Insulin Is Protein-Anabolic in Chronic Renal Failure Patients

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Abstract. To examine the protein anabolic actions of insulin in chronic renal failure, the authors measured four sets of whole body leucine fluxes during insulin alone and insulin with amino acid infusion in nine uremic patients before hemodialysis (B-HD). Seven were restudied 8 wk after initiation of maintenance hemodialysis (HD). Six normal subjects served as control (N). All values (μmol/kg/h, mean ± SEM) are presented in the sequence of B-HD, HD, and N, and only P < 0.05 are listed. During Flux 1 (baseline), D (leucine release from body protein degradation) were 114 ± 7, 126 ± 4, and 116 ± 6, respectively. C (leucine oxidation rates) were 18 ± 2, 17 ± 2, and 21 ± 3, respectively. S (leucine disappearance into body protein [index of protein synthesis]) were 96 ± 6, 107 ± 4, and 94 ± 4, respectively, and balances (net leucine flux into protein [values were negative during fasting]) were −18 ± 2, −17 ± 2, and −21 ± 3, respectively. During Flux 2 (low-dose insulin infusion), D were 89 ± 3, 98 ± 6, and 94 ± 5, respectively; C were 35 ± 4, 29 ± 5, and 39 ± 3, respectively; S were 105 ± 5, 145 ± 15, and 113 ± 6, respectively (P = 0.02), and balances were 32 ± 4, 38 ± 5, and 27 ± 3, respectively. These data show that B-HD and HD patients were as sensitive as normal subjects to the protein anabolic actions of insulin. Insulin alone reduced proteolysis and leucine oxidation, and insulin given with amino acids increased net protein synthesis.

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recording. Menus were subsequently created according to the subjects’ preference. The CRC kitchen prepared the food, subjects consumed the diet for 6 d, and the experiment was performed on day 7. Food not eaten was returned for measurement. While there was no restriction imposed on the controls, CRF patients had some restriction: salt, 5 g/d; phosphorus, 10 to 13 mg/kg/d; and fluid, 1500 ml/d for HD patients. The reason for eating a constant diet was to ensure that participants were in a stable nutritional state. For the seven patients who were studied twice, diet was identical during the two periods.

**Experimental Procedures.** Study subjects were admitted to the CRC the afternoon before the study, an evening snack was served at 7:00 p.m., and no additional food was allowed, except water, until the end of the experiment. The experiment was started at 7:00 a.m. the next day. Four in vivo whole-body leucine fluxes were measured (Figure 1). Flux 1 was the baseline study. Flux 2 and Flux 3 were measured during hyperinsulin-euglycemic clamps using insulin doses of 40 and 100 mU/m²/min, respectively. Flux 4 was measured during infusion of 40 mU/m²/min of insulin and 0.02 ml/kg/min of 10% Trasavsol (Baxter Health Care Corporation, Deerfield, IL). The latter contains 55.6 μmol/ml of leucine, giving a leucine infusion rate of 67 μmol/kg/h. Leucine Flux 1 was performed in 120 min, the other three fluxes lasted 140 min each. Total infusion time was 540 min. Tessari et al. (17) used similar successive short infusion protocol to study the dose-response relationship of insulin on leucine kinetics in humans. Solutions were infused through a venous catheter in one forearm, and blood samples were collected from a vein in the contralateral arm. In CRF patients, the arm with the vascular access was used for blood sampling. For HD patients, the study was performed on a mid-week nondialysis day, generally 14 h after the last hemodialysis.

**Leucine Flux Measurements.** Whole-body leucine flux was measured using a primed-constant infusion technique. The priming solution consisted of 4.2 μmol/kg of L[1-13C] leucine and 0.11 mg/kg of NaH13CO3 and the constant infusion, 4.2 μmol/kg/h of L[1-13C] leucine, as described previously (18–21). Solutions were prepared aseptically the afternoon before the experiment. Blood and breath samples were taken, and VCO2 was measured at times listed in Figure 1. Breath samples were collected anaerobically, and plasma samples were frozen at −70°C until ready for measurement (20,21).

To account for the drift in breath 13CO2 due to the presence of natural 13C abundance in the dextrose and Trasavsol solutions, separate studies were performed in four subjects, two controls and two B-HD patients, in whom breath 13CO2 was measured during identical insulin and Trasavsol infusion, but without the stable isotopes. The magnitude of the drift in 13CO2 between the controls and the CRF patients was not different. Thus, mean breath 13CO2 abundance obtained from these four subjects at each time/period were subtracted from the breath 13CO2 enrichment of each study subject at corresponding times.

