

Nutrition and Chronic Renal Failure in Rats: What Is an Optimal Dietary Protein?

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Abstract. In chronic uremia (CRF), malnutrition is an important determinant of morbidity in adults and impaired growth in children. Causes of malnutrition include anorexia and abnormal protein and amino acid metabolism. To determine how different levels of dietary protein and CRF interact to influence growth and nutritional status, CRF and sham-operated, pair-fed control rats were fed isocaloric diets containing 8, 17, or 30% protein for 21 d to mimic dietary regimens recommended for CRF patients: the minimum daily requirement; the recommended daily allowance; or an excess of dietary protein. Serum creatinine did not differ between groups of CRF rats but blood urea nitrogen was lowest in CRF rats fed 8% protein ($P < 0.001$). CRF rats eating 30% protein gained less weight and length compared to their controls or CRF rats fed 8 or 17%

protein ($P < 0.05$); they also had acidemia. CRF rats fed 8% protein had the highest efficiency of utilization of protein for growth, while 17% protein promoted the highest efficiency of utilization of food and calories for growth. Notably, CRF rats eating 30% protein had the lowest protein efficiency; their calorie intake was also the lowest because of anorexia. Plasma branched-chain amino acids were progressively higher in control rats eating 8, 17, or 30% protein. CRF rats fed 8 or 17% protein had lower branched-chain amino acid concentrations compared with CRF rats fed 30% protein. In CRF, it is concluded that excessive dietary protein impairs growth but a low-protein diet does not impair nutritional responses and permits utilization of protein for growth if calories are sufficient.

Malnutrition in adults with chronic renal failure (CRF) is associated with excessive morbidity and mortality (1–3), and the poor growth of children with CRF can be related in part to nutritional abnormalities (4–7). Even though it has been known for more than a century that dietary protein restriction can ameliorate uremic symptoms (8), concern about nutritional influences of a low-protein diet causing malnutrition has led some to suggest initiating dialysis in lieu of dietary protein restriction in the treatment of CRF patients (2). Fortunately, low-protein diets do not lead to malnutrition in CRF patients when properly administered (9) and although controversial, there is evidence that low-protein diets can slow the progression of renal insufficiency in adults but not children (10–13). These considerations prompt interest in the design of the diet in CRF and how a restricted diet interacts with CRF to affect nutritional status.

In healthy adults, diets deficient in calories and/or protein can cause loss of weight and muscle mass; in children, such a diet impairs growth and produces a low weight/height ratio (14). The dietary protein requirements of children with CRF

are not well-defined, but modest restriction of protein does not impair growth while raising calorie intake can improve growth (6,7,13). In adults with CRF, dietary regimens that supply limiting amounts of protein will yield neutral nitrogen balance, but if calories are limited, nutritional status can be compromised (1).

Difficulties in providing an adequate diet for CRF patients include anorexia induced by mechanisms linked to accumulated toxins (15). An excess of dietary protein could also lead to metabolic acidosis, which stimulates the degradation of branched-chain and possibly other essential amino acids and protein leading to loss of lean body mass (16–18). Thus, a critical issue is how to balance the diet to provide sufficient calories and protein while limiting the intake of uremic toxins.

Earlier reports by Kleinknecht and associates showed that the efficiency of utilization of protein and calories for growth in rats will change as protein or calories are raised and these adaptive responses promote growth (19–23). To examine how different levels of dietary protein interact with chronic renal insufficiency to influence nutritional status, we studied the growth of CRF and sham-operated, pair-fed control rats. Iso-caloric diets were designed to supply low protein (8%), an adequate amount of protein to sustain growth (17%), or an excess of protein (30%) to mimic diets of CRF patients that are directed at supplying dietary protein at the minimum daily requirement level (0.6 g/kg per d), the recommended daily allowance level (0.8 g/kg per d), or an excess (≥ 1.0 g/kg per d). Our goal was to examine how these diets affect the efficiency of utilization of protein and calories for growth.

