Urea and Protein Metabolism in Burned Children: Effect of Dietary Protein Intake

Bruce W. Patterson, Thuan Nguyen, Edgar Pierre, David N. Herndon, and Robert R. Wolfe

The response of urea metabolic kinetics, the rate of whole-body protein breakdown, and muscle and skin protein synthesis rates to dietary protein intake (1.15 to 2.92 g/kg/d) was assessed in children with 20% to 40% total body surface area burn injury using a primed continuous infusion of $^{15}N_2$-urea and L-$^{13}C_6$-phenylalanine. Plasma urea concentration, production, and excretion rates increased with dietary protein intake without evidence of approaching maximum plateau values. There was no consistent evidence of urea recycling in these subjects (urea production = excretion) at any level of protein intake. The rate of appearance (Ra) of phenylalanine (an index of whole-body protein breakdown) and rate of muscle protein synthesis were independent of dietary protein, whereas there was a significant increase in skin protein synthesis with higher protein intake. We conclude that there seems to be little benefit of high protein intake on whole-body protein breakdown and muscle protein synthesis rates in these burn patients, although high-protein diets may enhance wound healing.

PATIENTS WITH BURN TRAUMA are in a hypercatabolic metabolic state that results in net muscle protein loss. This protein catabolic state may result in impaired ventilatory function and infection-control mechanisms, and may delay wound healing. Furthermore, the return to normal physiological function after recovery is prolonged by loss of muscle mass.[1] Protein catabolism may persist for months following burn injury.[2] Rigorous nutritional therapy alone is ineffective at completely reversing this process,[3] although we have recently demonstrated that either exogenous insulin at high dose[4] or human growth hormone[5] can neutralize the catabolic effect of burn injury on muscle.

It has become common clinical practice to provide high protein intake to patients with burn injuries, particularly children. For example, Alexander et al[6] have advocated a rate of protein intake of greater than 4 g protein/kg/d in children with burns, which is three to four times the requirement for normal, unburned children. The goal of such a high rate of protein intake is presumably to stimulate protein synthesis sufficiently to offset the catabolic response, yet there is little evidence that amino acids alone are effective in this regard. Nitrogen (N) balance may be improved with high protein intake, but this is of questionable physiological significance, since even in normal volunteers the increase in nitrogen balance observed when N intake is greater than required does not correspond to improvement of any other indices of protein synthesis or breakdown.[7] In burn patients, increased N intake leads to increased urea production and plasma urea concentration.[8-10]

Whereas the general relation between high protein intake and urea production in burn patients is clear, several aspects of urea kinetics are unknown. Thus, there is much evidence that urea can recycle through gut flora urease, with the ammonia N being used for amino acid production rather than incorporation into urea.[11-13] Using ^{15}N-ammonia, we recently demonstrated that greater than half of the tracer that appears in plasma components is initially incorporated into amino acids rather than urea.[14] If this pathway of N salvaging exists in burn patients, it could significantly affect total N requirements. Furthermore, in normal subjects, the extent of recycling of urea N is dependent on the level of protein intake.[15] Therefore, it was our goal to quantify urea N recycling in burn patients on diets used in the clinical setting that provide different levels of protein intake. We further wished to examine the relationships between urea production, excretion, and concentration during different levels of protein intake to assess the ability of the kidneys of burn patients to maintain a normal blood urea concentration. To assess the effects of protein intake on urea kinetics in relation to the potential benefits of a high protein intake, we have also quantified the fractional synthetic rates (FSRs) of both muscle and skin protein. Whereas whole-body data indicate no beneficial effects of extremely high protein intake, specific tissues may respond positively.

SUBJECTS AND METHODS

Clinical Studies

Subjects aged 4 to 18 years with 20% to 40% total body surface area burn injury were recruited from patients admitted to the Shriners Burns Institute, Galveston, TX. Patients were excluded from study if they were receiving complete antibiotic therapy to control sepsis to minimize the impact on urease-positive colonic bacteria. Informed consent was obtained from parents or guardians. Studies were approved by the Institutional Review Board of the University of Texas Medical Branch.