**Hyperinsulin-Euglycemic Clamps.** Priming insulin was administered in a logarithmically falling manner over 10 min (22), followed by a continuous infusion at the calculated dose. Glucose was initially infused at 2.0 and then 2.5 mg/kg/min for the first 10 min. After that, the infusion rate was adjusted to maintain blood glucose at the individual’s baseline value. Blood glucose was measured every 5 min using a bedside glucose analyzer (Yellow Springs Instrument Co., Inc, Yellow Spring, OH). Insulin (Humulin Regular U-100; Eli Lilly, Indianapolis, IN) was diluted to 500 mU/ml in normal saline to which 1 ml of the subject’s blood was added for every 50 ml of infusate. Because insulin is partly degraded by the kidneys, doses in the CRF patients were arbitrarily reduced by 10%.

**Analytical Determinations.** Mole % enrichment of L[1-13C] leucine and L[1-13C] KIC in the plasma were quantitated using gas chromatography–mass spectrometry (Hewlett-Packard 5988A GC/MS) and breath 13CO2, dual-inlet gas-isotope ratio mass spectrometer (VG-Isogas SIRA II). Plasma leucine was converted to its heptafluorobutyryl n-propyl ester derivative, and 13C abundance was determined using either positive chemical ionization mass spectrometry with selected ion monitoring of mass-to-charge (m/z) ratio 371 and 370 or in the negative chemical ionization mode monitoring ions m/z 350 and 349. The tri-methylsilyl-quinoxalinol derivative of plasma KIC was prepared and 13C abundance was determined using electron-impact ionization mass spectrometry with selected ion monitoring of m/z ratio 233 and 232.

Plasma leucine concentration was determined by adding 50 nmol of d3-leucine (Cambridge Isotopes Laboratories, Andover, MA) to 1 ml of plasma. Plasma leucine was chemically derivatized to its heptafluorobutyryl n-propyl ester, and leucine concentration was measured using selected ion monitoring-gas chromatography-negative chemical ionization-quadrupole mass spectrometry. Signal areas for the selected ions (m/z 349 and 352) were quantitated and used to calculate leucine concentration.

Plasma insulin concentration was measured by electrochemical luminescence immunoassay (Roche Diagnostics Corporation, Indianapolis, IN).

**Calculations**

In the quantitation of leucine kinetics (Q = I + D = C + S); Q is total flux, I and D are, respectively, leucine intake and leucine release from body protein degradation, and C and S are, respectively, leucine disappearance via oxidation and incorporation into body proteins. In the current work, during Fluxes 1, 2, and 3, D = Q as intake was 0. During Flux 4, D = Q − 67 μmol/kg/h, the amount of leucine administered. Detailed derivations were previously described (21,22).

**Statistical Analyses**

All values are presented as mean ± SEM. Differences of the kinetic parameters among the three study groups, the four fluxes, as well as
the plasma and breath enrichment within the same flux of each study group were assessed by one-way ANOVA. Differences between any two groups were further assessed by multiple comparisons using Student-Newman-Keuls test. Paired *t* tests were performed on the seven CRF patients who were studied before and after HD. The significant level accepted to reject the null hypothesis is *P* < 0.05. Analyses were performed using statistical software package Sigma Stat (Jandel Scientific Software, San Rafael, CA).

**Results**

Nine B-HD, seven HD, and six N subjects completed the studies. Table 1 lists their demographic and baseline status. All participants had normal body mass index. Calorie and protein intakes were not different. Acidosis was corrected in all CRF patients. HD patients were mildly alkalotic; mean blood pH and total CO2 were 7.44 and 31 mmol/L, respectively. The B-HD group had higher blood urea nitrogen. Serum albumin and total CO2 were 7.44 and 31 mmol/L, respectively. The control values were 4.61 ± 0.09, 4.90 ± 0.07, 5.14 ± 0.10, and 2.57 ± 0.01, respectively. Note that plasma enrichment of the isotopes rose to Flux 4 were 3.66 ± 0.08, and control values were 4.56 ± 0.08, 5.24 ± 0.09, and 2.94 ± 0.03. For the HD group, values were 3.54 ± 0.13, 4.22 ± 0.07, 5.14 ± 0.10, and 2.57 ± 0.01. The control values were 3.56 ± 0.01, 4.34 ± 0.06, 4.87 ± 0.02, and 2.72 ± 0.11, respectively. Note that plasma enrichment of the isotopes rose during Fluxes 2 and 3 (insulin) and declined in Flux 4 (insulin and amino acid). The pattern of response was identical in the three study groups. Breath 13CO2 enrichment (values ×10−3) for the B-HD patients from Flux 1 to Flux 4 were 7.0 ± 0.04, 5.7 ± 0.09, 4.9 ± 0.09, and 9.2 ± 0.10. For the HD group, values were 5.4 ± 0.03, 4.4 ± 0.08, 3.5 ± 0.05, and 5.8 ± 0.08, and control values were 9.1 ± 0.03, 8.4 ± 0.02, 6.7 ± 0.05, and 9.9 ± 0.15. Again, the pattern of response was similar in all three study groups. Insulin alone reduced breath 13CO2 enrichment, whereas insulin with amino acid increased it.

