Effects of Insulin and Amino Acids on Glucose and Leucine Metabolism in CAPD Patients

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Abstract. This study investigates the basal and insulin-stimulated glucose metabolism, substrate utilization, and protein turnover in eight patients maintained on continuous ambulatory peritoneal dialysis (CAPD) (mean age 39 ± 5 yr, body mass index [BMI] 108 ± 6) and 14 control subjects (mean age 33 ± 4 yr, BMI 103 ± 3). Euglycemic insulin clamp studies (180 min) were performed in combination with continuous indirect calorimetry and 1-14C leucine infusion (study I). Postabsorptive glucose oxidation was higher (1.75 ± 0.18 versus 1.42 ± 0.14 mg/kg per min) and lipid oxidation was lower (0.43 ± 0.09 versus 0.61 ± 0.12 mg/kg per min) in CAPD patients than in control subjects (P < 0.05 versus control subjects). During the last 60 min of euglycemic hyperinsulinemia, the total rate of glucose metabolism was similar in CAPD and control subjects (6.33 ± 0.08 and 6.54 ± 0.62 mg/kg per min). Both insulin-stimulated glucose oxidation (2.53 ± 0.27 versus 2.64 ± 0.37 mg/kg per min) and glucose storage (3.70 ± 0.48 versus 3.90 ± 0.58 mg/kg per min) were similar in CAPD and control subjects. Basal leucine flux (an index of endogenous proteolysis) was significantly lower in CAPD patients than in control subjects (1.21 ± 0.15 versus 1.65 ± 0.07 μmol/kg per min). Leucine oxidation (0.13 ± 0.02 versus 0.26 ± 0.02 μmol/kg per min) and nonoxidative leucine disposal (an index of protein synthesis) (1.09 ± 0.16 versus 1.35 ± 0.05 μmol/kg per min) were also reduced in CAPD compared with control subjects (P < 0.01 versus control subjects). In response to insulin (study I), endogenous leucine flux decreased to 0.83 ± 0.08 and 1.05 ± 0.05 μmol/kg per min in CAPD and control subjects, respectively (all P < 0.01 versus basal). Leucine oxidation declined to 0.06 ± 0.01 and to 0.19 ± 0.02 μmol/kg per min in CAPD and control subjects, respectively (P < 0.01 versus basal). A second insulin clamp was performed in combination with an intravenous amino acid infusion (study II). During insulin plus amino acid administration, nonoxidative leucine disposal rose to 1.23 ± 0.17 and 1.42 ± 0.09 μmol/kg per min in CAPD and control subjects, respectively (both P < 0.05 versus basal, P = NS versus control subjects), and leucine balance, an index of the net amino acid flux into protein, become positive in both groups (0.30 ± 0.05 versus 0.40 ± 0.07 μmol/kg per min in CAPD and control subjects, respectively) (both P < 0.01 versus basal, P = NS versus control subjects). In summary, in CAPD patients: (1) basal glucose oxidation is increased; (2) basal lipid oxidation is decreased; (3) insulin-mediated glucose oxidation and storage are normal; (4) basal leucine flux is reduced; (5) the antiproteolytic action of insulin is normal; and (6) the anabolic response to insulin plus amino acid administration is normal. Uremic patients maintained on CAPD treatment show a preferential utilization of glucose as postabsorptive energy substrate; however, their anabolic response to substrate administration and the sensitivity to insulin are normal.

Uremia is characterized by a variety of metabolic abnormalities, including altered plasma and intracellular amino acid levels (1), impaired protein anabolism (2), insulin resistance, and glucose intolerance (3). In recent years, the use of continuous ambulatory peritoneal dialysis (CAPD) to treat patients with end-stage renal failure has grown considerably. However, little is known about the effects of CAPD treatment on the above-mentioned metabolic disturbances (4,5). In particular, uremic patients on CAPD are known to have higher fasting insulin levels, and a pronounced insulin response to each dialytic exchange and an elevated 24-h plasma insulin profile compared with control and hemodialysis subjects (5). Thus, although insulin plays a pivotal role in glucose and amino acid metabolism, its effects in CAPD patients have been poorly investigated.

