0)</th>
<th>(B)</th>
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<td>6.90</td>
<td>11.70</td>
<td>8.70</td>
<td>8.69</td>
<td>0.63</td>
<td>0.70</td>
<td>-1.29</td>
<td>1.95</td>
<td>-1.14</td>
<td>0.36</td>
</tr>
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<td>2</td>
<td>5.86</td>
<td>9.30</td>
<td>6.25</td>
<td>6.51</td>
<td>1.03</td>
<td>0.61</td>
<td>-0.69</td>
<td>1.07</td>
<td>-0.72</td>
<td>1.12</td>
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<tr>
<td>3</td>
<td>5.41</td>
<td>9.37</td>
<td>6.27</td>
<td>6.63</td>
<td>0.59</td>
<td>0.32</td>
<td>-0.81</td>
<td>1.01</td>
<td>-0.63</td>
<td>1.41</td>
</tr>
<tr>
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<td>5.85</td>
<td>9.91</td>
<td>5.10</td>
<td>5.72</td>
<td>1.79</td>
<td>1.67</td>
<td>0.15</td>
<td>0.61</td>
<td>-1.18</td>
<td>1.91</td>
</tr>
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<td>5</td>
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<td>6.04</td>
<td>6.15</td>
<td>1.20</td>
<td>1.31</td>
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<td>-0.05</td>
<td>-1.55</td>
<td>1.03</td>
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<td>7.15</td>
<td>11.59</td>
<td>8.50</td>
<td>7.32</td>
<td>0.91</td>
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<td>Mean</td>
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<td>10.07</td>
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<td>6.84</td>
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<td>0.99</td>
<td>-0.64</td>
<td>0.87</td>
<td>-1.11</td>
<td>1.38†</td>
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<tr>
<td>SEM</td>
<td>0.31</td>
<td>0.53</td>
<td>0.59</td>
<td>0.43</td>
<td>0.18</td>
<td>0.21</td>
<td>0.20</td>
<td>0.26</td>
<td>0.15</td>
<td>0.30</td>
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</table>

* \(\lambda\), nitrogen intake; \(\mu\), total urinary nitrogen; \(\nu\), fecal nitrogen; \(\epsilon\), estimated daily change in the nitrogen pool; \(B_0\), nitrogen balance. 0.6 and 1.0 refer to the appropriate dietary protein intake (g kg\(^{-1}\) day\(^{-1}\)).

* \(P < 0.05\), † \(P < 0.01\) compared to values obtained on 0.6 g kg\(^{-1}\) day\(^{-1}\) dietary protein intake.
In Figure 2. Basal and fasting plasma insulin levels (measured by radioimmunoassay, Incstar, Stillwater, MN) were consistently higher in the CRF patients at both dietary protein levels. Basal insulin levels were significantly lower in the CRF patients during the LP compared to the HP period (P < 0.01). The insulin response to feeding was similar in both groups at both dietary levels.

Nitrogen Balance

Tables 2 and 3 present individual values for dietary nitrogen intake (IN), urinary nitrogen (UN), fecal nitrogen (FN), the daily change in the body urea nitrogen pool (ΔN), and nitrogen balance (BN) for CRF patients and control subjects. Nitrogen balance was positive in both groups during the HP period but negative during the LP period. Urinary nitrogen was significantly increased by the HP diet in control subjects in contrast to CRF patients. In the latter group, urea nitrogen retention increased significantly.

Plasma Leucine and KIC Enrichments

The enrichments of plasma leucine and KIC were measured by total [13C]leucine (the sum of L-[1-13C]leucine and L-[15N,1-13C]leucine, by L-[13C]leucine, by L-[15N,1-13C]leucine, and by [1-13C]KIC. In both groups, leucine enrichment by total [13C]leucine was consistently (P < 0.01) greater than enrichment of plasma KIC by [1-13C]KIC. Enrichment of plasma KIC by [1-13C] was consistently greater than enrichment of plasma leucine by L-[1-13C]leucine, (P < 0.01; for both groups). The coefficients of variation (CV) for the isotopic "steady state" plasma enrichments of leucine and KIC by total [13C]leucine, L-[1-13C]leucine, L-[15N,1-13C]leucine and [1-13C]KIC are shown in Figure 3. Data from fasting and fed states, on HP and LP, have been combined because there were no significant differences between values from these two states. In both groups, the CV was highest for L-[15N,1-13C]leucine and lowest for [1-13C]KIC; the CV for total [13C]leucine was higher than for [1-13C]KIC in CRF patients and normal subjects (P < 0.01 and P = 0.06 respectively). These results reinforced our decision to use KIC enrichment to measure leucine flux.

