Abstract  Fasting plasma concentrations of branched-chain amino acids (BCAA) valine, leucine, and isoleucine were measured in 20 young patients (aged 18±2 years) with end-stage renal disease just before initiation of dialysis and compared with 7 healthy controls (aged 19±1 years). Plasma valine, leucine, and isoleucine were all lower than control values (P<0.01 in all 3 cases). Plasma valine, but not leucine and isoleucine, correlated with venous pH (P<0.02). Plasma valine, leucine, or isoleucine did not correlate with blood urea nitrogen or serum creatinine. Seven patients (aged 18±1 years) on maintenance hemodialysis with metabolic acidosis were then studied before and after 2 weeks of oral sodium bicarbonate (NaHCO3 ) treatment to correct the acidosis. To control for the effect of additional sodium, they were also studied after 2 weeks of an equivalent amount of oral sodium chloride (NaCl). Oral NaHCO3 treatment led to significant increases in venous pH and serum bicarbonate concentrations, but no significant change in total and ionized calcium, phosphate, sodium, potassium, creatinine, blood urea nitrogen, and intact parathyroid hormone concentrations. Oral NaCl did not change any of the biochemical parameters. Fasting plasma concentrations of BCAA were measured. Before treatment of acidosis, uremic patients had low plasma concentrations of valine, leucine, and isoleucine compared with controls. Following 2 weeks of NaHCO3 treatment, there were significant increases in the plasma concentrations of valine and leucine (P<0.01), although the values did not normalize. There were no changes in plasma concentrations of valine and leucine following 2 weeks of NaCl. The plasma concentration of isoleucine was not different during baseline (acidotic) and treatment periods with NaHCO3 and NaCl. Thus treatment of metabolic acidosis ameliorated abnormalities in plasma concentrations of valine and leucine in patients with uremia on hemodialysis.

Key words  Acidosis · Branched-chain amino acids · Leucine · Valine · Uremia · Hemodialysis

Introduction  Malnutrition and wasting are devastating complications of uremia and directly influence overall morbidity and mortality [1]. There is abundant evidence in uremic patients that protein metabolism is impaired, resulting in negative nitrogen balance, loss of lean body mass, and subnormal plasma concentrations of most essential amino acids [2, 3]. Inadequate dietary intakes of protein and energy secondary to anorexia, as well as other catabolic stimuli, in uremic patients may result in increased essential amino acid oxidation, increased protein degradation, and decreased protein synthesis [4]. Counahan et al. [5] reported low plasma concentrations of branched-chain amino acids (BCAA) valine, leucine, and isoleucine in adolescent patients with uremia on hemodialysis (HD). These authors postulated that the low plasma BCAA concentrations were due to increased oxidation to provide alternative sources of energy in uremia.

There is evidence that metabolic acidosis is a catabolic factor. Infants with metabolic acidosis grow poorly and have increased nitrogen excretion [4]. Children with renal tubular acidosis show catch-up growth when their acidosis is corrected by sodium bicarbonate (NaHCO3) [6]. Normal adults fed ammonium chloride to induce metabolic acidosis have increased oxidation of leucine and increased protein degradation [7]. A strong linear relationship has been demonstrated between the plasma bicarbonate concentration and free valine concentrations in muscle biopsies from uremic patients on HD [8]. Papadoyannakis et al. [9] reported improvement of nitrogen and protein balance in patients with chronic renal failure after correction of metabolic acidosis, but did not study BCAA metabolism. The present study examines the effect of metabolic acidosis on plasma BCAA concentrations in patients with end-stage renal disease.

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Patients and methods

In a preliminary study, fasting BCAA concentrations were measured in 20 patients (8 male, 12 female) with end-stage renal disease just prior to the initiation of dialysis. The underlying diagnosis included renal dysplasia, obstructive uropathy, reflux nephropathy, chronic glomerulonephritis, prune-belly syndrome, and unknown etiology. None of the patients had clinical signs of malnutrition or wasting. Their body weights were within 10% of the ideal weight for their height and were stable for at least 6 months prior to the study. Their dietary intakes of sodium (2 g/day) potassium (2 g/day), and phosphorus (800 mg) were restricted, but the intake of protein was not. Medications included dihydrotachysterol, calcium carbonate, water-soluble vitamins (Nephrovite, R and D Laboratories, Marina Del Rey, Calif., USA), and antihypertensives in the form of nifedipine and enalapril. Controls comprised 7 healthy subjects (aged 19±1 years) (3 male, 4 female) consuming regular weight-maintaining diets and taking no medications.