In previous reports, we demonstrated that the ability of amino acids to promote net protein anabolism is impaired in chronic renal failure (CRF) subjects (2), and it is not restored to normal by chronic hemodialysis treatment in diabetic uremic subjects (6). The effects of amino acid administration on protein metabolism in CAPD are unknown. In the present study, we used the euglycemic insulin clamp technique and amino acid administration in combination with 1-14C leucine infusion.
and indirect calorimetry to examine the effects of insulin and substrate availability on glucose and leucine metabolism in patients on CAPD treatment.

Materials and Methods

Patient Population

Fourteen healthy volunteers (10 men and four women) and eight patients with end-stage renal failure (six men and two women) maintained on CAPD treatment participated in the study protocol. Fourteen control subjects participated in study I, and 12 were reevaluated in study II. All CAPD subjects participated in both study I and study II.

All subjects were within 20% of their ideal body weight (control subjects 102 ± 3; CAPD patients 106 ± 5) based on the medium frame of the Metropolitan Life Insurance Table 1983. Their body mass index (BMI) values were: control subjects 22 ± 2; CAPD 23 ± 2. The mean age was 33 ± 4 and 39 ± 5 yr for control subjects and CAPD, respectively. Except for the presence of end-stage renal failure in the CAPD group, no subject had any evidence of endocrine or other major organ system disease. Liver function tests were normal in all of the uremic patients. Other than vitamin, bicarbonate, and phosphate binder supplementations in the uremic group, subjects were not taking any medication. There was no family history of diabetes in any of the subjects. The etiology of renal failure in the uremic group was as follows: chronic glomerulonephritis (n = 3), membranous nephropathy (n = 2), interstitial nephritis (n = 1), unknown (n = 2). The mean duration of CAPD therapy was 3 ± 1 yr. At the time of the study, the mean serum urea nitrogen and creatinine were 74 ± 6 and 14 ± 2 mg/dl, respectively. Serum electrolytes were as follows: sodium 134 ± 4, potassium 4.6 ± 0.3, bicarbonate 24 ± 2 mEq/L. For at least 3 d before the study, all subjects consumed a weight-maintaining diet providing at least 250 g of carbohydrate and between 50 and 80 g of protein. In control subjects, dietary protein intake was estimated from 24-h urinary nitrogen excretion. In CAPD patients, dietary protein intake was calculated from both a dietary history and 24-h nitrogen excretion in dialysate effluent and urine. Estimated protein intake ranged from 0.9 to 1.2 g/kg per d in control subjects and from 0.8 to 1.1 g/kg per d in CAPD patients.

The purpose and potential risks of the study were explained to all subjects, and their voluntary written consent was obtained before their participation. The study protocol was reviewed and approved by the Human Investigation Committee of the Yale University School of Medicine and by the Institutional Review Board of the University of Texas Health Science Center at San Antonio.

Experimental Protocol

All tests were performed in the postabsorptive state beginning at 8 a.m. after a 12-h overnight fast. The CAPD patients performed their last dialytic exchange at 10 p.m. the night before each study. The peritoneal cavity was drained and was maintained empty until completion of the protocol. Control subjects and CAPD patients participated in two experimental protocols that were performed in random order at 10- to 15-d intervals. In each study protocol, a small polyethylene catheter was inserted into an antecubital vein for the infusion of isotope. A slight negative pressure was maintained in the canopy to avoid loss of the expired air. The carbon dioxide and oxygen content of the expired air were continuously measured by a Deltatrac Metabolic Monitor (Sensoxomec, Anaheim, CA).

In control subjects, nitrogen excretion was calculated from urinary collections. In CAPD subjects, nitrogen excretion was estimated from the nitrogen content of dialysate effluent and residual diuresis. Data on nitrogen excretion allow estimation of nonprotein VO2 and VCO2. Glucose and lipid oxidation are then calculated from NPVO2 and NPVCO2 using standard formulas derived from Lusk Table (10).