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Materials and Methods

After anesthesia with ether, male Wistar rats weighing 40 g underwent a two-stage, subtotal nephrectomy. In the first stage, the two poles of the right kidney were excised; hemostasis was achieved by pressure. The rats were allowed to recover for 7 d while eating 22% protein chow (Nuvilab, Colombo, Parana-Brazil). The left kidney was then removed and the rats were fed a 17% protein diet *ad libitum* for 7 d. CRF rats were then randomly selected and paired by weight with sham-operated, control rats for pair feeding as described (17,24). Rats were housed in individual cages in a room with constant temperature and a 12-h light/dark cycle. All experiments followed the National Research Council's Guide for the Care and Use of Laboratory Animals.

Three diets were formulated according to the American Institute of Nutrition Rodent Diets, AIN-93 (25) for young rats: 8% protein (low protein), 17% protein (adequate protein to support growth), or 30% protein (high protein) (Table 1). All diets had the same content of energy (3.5 kcal/g), vitamins (AIN93-VX; ICN Biomedicals, Aurora, OH), and mineral mix (AIN93-G; ICN Biomedicals). Water was provided *ad libitum*.

Control rats were pair-fed with CRF rats for 21 d, and weight and food intake were measured daily. Length (nose-tail distance) was measured after light ether anesthesia at days 0, 7, 14, and 36. At the end of the study, rats were anesthetized with ether and aortic blood was obtained to measure pH, blood urea nitrogen (BUN), creatinine, and plasma concentrations of branched-chain amino acids (BCAA).

To measure BCAA, plasma samples were deproteinized by adding perchloric acid (10% final concentration) and neutralized by adding 3-*N* morpholinopropanesulfonic acid and sodium hydroxide to the supernatant; *o*-fluorophenylalanine was added as an internal standard (16,26,27). Amino acids were derivatized with *o*-phthalaldehyde (Fluoraldehyde Reagent, Pierce Chemicals, Rockford, IL) and separated by reverse-phase HPLC on a 3.0 × 150 mm C18 Nova-Pak column and a 1 ml/min solvent flow rate. Mobile phase A solvent consisted of 60 mM phosphate buffer (pH 6.65), and mobile phase B solvent consisted of 20% methanol, 20% acetonitrile, 20% α -propanol, and 40% 60 mM phosphate buffer (pH 6.65). Initially, a solvent mixture of 82% A/18% B was maintained for 20 min. The proportion of solvent B was then increased using a linear gradient to 24% over 10 min, then to 50% B over 6 min, then to 66% B over 10 min, then to

68% B over 3 min, and finally to 100% B over 6 min. Derivatized amino acids were measured by fluorescence spectroscopy (excitation 338 nm, emission 425 nm).

Serum creatinine and BUN were determined with an autoanalyzer RA-XT (Bayer, Elkhart, IN). Arterial blood pH was determined with an Omni-AVL analyzer.

Statistical Analyses

Values are reported as mean \pm SEM. Serum creatinine, weight, length, food, protein, and caloric intake and BCAA levels were compared by one-way ANOVA, followed by *post hoc* pairwise comparisons using the Duncan test. Results were considered significant at $P < 0.05$. BUN, food efficiency, protein, and caloric efficiency were compared using Kruskal–Wallis test. A two-tailed Mann–Whitney *U* test was used to compare the differences between two groups. The differences were considered as significant at $P < 0.05$.

Results

BUN values were higher in all of the CRF groups compared to the respective control groups ($P < 0.001$). The highest values were in CRF rats fed 30% protein; CRF rats fed 8% protein had the lowest values ($P < 0.001$) (Table 2). Serum creatinine values were also higher in CRF rats compared with their respective controls ($P < 0.05$), but there were no statistical differences among the CRF groups (Table 2). We do not have creatinine clearances, but since serum creatinine was the same in the different groups, the degree of renal insufficiency must have been similar (if, for example, dietary protein increased creatinine production, then CRF rats fed 30% protein would have had the least severe renal insufficiency). The blood pH was lower in each of the CRF rat groups *versus* control rats, but the difference was statistically significant only in CRF rats eating 30% protein ($P < 0.05$) (Table 2).

Initial values of weight and length were similar in randomly chosen rats assigned to different dietary groups (data not shown). The weight gain of CRF rats fed 8 or 17% protein was not statistically different from that of the respective pair-fed control rats, but rats fed 17% protein gained more weight than rats fed 8 or 30% protein ($P < 0.05$) (Figure 1). Notably, CRF rats eating 30% protein gained less weight than pair-fed control rats given 30% protein or CRF rats eating a low (8%) or moderate (17%) protein diet ($P < 0.05$).