The ratio of KIC enrichment by [1-13C]KIC to leucine enrichment by total [13C]leucine was 0.73 ± 0.03 in fasting CRF patients and 0.70 ± 0.01 in fasting control subjects. In fed CRF patients, it was 0.77 ± 0.04 compared to 0.75 ± 0.03 in fed control subjects. There was no significant difference in the ratio between the groups for either the fed or fasting periods, but in both groups there was a small increase in the ratio with feeding; this was statistically significant in CRF patients (P < 0.01).
TABLE 4. Components of leucine flux in chronic renal failure patients and normal, healthy control subjects fed two levels of dietary protein

<table>
<thead>
<tr>
<th></th>
<th>Chronic Renal Failure</th>
<th>Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High protein</td>
<td>Low protein</td>
</tr>
<tr>
<td>Leucine carbon flux (Qc)</td>
<td>94.36    113.36*</td>
<td>95.84    103.28</td>
</tr>
<tr>
<td>±0.33    ±7.09</td>
<td>±10.00    ±8.48</td>
<td>±9.65    ±8.96</td>
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<tr>
<td>Leucine nitrogen flux (QN)</td>
<td>155.45   202.39*</td>
<td>171.60   191.44*</td>
</tr>
<tr>
<td>±12.06   ±9.59</td>
<td>±15.78    ±12.92</td>
<td>±17.94   ±35.44</td>
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<tr>
<td>Leucine disappearance into proteins (PS)</td>
<td>79.83    82.96</td>
<td>83.23    80.85</td>
</tr>
<tr>
<td>±6.55    ±7.49</td>
<td>±9.26     ±9.44</td>
<td>±8.46    ±9.70</td>
</tr>
<tr>
<td>Leucine appearance from proteins (PD)</td>
<td>94.36    57.50*</td>
<td>95.84    69.57*</td>
</tr>
<tr>
<td>±0.83    ±2.73</td>
<td>±1.10     ±2.34</td>
<td>±1.37    ±1.59</td>
</tr>
<tr>
<td>Leucine oxidation (O)</td>
<td>14.52    30.41*</td>
<td>12.62    22.43*</td>
</tr>
<tr>
<td>±0.83    ±2.73</td>
<td>±1.10     ±2.34</td>
<td>±1.37    ±1.59</td>
</tr>
<tr>
<td>Reamination of KIC (XN)</td>
<td>61.10    89.03*</td>
<td>75.76    88.16*</td>
</tr>
<tr>
<td>±15.29   ±11.81</td>
<td>±18.14    ±13.24</td>
<td>±19.20   ±28.75</td>
</tr>
<tr>
<td>Leucine deamination (X0)</td>
<td>75.62    119.43*</td>
<td>88.39    110.59*</td>
</tr>
<tr>
<td>±15.79   ±12.61</td>
<td>±14.18    ±9.82</td>
<td>±9.91    ±27.30</td>
</tr>
</tbody>
</table>

*a Values (μmol kg⁻¹ h⁻¹) are mean ± SEM measured while subjects were eating 1.0 g (high protein) and 0.6 g protein kg⁻¹ day⁻¹ (low protein).

*P < 0.05; †P < 0.01 vs. fasting; *P < 0.05 vs. same value measured in normal subjects.

Leucine Flux

Leucine carbon flux (Qc) and leucine oxidation (O), measured in the fasting and fed states after 6 days of HP or LP diets are presented in Table 4. In both groups Qc increased significantly with feeding the HP diet, but there were no differences between CRF patients and control subjects. Leucine oxidation, measured in the fasting state, was virtually identical for both diets. When the two diets were compared, LP was associated with a lower value for meal-induced amino acid oxidation; the decline in oxidation while feeding the LP diet compared to the HP diet was similar in both groups (-26%, CRF vs. -33%, C; P = NS). The average increase in 13CO₂ enrichment with feeding during the sham infusion was 0.0017 ± 0.0002 APE. The extent to which oxidation could have been overestimated if this had not been taken into account was 9.4 ± 1.9%.