Analytical Determination

Plasma leucine and α-KIC specific activities were measured as described previously (9). Plasma leucine concentration was determined using an amino acid analyzer (System 6300; Beckman, Anaheim, CA). To precipitate plasma proteins, 2.5 ml of 10% sulfosalicylic acid was added to 2.5 ml of plasma, and a 1-ml aliquot of the supernatant was analyzed in duplicate for plasma amino acid concentration. One milliliter of the remaining supernatant was placed in duplicate on a Dowex 50 G cation exchange resin column (Bio-Rad Laboratories, Richmond, CA), and the free amino acid fraction was eluted with 4N NH4OH, subsequently dehydrated, and reconstituted in water. Scintillation fluid (10 ml) was added to each vial, and 14C radioactivity was measured in a Packard TriCarb scintillation counter (Packard Instruments, Dower Grove, IL). Plasma (1 ml) was placed in duplicate on a Dowex 50 G cation exchange resin column (Bio-Rad Laboratories), and the free α-ketoacid fraction was eluted with 4 ml of 0.01N HCl in 50-ml culture tubes. Methylen chloride (35 ml) was added, and after shak-
ing vigorously for 1 min, the tube was centrifuged for 5 min at 2000 rpm to extract the free α-ketoacid fraction from plasma. After decantation of the supernatant, the α-ketoacid was extracted in 350 μl of 0.2 M NaH₂PO₄ at pH 7. After a brief centrifugation, 200 μl of the supernatant was injected into an HPLC system. The system uses a C₁₈ reversed-phase column (Waters Nova-Pak, 0.3 × 30 cm) that was eluted with 2% acetonitrile in 0.1 NaH₂PO₄ buffer, pH 7.0, at a rate of 1.4 ml/min. Absorbance of α-KIC was monitored at 206 nm. Radioactivity eluting with the α-KIC peak was measured by scintillation counting. The interassay and intra-assay variations for the determination of [¹⁴C] leucine specific activity were 4 ± 2 and 5 ± 2%, respectively. More than 98% of the radioactivity collected in the amino acid fraction was in the leucine peak after separation by ion exchange chromatography. The interassay and intra-assay variations for the determination of [¹³C] α-KIC specific activity were 5 ± 2 and 5 ± 3%. The recovery of [¹⁴C] α-KIC was 68 ± 4%. Plasma insulin and glucagon concentrations were measured with standard RIA techniques. Plasma glucose concentration was determined by the glucose oxidase method.

Calculations

Protein Metabolism

Whole body leucine flux was calculated with a stochastic model for protein metabolism. The analysis assumes near steady-state conditions. The validity and assumptions of the model have been discussed previously in detail by Golden and Waterlow (12). Briefly, the model generates the following equations in which total leucine turnover or flux equals $Q = S + C = B + I$, where $S$ is the total rate of leucine incorporation into protein (or nonoxidative leucine disposal), $C$ is the rate of leucine oxidation, $B$ is the rate of leucine release from protein (endogenous leucine appearance), and $I$ is the rate of exogenous leucine input. The rate of leucine turnover ($Q$) is calculated as follows: $Q = F/Leu_{sp,act}$, where $F$ is the infusion rate of [¹⁴C]leucine (in disintegrations per min [dpm]), and $Leu_{sp,act}$ is the specific radioactivity of leucine in the plasma compartment under steady-state conditions. The leucine oxidation rate is calculated as follows: $C = O/(K \times Leu_{sp,act})$, where $O$ is the rate of appearance of [¹⁴C]CO₂ in the expired air (dpm/min), and $K$ is a correction factor (0.81) that takes into account the incomplete recovery of labeled [¹⁴C]CO₂ from the bicarbonate pool. An estimate of the rate of leucine incorporation into protein ($S$) can be calculated as follows: $S = Q - C$. An estimate of the rate of leucine release into the plasma space from endogenous protein ($B$) can be calculated as follows: $B = Q - I$. When subjects are in the postabsorptive state, leucine intake ($I$) equals 0 and $B = Q$. During the amino acid infusion, $I$ equals the rate of leucine administration.

To calculate rates of leucine turnover and oxidation, we have used the plasma α-KIC specific activity because it has been suggested that the plasma α-KIC specific activity, the transaminated product of leucine, may provide a better estimate of the specific activity in the intracellular mixing pool (13).

Glucose Metabolism

During the insulin clamp studies, the glucose infusion rate was calculated at 20-min intervals, and a space correction was applied for over- or underfilling of the glucose space when appropriate (7). For data presentation, the mean of the three 20-min intervals from 120 to 180 min is given. Hepatic glucose production was not determined in the present study. However, we have previously shown that both in normal and in uremic subjects a similar level of hyperinsulinemia suppresses hepatic glucose production by > 90% (3). Rates of glucose and lipid oxidation and energy expenditure were calculated from indirect calorimetric measurements that were averaged over 5-min intervals during the basal state and during the insulin clamp as described previously (9,10).

