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BRANCHED CHAIN AMINO ACID METABOLISM IN CHRONIC RENAL FAILURE AND HAEMODIALYSIS

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Summary

The rates of protein turnover have been quantified by utilizing (1-^{13}C) leucine tracer as a prime constant rate infusion for a period of six hours in five chronic renal failure (CRF) (creatinine 7.8mg/dl, BUN 87mg/dl), six normal, and two patients on regular haemodialysis. ^{13}C enrichment of plasma leucine and of expired CO_2 were measured by mass spectrometry. The rate of CO_2 production and O_2 consumption were calculated by respiratory calorimetry. While leucine turnover was not significantly different between patients with CRF and normals, the two patients on haemodialysis demonstrated increased leucine turnover rates. There was a significant reduction in leucine oxidation in patients with CRF compared with normals while patients on haemodialysis tended to have less dramatic reductions.

Introduction

While abnormalities in protein metabolism in CRF have been reported, the exact mechanism remains unclear. Altered amino acid stores, increased protein catabolism, decreased lean body mass, carbohydrate intolerance with insulin resistance, and hyperinsulinaemia and hyperglucagonaemia have been reported in uraemia [1]. The altered protein metabolism in uraemia might promote increased protein catabolism and therefore increase nitrogen requirements beyond those of normal subjects. If these demands are not met by dietary intake, protein malnutrition will ensue.

Essential amino acids and their keto analogues have been utilized to increase protein stores in patients with CRF [2], lower the generation of urea, and promote protein synthesis by lowering nitrogen losses. The specific concentrations of essential amino acids utilized as well as the type of keto analogues, may modulate the success of dietary therapy. Since losses of essential amino acids may occur with dialysis [3] resulting in greater negative nitrogen balance, specific dialysis therapies may offer advantages in different patients.

Branched chain amino acids (leucine, isoleucine, valine) are substrates for protein synthesis and are important precursors and regulators of a number of metabolic reactions. The availability of amino acids derived from breakdown of tissue proteins constitutes a rate limiting step in urea synthesis as a positive correlation exists between plasma amino acids and urea generation. Therefore a more thorough understanding of the relation between dietary therapy (keto analogues, protein restriction) and dialysis on protein metabolism is essential in approaching treatment of CRF.

Leucine, the essential branched chain amino acid studied in greatest detail, is considered an important regulator of protein metabolism by stimulating muscle protein synthesis and possibly decreasing protein breakdown. In the present study, stable isotopic tracer techniques were employed to quantify leucine turnover, oxidation, and to relate them to protein synthesis and degradation. Based on the belief that increased protein catabolism exists in uraemia, we had anticipated this would be reflected by an increase in leucine turnover and oxidation.

Methods

Patients Five chronically uraemic patients and two haemodialysis patients were recruited from the Cleveland Clinic, Department of Hypertension and Nephrology. Normal volunteers were selected from hospital personnel. The clinical characteristics of the study population are depicted in Table I. Patients discontinued

TABLE I. Branched chain amino acid metabolism in CRF and haemodialysis

	Clinical characteristics		
	Normal	CRF*	Haemodialysis
Age	41.6 (26–60)	49.2 (23–65)	25.5 (23–28)
Sex	Males: 3	3	2
	Females: 2	2	–
Creatinine (mg/dl)†	–	7.96±1.8	16.4±7.3
BUN (mg/dl)	13±2.3	87±10.9	51.0±9.9

* Non-dialysed, chronic glomerulonephritis or nephrosclerosis

† mean ± SD

all medications the day before the study and underwent a 12-hour overnight fast. The studies were conducted at the Metabolic Unit of the Cleveland Metropolitan General Hospital. Written informed consent was obtained from each patient after fully explaining the procedure. The protocol was approved by both institutional review committees on human investigation from the Cleveland Clinic and Cleveland Metropolitan General Hospital. The patients were not taking any medication which could potentially affect protein metabolism such as steroid therapy. All uraemic patients were non-diabetic and stable without nausea or vomiting.

Two principal methods were employed in the current study, 1) leucine turnover and oxidation, and 2) respiratory calorimetry.

Leucine turnover/oxidation The turnover rate of leucine was quantified during steady state by measuring the isotope enrichment of the substrate in the plasma and thus calculating the dilution of the infused tracer. The rate of CO_2 production and ^{13}C enrichment of expired CO_2 were measured to calculate the rate of leucine oxidation and protein catabolism.

After an overnight fast, an indwelling needle was placed in the superficial vein of each hand for infusion of the isotope tracer and for the drawing of blood samples. [1-^{13}C] leucine dissolved in isotonic saline was administered as a prime-constant rate infusion. A priming dose of 4μM/kg was followed by a constant infusion of [1-^{13}C] leucine at a rate of 5μM/kg/hr for six hours. Arterialized blood samples were drawn from a heated vein of the hand at 30 minute intervals during the study. Plasma leucine enrichment was determined on a Hewlett Packard GC Mass Spectrometer using selected ion monitoring [4]. Samples of expired air were obtained and respiratory calorimetry measurements were performed at 30 minute intervals. Expired air was collected in a 5 litre anaesthesia bag through a one-way breathing valve (Rudolph valve); an aliquot of this air was used to separate carbon dioxide by cryogenic distillation in vacuum. The $^{13}C/^{12}C$ ratio of the CO_2 was measured on a ratio mass spectrometer [5]. The contribution of leucine to expired CO_2 was measured by comparing the ^{13}C enrichment of expired CO_2 and the ^{13}C enrichment of plasma leucine at steady state. From these data and from the rate of CO_2 production, the rate of leucine oxidation was calculated.