Statistical analysis of the plasma and breath enrichment at 90, 100, 110, and 120 min during Flux 1 and at 110, 120, 130, and 140 min during Fluxes 2, 3, and 4 showed no differences among these values within the same flux period in all three study groups. Greater than 90% of the *P* values were > 0.80. By contrast, both plasma and breath enrichment were markedly different from one flux to the other within each study group. The *P* values derived from one-way ANOVA of plasma 13C leucine, plasma 13CKIC, and breath 13CO2 enrichment among Fluxes 1, 2, 3, and 4 were < 0.0001 in all three study groups. Furthermore, multiple comparisons using Student-Newman-Keuls test showed significant differences (*P* < 0.05) comparing Flux 1 versus 2, Flux 1 versus 3, Flux 1 versus 4, Flux 2 versus 3, Flux 2 versus 4, and Flux 3 versus 4. Thus, we have successfully created the planned metabolic perturbations while simultaneously achieving a steady-state condition by the end of each period.

Referring again to Figures 2, 3, and 4, one observes that,
In the HD patients, D KIC from Flux 1 to Flux 4 were 126, Flux 2 and 3, respectively, and increased markedly to Flux 1, became less negative, /H11002/H11006 remained low at 73.

Table 2 summarizes the kinetic data in the three study groups during each of the four fluxes. Multiple comparisons using the Student-Newman-Keuls method yielded P < 0.0001 for all the three study groups. Multiple comparisons using the Student-Newman-Keuls method yielded P < 0.05 comparing Flux 1 versus 2, Flux 1 versus 3, Flux 1 versus 4, Flux 2 versus 3, Flux 2 versus 4, and Flux 3 versus 4. Of note, the plasma 13C KIC enrichment of the three study groups within the same flux period were not different from one another.

...although plasma 13C leucine and 13C KIC enrichment were not statistically different among the three study groups during each flux, breath 13CO2 enrichment, on the other hand, was different in the three study groups, being consistently lower in CRF patients, especially marked in the HD group. One-way ANOVA comparing breath 13CO2 enrichment between the patients, especially marked in the HD group. Group was reduced in Flux 2: 86, 90, 98, and 111, respectively. During Flux 3, the values were 69, 73, 75, and 79, respectively. During Flux 4, plasma leucine levels were comparably increased: 238, 228, 234, and 246, respectively.

Figure 5 schematically depicts kinetic parameters during Fluxes 1, 2, and 4; data from Flux 3 were not plotted because they are similar to those of Flux 2. Panel A depicts protein degradation rates. Insulin alone and insulin plus amino acids reduced protein degradation to comparable degrees in all
groups. Panel B represents leucine oxidation rates, which were reduced by insulin alone and increased by insulin and amino acids. Oxidation rates were lower in the B-HD and HD groups. Panel C presents protein synthesis showing mild reduction during fasting. When amino acids and insulin were administered together, leucine balance became positive in all groups.