Nonoxidative glucose disposal was calculated by subtracting the glucose oxidation rate from the rate of total body glucose uptake during the 120- to 180-min interval of the insulin clamp.

Statistical Analyses

All values are expressed as means ± SEM. Comparisons between the basal and the infusion periods were performed using the t test for paired data. Intergroup analysis was performed by one-way ANOVA.

Results

Glucose and Lipid Metabolism

In CAPD patients, the rate of glucose infusion required to maintain euglycemia (6.33 ± 0.62 mg/kg per min) during the 120- to 180-min interval of the euglycemic insulin clamp was similar to that of control subjects (6.54 ± 0.51 mg/kg per min). Basal glucose oxidation was higher in CAPD subjects than in control subjects (1.75 ± 0.18 versus 1.42 ± 0.14 mg/kg per min in CAPD and control subjects, respectively) ($P < 0.05$ versus control subjects). During the last hour of the insulin clamp study, glucose oxidation rose significantly and similarly in both groups and averaged 2.53 ± 0.27 versus 2.64 ± 0.37 mg/kg per min in CAPD and control subjects, respectively ($P < 0.01$ versus basal, $P = NS$ versus control subjects). The rate of nonoxidative glucose disposal (total glucose uptake minus glucose oxidation) during the 120- to 180-min interval of the insulin clamp study was similar in CAPD and control subjects (3.70 ± 0.58 versus 3.90 ± 0.68 mg/kg per min in CAPD and control subjects, respectively) ($P = NS$ versus control subjects) (Figures 1 and 2).

The basal rate of lipid oxidation was lower in CAPD compared with control subjects (0.43 ± 0.09 versus 0.61 ± 0.12 mg/kg per min in CAPD and control subjects, respectively) ($P < 0.05$ versus control subjects). During the insulin clamp, lipid oxidation declined significantly in both groups and averaged 0.18 ± 0.07 versus 0.19 ± 0.05 mg/kg per min in CAPD and control subjects, respectively ($P < 0.01$ versus basal, $P = NS$ versus control subjects).

In the postabsorptive state, energy expenditure was similar in control subjects and CAPD subjects and averaged 1.02 ± 0.03 versus 1.04 ± 0.05 kcal/min ($P = NS$ versus control subjects) and rose to 1.13 ± 0.02 versus 1.07 ± 0.06 kcal/min during hyperinsulinemia in the two groups (control subjects $P < 0.05$ versus basal). Basal nonprotein respiratory quotient (NPRQ) was higher in CAPD than in control subjects (0.88 ± 0.03 versus 0.84 ± 0.02) ($P < 0.05$ versus control subjects). In the 120- to 180-min of hyperinsulinemia, the NPRQ rose in both groups and averaged 0.95 ± 0.02 versus 0.97 ± 0.01 in CAPD and control subjects, respectively ($P < 0.01$ versus basal, $P = NS$ versus control subjects).

Plasma Glucose and Hormone Concentrations

In the control group, fasting plasma glucose averaged 86 ± 3 and 85 ± 4 mg/dl in study I and study II and did not change
significantly during the euglycemic insulin clamp studies (85 ± 5 and 84 ± 4 mg/dl in studies I and II, respectively); the coefficient of variation was 4 ± 1% in both studies. In CAPD, fasting plasma glucose averaged 85 ± 3 and 85 ± 4 mg/dl in study I and study II, respectively, and did not change significantly during the euglycemic insulin clamp studies (85 ± 4 and 84 ± 3 mg/dl, respectively, with a comparable coefficient of variation of 4 ± 1%) (Table 1).

Basal plasma insulin levels were higher in CAPD (14 ± 4 μU/ml) than in control subjects (7 ± 2 μU/ml) (P < 0.01 versus control subjects) and rose to 84 ± 4 and 75 ± 5 in study I and to 79 ± 6 and 74 ± 5 μU/ml in study II in CAPD and control subjects, respectively (all P < 0.01 versus basal).

Plasma Amino Acids and α-KIC Concentrations
In the control group, insulin (study I) caused a consistent decline in most plasma amino acid levels. Total plasma amino acid concentration decreased from 1963 ± 66 to 1452 ± 51 μmol/L and branched chain amino acid concentration decreased from 398 ± 25 to 221 ± 8 μmol/L; plasma leucine and α-KIC levels decreased from 109 ± 6 to 56 ± 7 and from 29 ± 5 to 18 ± 2 μmol/L, respectively (all P < 0.01 versus basal).