Subjects were administered continuous enteral feeding with one of three enteral diets for 4 days before the metabolic tracer study. Two diets typically used in the clinical setting included a "high-protein" diet (mean intake, 1.84 g protein/kg/d; Prosobee (Mead Johnson, Evansville, IN): 40% of calories as carbohydrate, 48% as fat, and 12% as protein) and a "very–high-protein" diet (mean intake, 2.92 g protein/kg/d; Prosobee supplemented with ProMod (Mead Johnson), 22 g ProMod added per liter of Prosobee: 37% of calories as carbohydrate, 44% as fat, and 19% as protein). A "normal" diet was used for comparison in three

From the Metabolism Unit, Shriners Burns Institute, Galveston; and the Department of Surgery, University of Texas Medical Branch, Galveston, TX.

Submitted September 6, 1996; accepted October 26, 1996.

Supported by Grants No. 15856 and 15849 from the Shriners Hospitals for Crippled Children.

Presented previously in abstract form at the 27th Annual Meeting of the American Burn Association, April 19-22, 1995, Albuquerque, NM.

Present address: B.W.P., Department of Internal Medicine, Washington University School of Medicine, 660 S Euclid Ave, Box 8127, St Louis, MO 63110-1093; T.N., Department of Surgery, University of Southern California.

Address reprint requests to Robert R. Wolfe, PhD, Metabolism Unit, Shriners Burns Institute, 815 Market St, Galveston, TX 77550.

0026-0495/97/4605-0018$03.00/0

subjects (mean intake, 1.15 g protein/kg/d; Suplena (Ross Laboratories, Columbus, OH): 51% of calories as carbohydrate, 43% as fat, and 6% as protein). All subjects were administered an enteral diet at the rate of 1,500 kcal/m^2 total body surface area plus 1,500 kcal/m^2 body surface area burned as typically used in our clinical setting. Subjects were placed on the prescribed diet upon recovery from the first surgical procedure for wound closure, and were maintained on the diet for 4 days before and throughout the tracer infusion protocol. One subject was studied in a crossover design, first on the high-protein diet followed by the very–high-protein diet. One subject was studied twice on the same diet (very–high-protein) to assess reproducibility (results from the two studies were averaged together to represent this subject for intergroup comparisons).

The tracer protocol consisted of a 4-hour primed continuous infusion of $^{15}N_2$-urea (84 μmol/kg prime and 0.15 μmol/kg/min infusion, 99.4 atom % ^{15}N; Isotec, Miamisburg, OH) and L-ring-$^{13}C_6$-phenylalanine (2.4 μmol/kg prime and 0.06 μmol/kg/min infusion, 99 atom % ring-^{13}C; Isotec). Tracer was infused into a central venous catheter already used for clinical management. Blood samples (2 mL each) were obtained from a peripheral venous catheter before tracer infusion and then at 10-minute intervals from 3.5 to 4 hours to define the plasma urea isotopic enrichment plateau. Plasma was immediately separated and stored at −10°C until processed. Urine was quantitatively collected over the duration of the infusion period. The time and volume of individual urine voids were recorded, and 2-mL aliquots were stored at −10°C. The remaining portions of individual urine voids were combined to obtain a pooled urine sample, from which another 2-mL aliquot was stored at −10°C. Biopsies of thigh muscle were obtained with a 4-mm Bergström needle, and samples of skin from the muscle biopsy site were obtained from separate sites at 1 and 4 hours into the infusion protocol.[4,16,17] Blood, visible fat, and connective tissue were removed, and the samples were immediately frozen in liquid nitrogen and stored at −80°C.