The linear growth of the CRF and respective pair-fed control rats given 8 and 17% protein did not differ statistically over the 21 d, but in CRF rats eating 30% protein, the increase in length was less than that of the respective pair-fed control rats ($P < 0.05$) (Table 3). CRF rats fed 17% protein gained more length than the respective CRF rats eating 8 or 30% protein ($P < 0.05$) (Table 3).

CRF rats fed 8% protein had the highest food and caloric intake ($P < 0.05$) (Table 4). However, the protein intake was highest in CRF rats eating 30% protein ($P < 0.05$) but the spontaneous caloric intake was lowest in this group ($P < 0.05$) (Table 4). Because control rats were pair-fed, their food, calories, and protein intake were identical to the respective dietary protein group of CRF rats (Table 4).

The relationships between weight gain and caloric intake expressed as the efficiency of utilization of calories for growth

Table 1. Composition of the experimental diets^a

Diet	Protein		
	8%	17%	30%
Cornstarch	63.149	51.978	35.949
Casein	9.8	20.97	37.0
Sucrose	10.0	10.0	10.0
Soybean oil	7.0	7.0	7.0
Fiber	5.0	5.0	5.0
Mineral mix	3.5	3.5	3.5
Vitamin mix	1.0	1.0	1.0
L-cystine	0.3	0.3	0.3
Choline bitartrate	0.25	0.25	0.25
Tert-butylhydroquinone	0.0014	0.0014	0.0014

^a Formulated according to the American Institute of Nutrition Rodent Diets, AIN-93. Values are given as g constituent per 100 g of the diet. The protein content (81.1%) of casein was used to calculate the protein content of the different diets.

Table 2. Biochemical data from CRF and control rats fed 8, 17, and 30% protein for 21 d^a

Group/Dietary Protein	Blood Urea Nitrogen (mg/dl)	Serum Creatinine (mg/dl)	Blood pH
CRF /8%	41.01 ± 2.96 ^b (16)	0.96 ± 0.04 (16)	7.36 ± 0.02 (15)
Control /8%	6.04 ± 2.94 ^c (16)	0.54 ± 0.02 ^c (16)	7.40 ± 0.01 (16)
CRF /17%	69.59 ± 8.84 ^d (15)	0.87 ± 0.04 (15)	7.35 ± 0.02 (14)
Control /17%	9.65 ± 0.53 ^c (15)	0.52 ± 0.02 ^c (15)	7.40 ± 0.01 (14)
CRF /30%	124.03 ± 9.64 (13)	0.87 ± 0.06 (13)	7.26 ± 0.03 ^e (12)
Control /30%	16.95 ± 1.16 ^c (16)	0.42 ± 0.03 ^c (16)	7.38 ± 0.02 ^c (16)

^a Biochemical parameters from CRF and pair-fed, control rats were measured after 21 d. The number of rat samples measured is given in parentheses. CRF, chronic renal failure.

^b $P < 0.001$ versus CRF 17% or CRF 30%.

^c Control versus CRF, $P < 0.05$ (pH and serum creatinine), $P < 0.001$ (blood urea nitrogen).

^d $P < 0.001$ versus CRF 30%.

^e $P < 0.05$ versus CRF 17% or CRF 8%.