In CAPD subjects, total basal plasma amino acid concentration decreased from 2138 ± 120 to 1533 ± 90 μmol/L during the last hour of the insulin infusion (P < 0.01 versus basal and P = NS versus control subjects). Basal branched chain amino acid concentration was significantly lower than in control subjects (P < 0.01) and decreased from 244 ± 22 to 158 ± 10

Figure 1. Non-protein respiratory quotient (NPRQ), energy expenditure, Glucose oxidation, and lipid oxidation in control subjects (■) and CAPD subjects (□) during the basal period (BASAL) and the last hour of the insulin clamp (INS CLAMP). Values are mean ± SEM. *P < 0.05 versus control subjects; #P < 0.01 versus basal.

Figure 2. Whole body insulin stimulated glucose metabolism (M), glucose oxidation (OX), and nonoxidative glucose disposal (NON OX) in control (■) and CAPD subjects (□) during the last hour of the insulin clamp (study I). All values are mean ± SEM.
Table 1. Plasma glucose, total amino acid, leucine, α-ketoisocaproate levels, and energy expenditure during the last 60 min of the baseline and of the study periods in control subjects and CAPD patients

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<tr>
<th>Parameter</th>
<th>Control Subjects</th>
<th>CAPD</th>
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<tr>
<td></td>
<td>Study I</td>
<td>Study II</td>
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<td></td>
<td>Basal INS</td>
<td>Basal INS + AA</td>
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<tr>
<td>Plasma glucose (mg/dl)</td>
<td>86 ± 3</td>
<td>85 ± 4</td>
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<tr>
<td>Plasma insulin (μU/ml)</td>
<td>6 ± 2</td>
<td>8 ± 2</td>
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<tr>
<td>Total plasma AA (μmol/L)</td>
<td>1963 ± 66</td>
<td>1932 ± 69</td>
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<tr>
<td>Plasma leucine (μmol/ml)</td>
<td>109 ± 6</td>
<td>115 ± 6</td>
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<tr>
<td>Plasma KIC (μU/ml)</td>
<td>29 ± 5</td>
<td>32 ± 2</td>
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<tr>
<td>Energy expenditure (kcal/kg per min)</td>
<td>1.02 ± 0.03</td>
<td>1.04 ± 0.05</td>
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<tr>
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* Values are mean ± SEM. CAPD, continuous ambulatory peritoneal dialysis; INS, insulin; AA, amino acid; KIC, ketoisocaproate.

μmol/L during hyperinsulinemia (P < 0.01 versus basal, P = NS versus control subjects). Plasma leucine and α-KIC levels were also significantly lower than in control subjects and decreased from 71 ± 4 to 41 ± 3 and from 19 ± 2 to 13 ± 2 μmol/L, respectively (all P < 0.01 versus basal).

In the control group during study II, total plasma amino acid concentration increased from 1932 ± 69 to 2668 ± 109 μmol/L (P < 0.01 versus basal) and branched chain amino acid concentration from 407 ± 21 to 478 ± 18 μmol/L (P < 0.01 versus basal), plasma leucine and α-KIC levels went from 115 ± 6 to 137 ± 7 (P < 0.05 versus basal) and from 32 ± 3 to 34 ± 2 μmol/L, respectively.

In CAPD subjects during study II, total basal plasma amino acid concentration went from 2166 ± 122 to 2744 ± 98 μmol/L (P < 0.01 versus basal). Branched chain amino acid concentration went from 264 ± 26 to 336 ± 30 μmol/L (P < 0.01 versus basal), plasma leucine and α-KIC levels rose from 73 ± 4 to 88 ± 3 and from 18 ± 2 to 21 ± 2 μmol/L, respectively (all P < 0.05 versus basal). Steady-state plateau for plasma leucine and α-KIC concentrations and specific activities were achieved during the last hour of studies I and II in both control subjects and CAPD subjects.

In CAPD subjects, plasma leucine specific activity was constant during the last hour of the equilibrium period of study I and averaged 3.57 ± 0.2, 3.61 ± 0.2, and 3.72 ± 0.3 dpm/nmol at −60, −30, and 0 min, respectively. Leucine specific activity rose to a new steady-state plateau during insulin infusion and averaged 5.81 ± 0.4, 6.10 ± 0.5, and 6.03 ± 0.5 dpm/nmol at 150, 165, and 180 min, respectively.