Sample Analyses

Plasma samples were thawed and thoroughly mixed. Two 100-μL aliquots were placed into separate microfuge tubes. A 100-μL aliquot of either normal saline or 2.0-mmol/L $^{15}N_2$-urea as internal standard to quantify plasma urea concentration was placed into each tube. Samples were diluted with 300 μL normal saline and deproteinized, and plasma amino acids and urea were recovered by cation-exchange chromatography as previously described.[18] Samples were converted to *tert*-butyldimethylsilyl derivatives by addition of 50 μL *N*-methyl-*N*-*tert*-butyldimethylsilyl trifluoroacetamide (Pierce Chemicals, Rockford, IL) and 50 μL acetonitrile and heating at 70°C for 30 minutes. Isotopic enrichments of $^{15}N_2$-urea and $^{13}C_6$-phenylalanine from 1-μL aliquots were measured in triplicate by electron-impact ionization gas chromatography/mass spectrometry (GC/MS) as previously described,[19] using a VG 12-250 system (VG Biotech, Altrincham, UK). Ions at *m/z* 233/231 were used for urea and *m/z* 240/234 for phenylalanine. Tracer to tracee molar ratios (TTRs) were determined as $R - R_0$, where R and R_0 are the isotope ratios for enriched and unenriched (preinfusion) samples, respectively.[18] The total amount of urea in the plasma sample (unlabeled endogenous urea plus labeled tracer urea) was determined from the change in enrichment resulting from a known amount of internal standard tracer added to the sample; the endogenous unlabeled urea concentration was derived by subtracting the exogenous tracer content based on the TTR (approximately a 3% correction).

Urine samples were thawed and thoroughly mixed, and two 100-μL aliquots were placed into plastic 13 × 100-mm tubes. A 100-μL aliquot of either normal saline or 200-mmol/L $^{15}N_2$-urea as internal standard to quantify urinary urea concentration was placed into each tube. A 50-μL aliquot was then diluted with 450 μL normal saline and deproteinized, and urea was recovered by cation-exchange chromatography. One third of the recovered urea was then dried, derivatized, and analyzed by GC/MS.

Muscle and skin biopsy samples were thawed, weighed (typically 20 to 50 mg wet weight), and extracted three times with 14% perchloric acid (approximately 0.7 mL each wash) by grinding with a Teflon pestle in a microfuge tube. The protein precipitates were then washed three times with ethanol (1 mL each wash), followed by two washes with deionized water (1 mL each wash). All grinding and washing procedures were performed on ice. The protein precipitates were hydrolyzed at 110°C for a minimum of 24 hours in 6N HCl (ACS grade). A cation-exchange column (Dowex AG 50W-X8; Bio-Rad Laboratories, Richmond, CA) was used[18] to recover and purify the amino acids from a volume of protein hydrolysate that corresponded to 4 mg wet weight of starting tissue. *N*-heptafluorobutyryl-*n*-propyl ester derivatives were prepared by first heating the samples at 110°C for 1 hour with 0.5 mL 3.5N HBr in propanol (Alltech Associates, Deerfield, IL) in a screw-capped tube, then drying the sample under N_2, and heating at 60°C for 20 minutes with 0.1 mL heptafluorobutyric anhydride (Sigma Chemical, St Louis, MO). The samples were briefly evaporated to dryness under N_2 and dissolved in 0.1 mL ethyl acetate. One-microliter aliquots were analyzed by electron-impact ionization GC/MS on an MD800 (Fisons Instruments, Beverly, MA) GC/MS system. The TTR was obtained by comparing the m + 6/m + 4 isotope ratio of the samples against a set of calibration standards that ranged from 0% to 0.5% TTR using the *m/z* 91 ion from the phenyl-CH_2-side chain of phenylalanine as recently described.[4,20]

Calculations

The rate of appearance (Ra) of plasma urea and phenylalanine was determined from the isotopic steady-state equation, Ra (μmol/kg/min) = F/TTR, where F is the tracer infusion rate (micromoles per kilogram per minute) and TTR is the isotopic plateau TTR.[18] Urinary urea excretion rate was determined from the slope of the cumulative urea output versus time. The percent of urea that recycles was calculated as (Ra − excretion)/Ra, which accounts for the fraction of urea that recycles into products other than urea; this does not account for recycling of urea N back into urea production. However, we were not able to detect enrichment of singly labeled $^{15}N_1$-urea above background levels, such that corrections for recycling of ^{15}N tracer back into the resynthesis of urea[18,21] were not necessary; instead, all urea produced during the infusion protocol was unlabeled and therefore appropriately accounted for by the urea Ra determination.

Endogenous phenylalanine Ra was calculated by subtracting the dietary phenylalanine intake (assumed to be 100% absorbed) from the total Ra. Muscle and skin protein FSRs were determined from the rate of change of $^{13}C_6$-phenylalanine TTR between 1 and 4 hours, divided by the plasma $^{13}C_6$-phenylalanine plateau enrichment.[18] This calculation assumes that the intracellular precursor pool is at a constant isotopic enrichment reflected by the plasma plateau enrichment between muscle and skin biopsy samples.