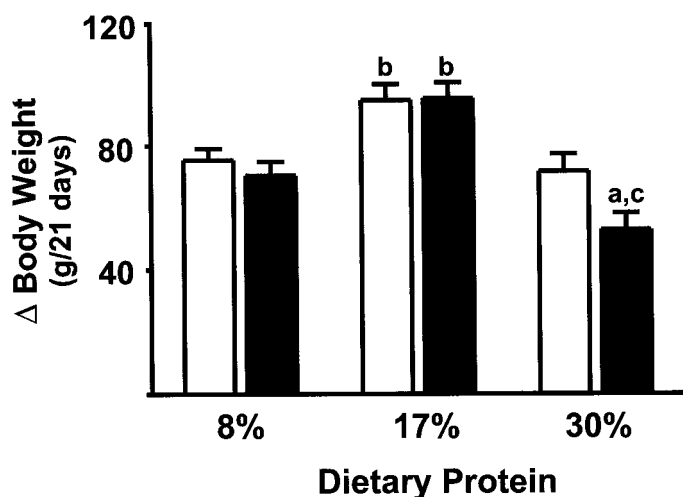


Figure 1. Change in body weight of rats with chronic renal failure (CRF) (■) and sham-operated, pair-fed rats (□) over 21 d. Rats were fed a diet containing 8% ($n = 19$), 17% ($n = 17$), or 30% ($n = 16$) protein. Results are expressed as mean ± SEM. ^a $P < 0.05$ for CRF 30% dietary protein rats versus pair-fed, control 30% protein rats; ^b $P < 0.01$ for 17% protein versus the corresponding 8 or 30% protein groups; ^c $P < 0.005$ for 30% versus the corresponding 17 or 8% protein groups.

(grams of weight gained per kilocalorie intake over 21 d) or food efficiency (grams of weight gained per gram of food intake per day) are shown in Figure 2. Control rats fed a barely adequate amount (8%) of protein had the lowest food and calorie efficiency compared with control rats fed 17 or 30% protein ($P < 0.005$) (Figure 2). CRF rats fed a moderate amount of protein (17%) had higher food and calorie efficiencies than CRF rats fed 8 or 30% protein ($P < 0.001$) (Figure 2).

Table 3. Influence of CRF and dietary protein on gain in body length for 21 d^a

Group/Dietary Protein	Initial Length (cm)	Final Length (cm)
Control/8% (18)	20.3 ± 0.1	35.9 ± 0.3
CRF/8% (19)	20.4 ± 0.2	35.7 ± 0.3
Control/17% (17)	20.5 ± 0.1	36.9 ± 0.4
CRF/17% (17)	20.3 ± 0.1	37.0 ± 0.5 ^b
Control/30% (16)	20.6 ± 0.2	36.6 ± 0.4
CRF/30% (16)	20.4 ± 0.1	35.0 ± 0.5 ^c

^a Rats were lightly anesthetized and their body lengths (nose to tail) were measured. Results are mean ± SEM, and the number of animals per group is indicated in parentheses.

^b $P < 0.01$ for 17% protein versus the corresponding 8 or 30% protein groups.

^c $P < 0.05$ between CRF and pair-fed control rats.

Notably, the food and calorie efficiencies were significantly reduced in CRF rats compared with pair-fed control rats that were eating 30% protein ($P < 0.001$).

The protein efficiency (grams of weight gained per gram of protein intake) was greatest in rats eating 8% protein, compared to the respective CRF and control rats eating 17 or 30% protein ($P < 0.001$) (Figure 2). CRF rats eating 17% protein had a higher protein efficiency compared with CRF rats fed 30% protein ($P < 0.001$). Differences between protein efficiencies of CRF and control rats occurred only in rats fed 30% protein: CRF rats had the lowest value of any group of rats ($P < 0.002$) (Figure 2).

The plasma concentrations of all three BCAA were lower in control rats eating 8% protein compared with the 17 or 30% protein groups ($P < 0.05$) (Table 5). Plasma leucine in control

Table 4. Dietary intakes of CRF and control rats fed different regimens for 21 d^a

Group/Dietary Protein	Food Intake (g/d)	Protein Intake (g/d)	Calorie Intake (kcal/d)
CRF /8% (19)	12.45 ± 0.38	0.99 ± 0.03	43.57 ± 1.20
CRF/17% (17)	11.79 ± 0.47	2.00 ± 0.08 ^b	41.26 ± 1.66
CRF/30% (16)	9.43 ± 0.38 ^c	2.83 ± 0.11 ^c	32.98 ± 1.32 ^c

^a The amount of the respective chow consumed daily was measured. Results are mean ± SEM, and the number of animals per group is given in parentheses.

^b $P < 0.05$ versus CRF 8%.