Respiratory calorimetry The rate of oxygen consumption and carbon dioxide production were quantified using an 'open circuit respiratory calorimetry system'. The subject's head was placed in a clear acrylic hood. Air leaked into the hood continuously. The flow of air through the hood was maintained by connecting it by a non-collapsible hose to a suction pump. A flow sensor and servocontrol device which measured flow rate were positioned in the line. A mixing chamber was placed between the acrylic hood and the flow controller. The mixing chamber was connected to a gas analyser (Perkin Elmer MGA 1100). The rates of oxygen consumption and CO_2 production were calculated from the gradient of these gases and the rate of flow of air across the face. The data were corrected using Haldane transformation.

Results

All subjects studied were stable and asymptomatic from their uraemia. The cause of renal failure was either nephrosclerosis or chronic glomerulonephritis. Patients were selected with the least number of medications, avoiding patients on medications which would adversely affect protein metabolism (i.e. diphenylhydantoin, corticosteroids, androgens, thyroid replacement, etc). The two dialysis patients were studied the day following a routine haemodialysis treatment.

TABLE II. Metabolic parameters in controls, CRF and haemodialysis patients

	Controls (6)	CRF (5)	Haemodialysis (2)
Leucine (μM/L)	128.4±19	117.96±30	–
Glucose (mg/dl)	88.2±14.9	88.4±3.4	86±5.6
Beta hydroxybutyrate (mM/L)	0.22±0.12	0.36±0.15	0.42±0.08
Respiratory quotients	0.81±0.05	0.80±0.02	0.85±0.07

The results of metabolic screening in each group are represented in Table II. Plasma leucine was not significantly different in the CRF and normal groups, basal glucose was similar. The increase in beta hydroxybutyrate signifies the increased utilization for energy of fat by patients with chronic renal failure. The two haemodialysis patients demonstrated increased respiratory quotient (RQ) values compared to controls. However, the RQ values were similar between the CRF patients and controls. This was possibly due to the delay in the reflection of changes in cellular metabolism upon respiratory gas exchange.

Table III summarizes the results of leucine and protein metabolism in the three study groups (normals, CRF and haemodialysis). While there was a significant decrease in leucine oxidation in patients with CRF, the rate of leucine turnover remained unchanged as compared to normal. Leucine turnover was greater in the two dialysis patients compared to CRF and normal study patients; the rate of leucine oxidation was relatively unchanged compared to normal but greater than CRF patients. From these data and assuming a leucine content of 590μM/g muscle protein, the rates of protein turnover were derived and represented in Table III. Protein catabolism and synthesis varied little within the CRF and normal groups while protein oxidation was significantly decreased in the CRF patients. Protein catabolism was significantly elevated in the two dialysis patients.

TABLE III. Protein metabolism in chronic uraemia utilizing [^{13}C]leucine tracer dilution

	Leucine*		Protein†		
	Turnover	Oxidation	Catabolism	Synthesis	Oxidation
Normals (6)	86.3 ±19.46**	12.42 ±3.29	3.54 ±0.79	3.03 ±0.68	0.53 ±0.18
CRF (5)	79.35 ±16.99	7.58 ±2.05	3.23 ±0.69	2.92 ±0.62	0.284 ±0.08
Haemodialysis (2)	109.92 ±0.09	13.16 ±2.3	4.57 ±0.11	4.04 ±0.01	0.54 ±0.09

*moles/kg/hr; †gm/kg/day; **mean ± SD

Discussion

Theoretically due to the insulin resistance and hyperglucagonaemia which characterizes states of chronic uraemia, alternate fuels for oxidative metabolism are utilized, resulting in increased protein catabolism and nitrogen wasting. Therefore, one expects to see an overall increase in protein turnover in chronic uraemia. However, in the present study, non-dialysed CRF patients failed to demonstrate an increase in protein turnover in a fasting state. Moreover, there was a significant decrease in protein oxidation in this study group. These alterations in protein metabolism potentially reflect an adaptive response to lower urea production while conserving protein stores in chronic uraemia. While these homeostatic mechanisms conserve nitrogen (protein) to avoid malnutrition, these same patients are unable to decrease urea production in the setting of decreased urea excretion.

The two patients undergoing routine haemodialysis demonstrated an increase in protein turnover compared to non-dialysed CRF patients and normals. Considering the small number of dialysis patients, these data warrant verification with larger patient numbers prior to making conclusions regarding the effect of dialysis on protein metabolism. While dialytic therapy is employed in the routine treatment of end-stage renal disease, continuous ambulatory peritoneal dialysis and haemodialysis may differ in their effect on protein turnover/oxidation and urea production.

Further studies evaluating the effect of feeding in patients with CRF need to be examined. The response to feeding in CRF patients may be significantly different than in normal individuals. Also the specific effects of ketoanalogues on urea production utilizing stable isotope techniques are warranted.

In conclusion, decreased protein oxidation while maintaining normal protein turnover may represent the body's response to conserve protein nitrogen in chronic renal failure.

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