The disappearance of leucine into protein (PS) was calculated as leucine carbon flux (Qc) minus leucine oxidation (O). There was no detectable change in PS with feeding, and there were no differences between the groups or the two diets (Table 4). Thus, the meal-induced stimulation of leucine flux was primarily due to an increased rate of leucine oxidation.

The rate of appearance of leucine from protein degradation (PD) was calculated as follows: in the fasting state PD is equal to leucine carbon flux (Qc) and in the fed state, PD equals Qc minus dietary leucine intake. Fasting did not cause differences between the groups on either diet (Table 4). In both C and CRF groups, feeding caused a significant decrease in PD; this was greater in the HP period. However, the decrease in PD with feeding was not different when control subjects and CRF patients were compared. In calculating protein turnover, we have not corrected for a "first-pass" loss of dietary leucine within splanchnic tissues. Based on our unpublished studies of this loss, it would increase our calculated values of leucine appearance from protein breakdown by 10% on the LP diet and 20% on the HP diet. Even if we use these figures in the calculation and assume no first pass effect in CRF patients, values of leucine appearance in C and CRF subjects would not differ statistically.

Leucine nitrogen flux (QN), during the HP period, was similar in normal subjects and CRF patients (Table 4). QN increased with feeding in both groups, but the change was statistically significant (P < 0.01) only in CRF patients. In the LP period, QN was higher in CRF patients, both in the fasting and fed state, but this was significant only in the fed state (P < 0.05).

The rates of KIC reamination to leucine (XN) and the leucine deamination to KIC (X0) are presented in Table 4. XN and X₀ in both groups increased with feeding in the HP period. When CRF patients were compared to normal subjects during the LP diet, XN and X₀ were higher in fed CRF patients (P < 0.05).

Isotopic Nitrogen Balance

We also calculated the difference between leucine disappearance from incorporation into protein and leucine derived from protein breakdown to estimate
Nutritional Adaptation in Chronic Renal Failure

![Graph](https://via.placeholder.com/150)

Figure 4. Nitrogen balance measured using classical and isotopic methods (see Methods).

...a daily protein and, hence, nitrogen balance. These calculated values together with those obtained directly from nitrogen balance measured in the "classical manner" (13, 14), are shown in Figure 4. With the isotopic method, the balance was more positive for both HP and LP. However, for both groups the difference in nitrogen balance between the HP and LP diets was the same, regardless of the method of calculation.

**DISCUSSION**

In the present study, we used amino acid turnover methodology to examine how uncomplicated, moderately severe CRF affects the ability of patients to adapt to dietary protein restriction. CRF patients and C subjects responded qualitatively and quantitatively the same to dietary protein restriction by limiting the meal-induced rise in amino acid oxidation and protein breakdown (Table 4). Although no defect in the ability to adapt was uncovered, it is possible that if uremia is more severe, and, especially, if metabolic acidosis complicates CRF, the responses would be impaired with stimulation of amino acid oxidation and protein catabolism (24, 25). It is also possible that results from a larger number of C subjects might reveal a difference even though the C subjects examined responded similarly to those studied previously (17). However, using the present estimates of variability, it can be calculated that over 50 subjects would be needed in each group to be 95% confident that the difference between the two groups in the critical measurement of leucine carbon flux was not significant at 5% significance levels. Moreover, we have calculated that between four and seven patients are needed in each group to detect differences in the various components of leucine kinetics of a magnitude of 20% (26). It is also possible that the results could have been influenced by the low-protein diet prescribed for CRF patients 1–4 before the study. We attempted to overcome this problem by randomly assigning the order of the HP and LP diets. If the CRF patients had already adapted before beginning the study, we would have expected measurements of leucine metabolism including oxidation and protein turnover to have differed sharply from C subjects. Since this did not occur, we conclude that either compliance with the prescribed diet was poor or that our randomization of the order of diets removed a potential source of bias from the previous dietary regimen.