^c $P < 0.05$ versus CRF 8% or CRF 17%.

rats eating 17% protein was lower than values in control rats fed 30% protein ($P < 0.05$). In comparing CRF with control rats eating 8 or 17% protein, the plasma BCAA levels were lower in control rats than in CRF rats ($P < 0.05$); BCAA levels were not different in control and CRF rats fed 30% protein. The differences in the plasma concentrations of valine or leucine among CRF rats fed different levels of protein were not statistically significant; plasma isoleucine was statistically lower in the plasma of CRF animals fed 30% protein compared with CRF rats eating 17 or 8% protein ($P < 0.05$) (Table 5).

Discussion

There are multiple potential causes of protein-energy malnutrition in uremic patients. Anorexia can result in an insufficient intake of nutrients to maintain protein balance. Uremia can also cause loss of muscle mass by inhibiting protein synthesis and/or accelerating protein catabolism due to resistance to insulin and growth factors, hyperglucagonemia, hyperparathyroidism, or metabolic acidosis (28). In children with CRF, growth retardation is a well-recognized problem that has been linked to reduced calorie intake, vitamin D deficiency, disturbances in growth hormone and insulin-like growth factor-1 levels, and acidosis (14,29). In rats with CRF, Kleinknecht *et al.* showed that CRF rats fed 17% protein had the least growth impairment compared with CRF rats fed 27 or 37% protein (30). Our results confirm that a similar protein content (*i.e.*, 14%) supports growth of CRF rats, whereas higher levels suppress their growth. We also studied how dietary protein interacts with CRF to change nutritional status. Food and calorie intake were greatest in CRF rats fed 8% protein and their BUN was the lowest, but their utilization of protein for growth was the highest. Despite this, their weight gain was lower than CRF rats fed 17% protein. Moderate protein restriction (17%) promoted the highest calorie- and food-growth efficiency, whereas rats fed 30% protein had impaired growth.

Anorexia is a major cause of impaired nutritional status in both adults and children with CRF. When anorexia reduces dietary calories, amino acids may be shifted away from protein synthesis to provide energy, offering an explanation for the observation that nitrogen balance improves as calorie intake increases in CRF patients eating a low-protein diet (1,31). For children with CRF, the minimum calorie RDA for height/age ratio is recommended (6,14,32); in adults, the calorie intake

should be 30 to 35 kcal/kg per d (31). Insight into the mechanisms causing anorexia in CRF was provided by Anderstam *et al.*, who reported that an intravenous infusion of a serum fraction from uremic patients inhibited the appetite of normal rats, suggesting that a uremic toxin suppresses appetite (15). They also found that infusion of amino acids inhibits food intake of normal rats by a mechanism involving changes in brain neurotransmitters and in peptides secreted by the gastrointestinal tract (33). Subsequently, they demonstrated that peritoneal dialysis with solutions containing amino acids also suppresses protein intake in normal rats and concluded that the appetite can vary with different dietary constituents (34). Our studies are consistent with these reports because the CRF rats fed 8% protein had a 25% higher calorie intake than CRF eating the 30% protein diet; the calorie intake of the 8% protein group was also higher compared with CRF rats fed 17% protein (Table 4). Taken together, these results indicate that an 8% protein diet approaches the minimal protein requirement that avoids anorexia and malnutrition in CRF rats.

Dietary protein requirements for healthy children vary with age (35); however, the requirements for children with CRF are not as well established (14). Diets prescribed for CRF children include the recommended safe allowance of 0.8 to 1.1 g protein/kg per d, which resulted in no statistical growth impairment (12,13). In contrast, when infants with CRF fed a diet with approximately 1.4 g protein/kg per d, their growth was less than that of CRF infants fed approximately 2.4 g protein/kg per d (36).

When adults with uncomplicated CRF were provided the minimal daily protein requirement of healthy adults (0.6 g/kg per d) or even more restricted regimens supplemented with essential amino acids or ketoacids, nitrogen balance was neutral (9). Despite these findings, it has been suggested that dietary protein of CRF patients should be raised above the minimal level to combat malnutrition (2). This debate prompted the present study, and our results indicate that this suggestion should be viewed with caution. CRF rats fed a high-protein diet (30%) exhibited a substantial limitation in their growth even though their protein intake was the greatest. In addition, this group utilized dietary protein and calories less efficiently than pair-fed control rats or CRF rats fed the low- or moderate-protein diet. Growth impairment in CRF rats fed 30% protein may have been a result of two complications: anorexia and acidosis. Anorexia is suggested by the finding