Table 3 presents data on glucose kinetics. Plasma insulin (µU/ml) levels were 15 ± 2 and 14 ± 2, respectively, in the B-HD and HD patients and 6 ± 1 in the normal controls (P = 0.007) during Flux 1. During Flux 2, insulin levels were increased to 96 ± 9, 94 ± 7, and 88 ± 5 in the B-HD, HD, and N groups, respectively. During Flux 3, insulin levels were further increased to 244 ± 23, 262 ± 40, and 220 ± 14, respectively. During Flux 4, insulin levels were 142 ± 17, 123 ± 9, and 108 ± 5, respectively. Plasma insulin levels were not different among the three groups during the last three fluxes. Plasma glucose concentration was normal and not different among the three groups during Flux 1 and was subsequently clamped at basal level during the entire experiment. Glucose disposal rate (mg/kg/min) was derived from the amount of glucose infused to maintain plasma glucose at baseline concentration. The rates during Flux 2 were 4.0 ± 0.4, 4.8 ± 0.6, and 5.9 ± 0.5 in the B-HD, HD, and C groups, respectively. Disposal rates during Flux 3 were 8.4 ± 0.7, 9.8 ± 0.7, and 11.6 ± 0.5 (P = 0.01). During Flux 4, disposal rates were 8.3 ± 0.6, 9.0 ± 0.6, and 10.2 ± 0.2 (P = 0.04). Glucose disposal was statistically lower in B-HD compared with controls. There were no significant statistical differences noted between the HD and the N groups.

Discussion

Despite the general belief that uremia is an insulin-resistant state, there are few papers addressing insulin action on protein metabolism in CRF patients. Using a femoral vein, brachial artery catheterization technique, Alverstrand et al. (8) noted that amino acid release from muscle declined similarly in control subjects and predialysis patients during hyperinsulinemia. Castellino et al. (9,10) did two studies. In one (9), they found that proteolysis and leucine oxidation are similarly reduced during hyperinsulinemia in normal subjects and CRF patients, but the ability of the body to increase protein synthesis is impaired in

<table>
<thead>
<tr>
<th>Group</th>
<th>Flux 1</th>
<th>Flux 2</th>
<th>Flux 3</th>
<th>Flux 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q_{KIC} (µmol/kg/h)</td>
<td>C_{KIC} (µmol/kg/h)</td>
<td>S_{KIC} (µmol/kg/h)</td>
<td>D_{KIC} (µmol/kg/h)</td>
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<tr>
<td>B-HD</td>
<td>114 ± 7</td>
<td>18 ± 2.3</td>
<td>96 ± 6</td>
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<tr>
<td>HD</td>
<td>126 ± 4</td>
<td>17 ± 2.1</td>
<td>107 ± 4</td>
<td>126 ± 4</td>
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<tr>
<td>N</td>
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<td>21 ± 2.6</td>
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<td>116 ± 6</td>
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<td>P</td>
<td>0.32</td>
<td>0.44</td>
<td>0.14</td>
<td>0.32</td>
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<tr>
<td>B-HD</td>
<td>89 ± 3</td>
<td>12 ± 1.4^b</td>
<td>77 ± 3.7</td>
<td>89 ± 3</td>
</tr>
<tr>
<td>HD</td>
<td>98 ± 6</td>
<td>11 ± 1.6^c</td>
<td>87 ± 5.3</td>
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<tr>
<td>N</td>
<td>94 ± 5</td>
<td>18 ± 1.1</td>
<td>76 ± 4.7</td>
<td>94 ± 5</td>
</tr>
<tr>
<td>P</td>
<td>0.39</td>
<td>0.021</td>
<td>0.22</td>
<td>0.39</td>
</tr>
</tbody>
</table>

* B-HD, HD, and N represent, respectively, predialysis, hemodialysis patients, and normal subjects. Q, D, C, and S represent, respectively, leucine flux, protein degradation, leucine oxidation, and protein synthesis. Balance represents net flux of leucine into protein. KIC indicates that calculations were derived from plasma 3\(^{13}\)C KIC enrichment. During Flux 1, 2, and 3, D = Q. During Flux 4, D = Q – 67 µmol/kg/h. VCO\(_2\) is CO\(_2\) production rate. All P values listed were derived from one-way ANOVA comparing the three study groups within each flux period.

^b P < 0.05 B-HD versus N.

^c P < 0.05 HD versus N.

^d P < 0.05 B-HD versus HD.
CRF patients during amino acid infusion. In the second study (10), they found that hyperaminoacidemia did increase net protein balance in CAPD patients. The difference between the two studies was the infusion of insulin together with the amino acids in the latter, suggesting that both substrate (i.e., amino acids) and insulin are needed to induce anabolism. Reachi et al. (11) found that protein degradation in predialysis patients declined with insulin administration irrespective of acid/base status. Luzi et al. (12) noted that insulin-dependent uremic patients have a blunted reduction in proteolysis and leucine oxidation during hyperinsulinemia. The reduction in proteolysis, but not leucine oxidation, was corrected in kidney transplant patients, and both abnormalities were corrected in combined kidney/pancreas transplant patients. These data suggest that uremia impaired the insulin-induced reduction in proteolysis. Garibotto et al. (13) measured forearm muscle kinetics and found that, although proteolysis was inversely related to plasma insulin concentrations in normal subjects, no such correlation exist in the CRF patients. These data, again, suggest impaired insulin suppression of proteolysis during CRF.