Although nitrogen balance has been the principle method of assessing the minimum dietary protein requirement, the technique has limitations (27). For instance, when dietary protein intake is reduced, urinary nitrogen can take several days to reach a new equilibrium (28). In the present study, we took this into account by correcting nitrogen balance for changes in the body urea nitrogen pool (13, 14). Our finding that both groups were in negative nitrogen balance during the initial 6 days of LP was somewhat unexpected since studies in both normal subjects and CRF patients suggest that the minimum daily dietary protein requirement for neutral nitrogen balance is approximately 0.6 g/kg (8, 10, 11, 29, 30). Possible explanations for the negative nitrogen balance include the short period of the study (31) and the energy intake (32). Assessment of nitrogen balances in normal subjects fed different levels of protein indicated that estimates of nitrogen balance are best made from measurements over about two weeks (31). The neutral nitrogen balance in CRF patients eating approximately 0.6 g kg⁻¹ day⁻¹ obtained by Ford et al (11) was performed over 6 to 55 days, and they noted that with time nitrogen balance seemed to become either less negative or even more positive. Kopple et al (10) reported that when protein intake was reduced from 40 to 20 g protein/day, nitrogen balance was negative, but it improved somewhat over the next 15 to 30 days. During the 1-wk period of the LP diet, we did not find any obvious tendency for nitrogen balance to improve. Thus, these previous reports and our data suggest that even when adaptive responses to a low-protein diet are intact, neutral nitrogen balance may not occur until the diet is eaten for longer than 7 days. On the other hand, the 7-day period of study was necessary to explore the process of adaptation in protein turnover as revealed by changes in leucine kinetics because these processes occur in relation to an acute reduction in dietary protein (6).

The mean energy intake of the CRF patients in our study (32.5 ± 3.5 kcal kg⁻¹ day⁻¹) is similar to that used by Kopple et al (36–38 kcal kg⁻¹ day⁻¹) (10, 33), Ford et al (23–59 kcal kg⁻¹ day⁻¹) (11), and Herndon et al (27–30 kcal kg⁻¹ day⁻¹) (12) in their studies that demonstrated neutral nitrogen balance in CRF patients eating approximately 0.6 g protein kg⁻¹ day⁻¹. It has been reported that increasing the energy intake will improve nitrogen balance in CRF patients, so it is possible that the energy intake of patients in this study was insufficient. However, the CRF patients
were in positive balance at the same caloric intake while eating 1.0 g protein kg$^{-1}$ day$^{-1}$ (Table 2), suggesting that when nitrogen intake is clearly adequate, 32 kcal kg$^{-1}$ day$^{-1}$ is sufficient to achieve nitrogen balance in normal and CRF patients. Regardless, the important finding was that both groups responded in a similar fashion to limiting dietary protein.

The adaptive mechanisms for responding to a low-protein diet cannot be examined by measuring nitrogen balance alone. The usefulness of measuring amino acid flux and oxidation has been criticized because of the assumptions used (34); the major assumption is that the enrichment of the amino acid in plasma accurately reflects enrichment of the intracellular amino acid. With leucine this problem has been minimized by using the enrichment of plasma KIC as an index of the enrichment of intracellular leucine (20, 21). Vazquez et al studied rats and showed that data obtained with plasma KIC yielded the same results for many aspects of amino acid metabolism as did those based on the labelling of intracellular leucine (35). Although this "reciprocal pool method" has not been examined in CRF patients, we found that the ratio of the enrichment of plasma KIC by [1-¹³C]KIC to enrichment of plasma leucine by total [¹³C]leucine did not differ when normal subjects and CRF patients were compared. This result suggests that the KIC enrichment technique, which was shown by Schwenk et al (21) to yield an accurate estimate of albumin synthesis and amino acid metabolism in normal subjects, should be valid in CRF patients. Our finding that the ratio of ¹³C-enrichment of KIC-to-leucine increases with feeding is not unexpected because leucine released from protein breakdown decreased with feeding. Finally, the variability of the steady state values of KIC enrichment by [1-¹³C]KIC was the lowest. Consequently, we used this value to obtain greater accuracy of flux measurements.