In study II, basal leucine specific activity averaged 3.4 ± 0.3, 3.4 ± 0.3, and 3.5 ± 0.3 dpm/nmol at −60, −30, and 0 min, respectively. During insulin plus amino acid infusion, leucine specific activity averaged 3.1 ± 0.3, 3.0 ± 0.2, and 3.2 ± 0.2 dpm/nmol at 150, 165, and 180 min, respectively.

In control subjects, leucine specific activity was constant during the last hour of study I and averaged 3.1 ± 0.3, 3.3 ± 0.3, and 3.2 ± 0.3 dpm/nmol at −60, −30, and 0 min, respectively. Leucine specific activity rose to a new steady-state during insulin infusion and averaged 4.7 ± 0.4, 4.7 ± 0.5, and 4.8 ± 0.4 dpm/nmol at 150, 165, and 180 min, respectively. In study II, basal leucine specific activity averaged 2.9 ± 0.3, 3.0 ± 0.3, and 3.0 ± 0.3 dpm/nmol at −60, −30, and 0 min, respectively. Leucine specific activity went to 2.2 ± 0.4, 2.3 ± 0.4, and 2.3 ± 0.4 dpm/nmol at 150, 165, and 180 min, respectively, of the insulin plus amino acid infusion.

Total and Endogenous Leucine Flux

In control subjects during study I, basal leucine turnover as estimated from plasma α-KIC specific activity averaged 1.65 ± 0.07 μmol/kg per min. Under conditions of euglycemic hyperinsulinemia, leucine flux declined by 37 ± 2% to 1.04 ± 0.05 μmol/kg per min. In study II, basal total leucine flux was 1.58 ± 0.07 and went to 2.00 ± 0.15 μmol/kg per min (P < 0.01 versus basal). The endogenous leucine flux declined to 1.12 ± 0.15 μmol/kg per min (P < 0.01 versus basal) (Figures 3 and 4).

In CAPD patients, basal leucine flux (1.21 ± 0.15 μmol/kg per min) was significantly lower than in control subjects (P < 0.01). In study I, the insulin infusion determined a 32 ± 3% decline in total leucine flux (P = NS versus control subjects) to 0.83 ± 0.08 μmol/kg per min (P < 0.01 versus basal). In study II, basal total leucine flux went to 1.43 ± 0.21 μmol/kg per min (P < 0.05 versus basal). During amino acid and insulin infusion, endogenous leucine flux was markedly decreased to 0.82 ± 0.15 μmol/kg per min (P < 0.01 versus basal, P = NS versus study I and control subjects).

In CAPD subjects, body fat content may be increased. To account for differences in body composition between control...
subjects and CAPD subjects, data were corrected for ideal body weight (IBW) according to the formula: Endogenous leucine flux \((ELF) \, (\mu\text{mol/min}) \times \text{IBW/bdy weight (kg)} \times 100\). Corrected fluxes averaged 1.67 ± 0.08 and 1.29 ± 0.12 \(\mu\text{mol/kg per min}\) in control subjects and CAPD, respectively \((P < 0.01 \text{ versus control subjects})\).

**Leucine Oxidation and Nonoxidative Leucine Disposal**

In control subjects, basal leucine oxidation (LOX) and nonoxidative leucine disposal (NOLD) were similar in study I and study II and averaged 0.26 ± 0.02 and 1.36 ± 0.05 \(\mu\text{mol/kg per min}\), respectively. In study I, both LOX and NOLD declined significantly in response to insulin \((0.19 ± 0.02 \text{ and } 0.85 ± 0.05 \mu\text{mol/kg per min}) \text{ (both } P < 0.01 \text{ versus basal). In study II during combined amino acid and insulin infusion, LOX averaged 0.59 ± 0.05 \mu\text{mol/kg per min} \text{ (} P < 0.01 \text{ versus basal), and NOLD was } 1.42 ± 0.09 \mu\text{mol/kg per min} \text{ (} P < 0.01 \text{ versus study I)}\).

In CAPD subjects, LOX and NOLD were similar in studies I and II and averaged 0.13 ± 0.02 and 0.89 ± 0.05 \(\mu\text{mol/kg per min}\), respectively \((P < 0.01 \text{ versus control subjects})\).