Seven patients (aged 18±1 years) (3 male, 4 female) on maintenance HD with metabolic acidosis were entered into a controlled study examining the effect of correction of acidosis on BCAA. Their underlying diagnoses included renal dysplasia, obstructive uropathy, reflux nephropathy, prune-belly syndrome, and unknown etiology. They had been stabilized on maintenance HD for at least 6 months prior to the study. The patients were dialyzed with CA 90 dialyzers with mean blood flows of 180±15 ml/min. There was no change in their dialysis prescription during the study. Their blood pressure was controlled on medications and stabilized for at least 2 months before the study. None of the patients had clinical signs of malnutrition or wasting. Their body weights were within 10% of the ideal weight for their height. These 7 patients had moderate metabolic acidosis detected on entry into the study. They underwent the initial metabolic studies whilst being acidic. Four were then treated first with oral NaHCO3 (3 mEq/kg per day) for 2 weeks and restudied; they were then treated with an equivalent amount of NaCl for another 2 weeks and studied again. The other 3 patients were first treated with NaCl and then with NaHCO3 and were studied in the same way. Other medications included dihydrotachysterol, calcium carbonate, antihypertensives in the form of long-acting nifedipine, water-soluble vitamins (Nephrovite), and intravenous erythropoietin during HD sessions. All the medications (other than NaHCO3 or NaCl) were continued and there were no dosage changes during the study. Their carbohydrate intake was limited to 150 g/kg per day and their body weights were stable for at least 2 months prior to the studies. Their dietary intakes of sodium (2 g/day excluding NaHCO3 or NaCl), potassium (2 g/day), and phosphorus (800 mg) were restricted, but the intake of protein was not. Caloric intake was monitored by 3-day diet diary during the different study periods. Protein intake was estimated from urea kinetics using the formula protein catabolic rate (g/day)=9.35×UGR (urea generation rate) (mmol/h) + 0.17×weight (kg) [10]. UGR was estimated from the increase in blood urea between dialytic sessions, assuming a volume of distribution of 0.6 l/kg body weight, plus the urinary excretion of urea, which was measured in a timed collection over the same interdialytic time period.

All patients had Tanner puberty scores of 5. None of the patients had diabetes mellitus nor was there a family history of diabetes mellitus. All patients and controls were free from infections at the time of the studies. The clinical studies were performed at Children’s Hospital of Los Angeles and Lucile Packard Children’s Hospital at Stanford, and were approved by the local institutional review board. The purpose and potential risks of the study were carefully explained to all patients and subjects and written informed consent was obtained before their participation.

Biochemical analysis

For fasting BCAA concentrations, 1 ml of plasma was deproteinized by addition of 0.1 ml of 45% sulfosalicylic acid and agitated using a vortex mixer. The deproteinized sample was centrifuged at 12,000 g and the protein-free supernatant was removed and stored at –70°C until analysis. The supernatant was analyzed using a Beckman Amino Acid Analyzer (Beckman Instruments, Palo Alto, USA). Serum biochemical analyses were carried out by standard methods on multichannel autoanalyzers. Intact serum parathyroid hormone (PTH) was measured by an immunoradiometric assay (Nichols, San Juan Capistrano, Calif., USA). The intraassay and interassay coefficients of variation for serum PTH were 7.2% and 9.3%, respectively.

Statistical analysis

All values are expressed as mean plus or minus standard error of the mean. The data were tested for normality using the chi-squared method. Student’s t-tests for paired and unpaired observations were used for analysis of the results. Statistical significance was recognized at the 5% level.

Results

Plasma concentrations of valine (152±19 µmol/l), leucine (78±9 µmol/l), and isoleucine (43±4 µmol/l) in the 20 patients with end-stage renal disease were all significantly lower than the corresponding values in controls (210±19, 105±13, 55±4 µmol/l, respectively) (P<0.01 in all 3 cases). Plasma valine, but not leucine or isoleucine, correlated with venous pH in these patients (r=0.88, P<0.01) (Fig. 1). Neither plasma valine, leucine nor isoleucine correlated with blood urea nitrogen or serum creatinine.

Serum biochemical data of the 7 patients on HD at baseline (whilst acidic) and during treatment with NaHCO3 and NaCl are presented in Table 1. Venous pH and serum bicarbonate increased significantly during NaHCO3 treatment (P<0.01 in both cases), but did not change during NaCl treatment. Fasting serum glucose decreased and fasting serum insulin increased following NaHCO3 treatment, but did not change following NaCl treatment. There were no significant changes in the other biochemical parameters. The patients remained moderately anemic despite erythropoietin treatment, and there were no changes in hematocrit values after either NaHCO3 or NaCl treatment. There were also no changes

![Fig. 1] Plasma valine versus venous pH in 20 patients with end-stage renal disease before initiation of dialysis