The principle changes in leucine kinetics induced by feeding in both CRF and control subjects included a decrease in leucine derived from protein breakdown and an increase in leucine oxidation (Table 4). It can be seen that the primary metabolic adaptation of both groups to a low protein diet was to decrease amino acid utilization. The absence of any change in protein synthesis with feeding is in agreement with some (36) but not all of our (7) previous studies and those of others. Rennie et al (37) fed 1.2 g protein kg$^{-1}$ day$^{-1}$ and measured leucine kinetics while the subjects were fasting and on another day while they were fed. There was a significant increase in leucine flux, protein synthesis, and amino acid oxidation but no change in protein breakdown with feeding. Clugston and Garlick (38), fed 1.0 g protein kg$^{-1}$ day$^{-1}$ and measured leucine kinetics during fasting and feeding on the same day. They found significant increases in amino acid flux, amino acid oxidation, protein synthesis and protein breakdown with feeding. Hoffer et al (36) fed 1.5 g protein kg$^{-1}$ day$^{-1}$ and reported an increase in amino acid flux, amino acid oxidation, and protein synthesis, and a decrease in protein breakdown with feeding. Motil et al (7), fed protein intakes of 1.5, 0.6, and 0.1 g kg$^{-1}$ day$^{-1}$ in different subjects on different days. With 1.5 g kg$^{-1}$ day$^{-1}$, leucine flux and oxidation were significantly higher and protein breakdown was lower, but there was no significant impact on protein synthesis. With a diet of 0.6 g protein kg$^{-1}$ day$^{-1}$, there were no significant changes in amino acid flux, amino acid oxidation, or protein synthesis; protein breakdown decreased significantly. Our results appear to agree most closely with those of Motil et al (7); however, our experimental design was most similar to that of Hoffer et al (36), except that the latter study used three times more protein than we used in our LP diet and was 50% more than contained in our HP diet. Thus, among other factors, variations in experimental design and quantity and rate of ingestion of protein during the infusion may be responsible for the differences between our results for protein synthesis during the fed state and those of other reports. Nevertheless, the responses of CRF and C subjects to the two different levels of dietary protein were the same.

We have not assessed whether abnormal gastric emptying influenced our results. If emptying were delayed, whole body PD during feeding would have been underestimated, but we suspect any such abnormality had a minimal impact since the liquid diet was fed over 6 h and the kinetics were measured only during the final hour. Moreover, the time course of the release of amino acids, including leucine, from the splanchnic tissues after ingestion of a mixture of amino acids simulating a 0.8 g protein/kg meal was more rapid or not different from that of control subjects (39). These results suggest that CRF does not greatly impair gastric emptying.

Nitrogen balance assessed from the isotope data was more positive than that observed with the classical method, but the difference between the balance on HP and LP was the same in each group using either method (Figure 4). The discrepancy between results using the two methods may be explained by our experimental design; the subjects were fed continuously for 6 h to obtain the isotopic results, while the normal intermittent feeding pattern over 5 days was used to measure "classical" nitrogen balance. It would appear, therefore, that continuous feeding, by suppressing protein degradation, may lead to more efficient nitrogen retention.

Finally, it is worth emphasizing that adaptations to a reduction in dietary protein occur within about 1 wk in normal, healthy subjects and lead to neutral nitrogen balance (6, 28, 29). Beyond 1 wk, additional
changes in nitrogen metabolism (40) and leucine kinetics may well occur (41), initiating responses to accommodate to an inadequate protein intake. Accommodation responses have been found to be associated with continued loss of body nitrogen (40) and a reduced rate of leucine incorporation into proteins (and, presumably, in protein turnover) (17, 41). Accommodation, therefore, involves metabolic responses different from those of adaptation, which our study was directed at investigating. Although it would have been of interest to study the mechanisms for accommodation, it would require study of the metabolic responses to a chronic, inadequate supply of dietary protein. Thus, it remains possible that CRF causes differences in the accommodation responses to dietary protein restriction, leading to more severe protein wasting.

In conclusion, the results suggest that the mechanisms for nutritional adaptation to a LP diet are similar in C and CRF and that the responses are not impaired by CRF. On the other hand, we did not study the influence of acidosis (24, 25) or other signs of severe uremia and, therefore, cannot extend these findings to all patients. Because the adaptive responses to dietary protein restriction are unimpaired, a protein intake sufficient for normal, healthy subjects should avoid malnutrition in clinically stable CRF patients.

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74 Volume 1 • Number 1 • 1990