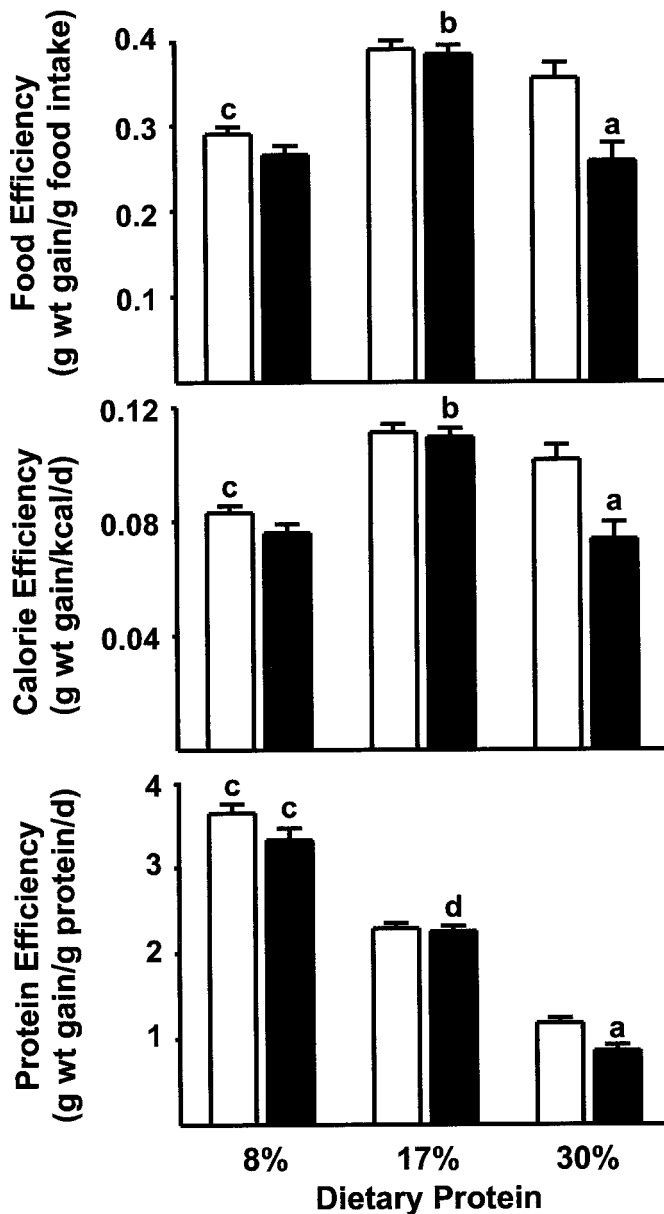


Figure 2. The effect of dietary protein on nutritional parameters. Protein efficiency, food efficiency, and calorie efficiency in rats with CRF (■) and sham-operated pair fed rats (□) over 21 d. Rats were fed a diet containing 8% ($n = 19$), 17% ($n = 17$), or 30% ($n = 16$) protein. Results are expressed as mean \pm SEM. ^a $P < 0.05$ for CRF 30% dietary protein rats versus pair-fed, control 30% protein rats; ^b $P < 0.01$ for 17% protein versus the corresponding 8 or 30% protein groups; ^c $P < 0.005$ for 8% versus the corresponding 17 or 30% protein groups; ^d $P < 0.001$ for CRF 17% protein rats versus CRF 30% protein rats.

that this group of CRF rats had the lowest calorie and food intake. This group also had the lowest blood pH because the amount of dietary protein is closely related to acid production. Acidosis could limit growth and stimulate loss of lean body mass. For example, children with metabolic acidosis from renal tubular disorders grow poorly, and their growth improves after correction of acidosis (37). Adults with metabolic acidosis

have increased catabolism of amino acids and protein, decreased insulin-like growth factor-1 response to growth hormone and thyroid hormone release in response to thyroid stimulating hormone, as well as a lower level of 1,25 dihydroxy cholecalciferol (28). Together, these abnormalities could impair the growth of children and contribute to metabolic abnormalities found in CRF patients.