In response to insulin (study I), LOX \((0.06 ± 0.01 \mu\text{mmol/kg per min})\) and NOLD \((0.69 ± 0.07 \mu\text{mol/kg per min})\) declined significantly \((P < 0.01 \text{ versus basal and } P = \text{NS CAPD versus control subjects})\).

In study II, LOX and NOLD went to 0.20 ± 0.04 and 1.23 ± 0.17 \(\mu\text{mol/kg per min}\), respectively \((P < 0.01 \text{ versus study I, LOX } P < 0.01 \text{ CAPD versus control subjects})\).

**Net Flux of Leucine into Protein**

The balance between NOLD and ELF represents the net flux of leucine into protein, and it provides an index of protein anabolism. In the postabsorptive state, the net flux of leucine into protein was negative in both control subjects and CAPD subjects and averaged \(-0.26 ± 0.13\) and \(-0.13 ± 0.03 \mu\text{mol/kg per min}\). In response to insulin, the leucine balance become less negative in both groups and averaged \(-0.19 ± 0.02\) and \(-0.06 ± 0.01 \mu\text{mol/kg per min}\) in control subjects and CAPD, respectively \((P < 0.01 \text{ versus basal})\).

In study II, the net balance of leucine into protein became positive in both groups and averaged \(0.30 ± 0.05\) and \(0.40 ± 0.07 \mu\text{mol/kg per min}\) in control subjects and CAPD, respectively \((P < 0.01 \text{ versus } P = \text{NS control subjects versus CAPD} )\).

**Discussion**

In the present study, we investigated glucose and amino acid metabolism in uremic patients maintained on CAPD. In the postabsorptive state, CAPD subjects showed a preferential utilization of glucose as an energy substrate and a lower rate of
lipid oxidation. Basal endogenous proteolysis, protein synthesis, and irreversible amino acid losses were lower than in control subjects. In response to insulin, the utilization of glucose and the inhibition of proteolysis were similar to control subjects. In addition, the anabolic response to a combined amino acid and insulin administration was similar in CAPD and control subjects.

In CAPD patients, basal insulin levels are increased, and each dialytic exchange is associated with a marked insulin response (5). As a result, integrated insulin profiles are significantly higher with respect to control subjects and hemodialysis patients. Glucose uptake from the dialysis fluid represents a significant portion of daily substrate uptake (5). Thus, the preferential utilization of glucose as an oxidative substrate may represent an adaptive response to the increased glucose absorption. Similar changes in the respiratory quotient and glucose oxidation are observed in healthy subjects maintained on an elevated dietary carbohydrate intake (10).

The ability of the body to metabolize an exogenous glucose load is dependent on the tissue sensitivity to insulin. In both uremic and control subjects, muscle is the primary tissue responsible for the removal of an infused glucose load under euglycemic hyperinsulinemic conditions (3,10). The glucose taken up by muscle undergoes one of two major metabolic fates: (1) oxidation to carbon dioxide and water; and (2) glycogen formation (14). In previous studies (2,3,15), we demonstrated that CRF subjects exhibit marked insulin resistance with a significant reduction of insulin-mediated glucose uptake, which is characterized by a reduction in insulin-mediated glucose storage (2). In contrast, insulin-stimulated glucose oxidation is affected only marginally by renal insufficiency (2). The present data show that the total rate of insulin-mediated glucose uptake is similar in CAPD patients and control subjects. In addition, both oxidative and nonoxidative pathways of glucose utilization are normal. It is concluded that CAPD entirely normalizes insulin-mediated glucose metabolism and restores the ability of insulin to stimulate muscle glycogen synthesis.

In the present study, hepatic glucose production was not measured, and its incomplete suppression in response to insulin cannot be ruled out. However, in previous studies we (3) have demonstrated that in CRF subjects, hepatic glucose production is normal and it is suppressed by more than 90% during hyperinsulinemia. Thus, under the steady-state conditions of the present work, glucose uptake by the entire body can be considered equal to the rate of exogenous glucose infusion, corrected for changes in the glucose space.

Branched chain amino acids were significantly lower in CAPD patients compared with control subjects. This observation is in agreement with previously published results by Berg-
strom et al. (16), who demonstrated that the uremic patients maintained on CAPD have decreased extra- and intracellular concentrations of many amino acids.