The current study is different from the above-cited works. We studied CRF patients immediately before initiating dialysis, so the degree of uremia was more profound. In the Castellino et al. (9) subjects, mean serum creatinine was 4.0 mg/dl; in our subjects, it was 10.2 mg/dl. This is the first time hemodialysis patients have been studied with regard to insulin actions on protein anabolism. Moreover, we studied the same CRF patients before and after initiation of maintenance hemodialysis. This allowed us to assess whether stabilization on maintenance dialysis altered insulin sensitivity. Lastly, we measured the effects of insulin alone and insulin plus amino acids all in one setting when the general health status of each participant was identical.

In normal subjects, insulin suppresses proteolysis in a dose-dependent manner (17,23). Castellino et al. (9) and Tessari et al. (17) found that insulin also suppresses leucine oxidation. Fukagawa et al. (23), on the other hand, found variable effects of insulin on leucine oxidation (23). There are few studies documenting enhanced protein synthesis, one of which is that of Castellino et al. (10) in CAPD patients. In the present study, we found that insulin alone consistently reduced protein degradation and suppressed leucine oxidation during fasting in all three study groups. When insulin was administered with amino acids, protein degradation declined, leucine oxidation rose, protein synthesis increased and, most importantly, net amino acid flux into body protein was enhanced.

Of interest, as shown in Table 2, is the observation that the two CRF groups, especially the hemodialysis patients, appeared to be more sensitive to the suppressive effect of insulin.
in reducing leucine oxidation, which was lower in the B-HD and HD groups during Fluxes 2 and 3. During amino acid infusion, leucine oxidation rose in all three study groups, but the increment was of lesser magnitude in HD patients, resulting in a higher protein synthesis rate. During that same period, leucine balance, an index of net protein synthesis, was markedly positive in all the three study groups, more so in the HD patients. Thus, both B-HD and HD patients behaved similarly to normal controls with regard to the anabolic effects of insulin.

The higher protein synthesis rate observed in the HD patients is likely related to the lower leucine oxidation rate. This could be attributed to a reduction in the magnitude of uremia. Alternatively, it may be related to the mild alkalosis. Blood pH and total CO₂ measured on the morning of the experiment were 7.44 and 31 mmol/L, respectively. Acidosis has been demonstrated to increase leucine oxidation in CRF patients and normal subjects (21,24).

In this study, we showed that glucose disposal was impaired in the B-HD patients and normal in the HD group. In the B-HD group, while glucose disposal was impaired, protein synthesis was normal. This dissociation between glucose disposal and protein anabolism suggests that pathways of insulin-mediated carbohydrate and protein metabolism might be different.

It should be noted that we measured whole-body protein flux and not regional kinetics. While the results indicate whole body anabolism, the data do not differentiate between splanchnic versus skeletal muscle compartments. Since skeletal muscle constitutes a major fraction of body protein, it is likely that there is anabolism in the muscle. We expressed all our results in μmol/kg/h and not per unit lean body mass because we did not measure body fat and did not calculate lean body mass. This, however, should not negate the validity of our findings because the body mass indices of the three study groups were similar (28, 26, and 27; P = 0.7; Table 1). It is extremely unlikely that any difference in the three groups was masked because of variation in lean body mass.

Our findings may not be applicable to the general end-stage renal disease population because some of these patients have concomitant illnesses. We purposely chose stable, well-nourished patients free of other illnesses to specifically study the effects of uremia and maintenance hemodialysis on insulin sensitivity.

The current study and many others have provided evidence that uremia per se is not a protein catabolic state as is believed traditionally. Our study further underscores that, despite the catabolic nature of the hemodialysis procedure (25,26), patients undergoing maintenance hemodialysis can achieve anabolism when given sufficient protein supply and when adequate insulin is present. The recent work of Pupim et al. (27) showing increased protein synthesis and net protein balance with intradialytic parenteral nutrition supports this notion.

Anorexia due either to uremia or concomitant illness may be an important factor contributing to malnutrition in the end-stage renal disease population. Our knowledge regarding anorexia is still very limited; therefore, nephrologists should pay attention to providing optimal dialysis and ensuring adequate nutritional intake of their patients.

Acknowledgments

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References