Does a low-protein diet impair the efficiency of utilization of protein and/or calories in CRF? The present results indicate that this is not the case because the efficiency of utilization of dietary protein for growth was highest among rats eating the low-protein (8%) diet. It is important that the only values of protein efficiency that differed between CRF and control, pair-fed rats were in the 30% protein group (Figure 2). These findings indicate that when dietary protein is excessive, anorexia will limit the calorie intake and reduce the efficiency of dietary protein utilization for growth.

In CRF, low levels of BCAA in plasma and muscle have been linked to accelerated oxidation of these amino acids (18,38,39). For this reason, we measured plasma levels of BCAA. Potential explanations for the plasma BCAA levels we found in CRF rats involve the diet and adaptive changes in BCAA metabolism. For example, in CRF rats eating 30% protein, the impact of CRF on BCAA catabolism may have been overcome by an excessive protein intake, since raising dietary protein of control rats led to a progressive increase in the plasma concentrations of valine, leucine, and isoleucine. The interpretation of changes in plasma BCAA levels is complicated by the finding that acidosis attenuates amino acid uptake by the liver and reduces the activity of the key enzyme in BCAA degradation, branched-chain α -ketoacid dehydrogenase, defects that would raise plasma BCAA levels (40,41). In fact, Hara *et al.*, found that there were no statistically significant decreases in plasma BCAA levels in CRF rats even though they discovered that BCAA catabolism in muscle is accelerated in acidotic, CRF rats (16). Another metabolic factor blunting the effect of accelerated BCAA catabolism on plasma BCAA levels is the increase in protein degradation that is stimulated by acidosis (17,18,24). In this case, the rate of BCAA appearance from accelerated protein degradation could exceed the rate of BCAA oxidation. Besides the proteolysis induced by acidosis, Ding *et al.* reported that there is accelerated muscle protein degradation associated with CRF even when acidosis is treated (42). If this proteolytic response were coupled with a concurrent reduction in BCAA oxidation due to reduced dietary protein (43) plus the suppressive effects of insulin on BCAA oxidation (16,26), there could be a rise in plasma BCAA levels. Indeed, this type of response occurred in CRF rats eating 8% protein (Table 5). In CRF rats fed 17% protein, the explanation for the higher BCAA levels compared to pair-fed, control rats was not obvious, but protein breakdown due to CRF could release BCAA, explaining this result. Together, these tissue-specific adaptations to acidosis could maintain plasma BCAA levels in CRF rats.

We conclude that a low-protein diet does not interfere with the utilization of protein for growth in CRF as long as calories are adequate. This diet does not impair appetite and limits the

Table 5. Plasma concentrations of the branched-chain amino acids in CRF and pair-fed control rats fed 8, 17, or 30% protein diets^a

Amount of Dietary Protein	Valine		Isoleucine		Leucine	
	Control	CRF	Control	CRF	Control	CRF
8% (17)	124 ± 4 ^b	177 ± 7 ^c	69 ± 2 ^b	112 ± 5 ^c	87 ± 4 ^b	129 ± 6 ^c
17% (14)	163 ± 11	185 ± 6 ^c	93 ± 5	111 ± 4 ^c	114 ± 7 ^d	138 ± 6 ^c
30% (15)	179 ± 6	175 ± 7	99 ± 3	98 ± 4 ^e	132 ± 4	131 ± 7

^a Values are reported as mean ± SEM of the plasma concentrations of each branched-chain amino acid (μmol/L) after 21 d of pair-feeding; the numbers of pairs of control and CRF rats studied from each dietary protein group are indicated in parentheses. Superscript letters indicate values of $P < 0.05$ and are considered statistically significant.

^b Control rats eating 8% protein versus control rats eating 17 or 30% protein.

^c Control versus CRF rats eating equal amounts of protein.

^d Control rats eating 17% protein versus control rats eating 30% protein.

^e CRF rats eating 30% protein versus CRF rats eating 8 or 17% protein.

rise in BUN induced by high-protein diets. This diet or a moderate level of dietary protein (17%) reduces the accumulation of nitrogenous products and prevents acidosis. In sharp contrast, a high-protein diet potentiates the adverse effects of uremia by increasing the BUN and the accumulation of acid. The result is impaired growth and nutritional status.

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