In the present study, we used labeled leucine to evaluate the rate of whole body protein metabolism. In patients with chronic renal failure, leucine flux is reported as either normal (16) or decreased (2). When acidosis is present, leucine turnover and oxidation are increased (17). Data on the effects of acute or decreased (2). When acidosis is present, leucine turnover and oxidation are increased (17). Data on the effects of acute or decreased (2).

In the present study, in nonacidotic CAPD subjects we observed a significant decline in fasting leucine flux (ELF; an index of proteolysis), leucine oxidation (LOX; an index of irreversible amino acid losses), and nonoxidative leucine disposal (NOLD; an index of protein synthesis). Possible explanations for the decline in ELF and NOLD are the following: (1) lower protein intake; (2) increased insulin levels; and (3) increased catecholamine concentrations compared with control subjects. Protein intake ranged from 0.8 to 1.1 g/kg per d in CAPD subjects and from 0.9 to 1.2 g/kg per d in control subjects. Goodship et al. (19) reported that in CRF subjects a decline in protein intake from 1.0 to 0.6 g/kg per d did not affect ELF or NOLD. Only a more pronounced restriction of protein intake (0.3 to 0.4 g/kg per d) is associated with a significant decline in ELF and NOLD in CRF patients (20).

Thus, it seems unlikely that the decline in proteolysis and protein synthesis in CAPD subjects can be ascribed to moderate and undetected changes in long-term dietary protein intake. High insulin and catecholamine levels are associated with low leucine fluxes (21,22). CAPD subjects are characterized by fasting hyperinsulinemia and increased epinephrine and noradrenaline concentrations (23), which may have contributed to the lower basal turnover rate. The role of hyperinsulinemia is supported by the normal sensitivity to insulin shown by CAPD subjects during study I. In fact, in response to physiologic euglycemic hyperinsulinemia, the percentage inhibition of ELF (32 versus 37%) was similar in CAPD and control subjects showing a normal sensitivity to insulin with respect to the proteolytic pathway. Thus, the insulin response induced by each dialytic exchange exerts an antiproteinolytic action, which reduces amino acid availability for intracellular oxidative pathways.

There is evidence that body fat content of CAPD subjects may be increased. To account for differences in body composition, ELF was normalized for ideal body weight. After normalization, ELF in CAPD subjects was still significantly lower than in control subjects. It should also be pointed out that the present experimental design does not allow us to evaluate the relative contribution of renal failure per se and CAPD treatment. A prospective study on CRF patients before and after the start of a renal replacement therapy would help to clarify this relevant issue.

Maintenance of long-term protein homeostasis is the result of a neutral balance between the catabolic state of fasting and the anabolic response to food ingestion. In this regard, we have previously demonstrated that amino acid availability exerts a dose-dependent stimulation of whole body protein synthesis and anabolism (24). To investigate the ability of CAPD patients to promote a net anabolic response, subjects who participated in study I were reevaluated with a combined amino acid, insulin, and glucose infusion to mimic the substrate availability and hormonal response that occurs following a mixed meal. The total dose of amino acids and glucose administered during the 180 min of study II was 0.2 g/kg body wt and 60 to 80 g, respectively. They correspond to the amino acid and glucose content of a light meal. The elevation in plasma amino acid concentration in study II was similar to what is seen after a light mixed meal (25).

In a previous study, we demonstrated that the whole body anabolic response to amino acid administration is reduced in CRF patients (2). Luzi et al. (6) reported that in diabetic uremic patients, the ability of a combined amino acid, glucose, and insulin infusion to promote protein anabolism is restored to normal by organ transplantation, but not by hemodialysis treatment. The anabolic response to amino acid administration in nondiabetic CRF patients on hemodialysis has not been investigated.

In study II, NOLD significantly increased compared with study I and, perhaps more importantly, the leucine balance (an index of the net flux of amino acid into protein) became positive in both CAPD and control subjects, thus demonstrating a similar anabolic response to amino acid administration in the two groups. Taken together, the present data provide experimental evidence to support the use of amino acid supplementation, either by oral or peritoneal routes, to stimulate a net protein anabolism in CAPD patients (26).

In conclusion, we have shown that in CAPD patients the action of insulin with respect to glucose metabolism and inhibition of proteolysis is restored to normal. In addition, the postabsorptive reduction of protein synthesis is reversed by amino acid administration, suggesting a normal anabolic response to protein feeding.

References