Statistical Analysis

Results are expressed as the mean ± SEM. Dietary groups were compared with a Student's two-tailed *t* test. A *P* value of .05 or less was taken as indicating a significant difference.

RESULTS

Subject characteristics are summarized in Table 1. There were no differences between dietary protein groups with respect to subject age, height, body surface area, weight, change in body weight, percent area burned, or caloric intake. Subjects on the normal protein diet happened to have a higher extent of third-degree burn. Whereas essentially all subjects lost body

Table 1. Subject Characteristics

	Protein Intake		
	Normal (n = 3 male)	High (n = 5 male)	Very High (n = 1 female and 3 male)
Age (yr)	10.8 ± 2.2	11.3 ± 2.0	12.6 ± 1.3
Height (cm)	132 ± 7.6	142 ± 8.6	148 ± 7.5
Body surface area (m^2)	1.02 ± 0.08	1.28 ± 0.15	1.44 ± 0.12
Admission weight (kg)	37.0 ± 9.1	43.3 ± 8.2	51.9 ± 7.2
% change in body weight	0.1 ± 3.3	−4.5 ± 2.1	−7.4 ± 2.3
Days postburn	10 ± 6	10 ± 2	11 ± 2
% burn	24 ± 4	27 ± 3	25 ± 1
% 3rd-degree burn	10 ± 3*	5 ± 2	1 ± 1*
Caloric intake (kcal/kg/d)	76.4 ± 18.9	62.5 ± 7.8	60.1 ± 4.6
Protein intake (g/kg/d)	1.15 ± 0.28*	1.84 ± 0.23†	2.92 ± 0.19*†

*$P < .05$, normal *v* very high.
†$P < .05$, high *v* very high.

weight between the time of admission and the date of study, there was a tendency for greater loss of body weight with higher protein intake, although the differences did not reach statistical significance.

Metabolic measures are summarized in Table 2. Plasma urea concentration, urea Ra, and urinary urea excretion increased as dietary protein intake increased, although differences between groups only reached statistical significance ($P < .05$) for urea Ra between the normal and very–high-protein groups. There was no evidence of urea recycling (urea excretion = urea Ra) in the normal and high-protein groups, whereas in the very–high-protein group the urinary excretion was greater than the Ra, resulting in an apparent "negative" recycling. There were no significant differences in endogenous phenylalanine Ra between groups, suggesting that the whole-body protein breakdown rate was unaffected by diet.

Muscle and skin protein FSR measurements are also summarized in Table 2 (samples were not obtained from a sufficient number of subjects from the normal protein group to enable valid group comparisons). The skin protein FSR was 3.2-fold faster, on average, than the muscle protein FSR. Muscle and skin protein FSRs were not significantly different between the very–high-protein and high-protein groups.

Although all subjects within a given dietary group received the same dietary intake based on total and burned body surface area, the range of burn size and body size between small and large subjects resulted in a wide range of dietary intake relative to body mass. It is therefore useful to examine the metabolic measurements as a continuous function of dietary protein intake per kilogram body mass. Selected urea metabolic parameters and endogenous phenylalanine Ra as a function of protein intake are shown in Fig 1. Plasma urea concentration (A) and urea Ra (B) were linearly correlated with dietary protein intake. Neither urea concentration nor urea Ra showed any evidence of approaching a plateau value up to 3.7 g/kg/d protein intake. In contrast to the urea metabolic parameters, the Ra of endogenous phenylalanine into plasma was not correlated with protein intake (C). Thus, there is no evidence that whole-body protein breakdown was reduced by increasing protein intake.

The rate of urea excretion and urea Ra were linearly correlated with the plasma urea concentration, with high correlation coefficients ($R^2 > .9$; Fig 2); there was no evidence that either excretion or Ra were approaching a limiting plateau value. The urinary excretion rate had an intercept that was not significantly different from zero; thus, urinary excretion was a

Table 2. Urea, Phenylalanine, and Protein Synthesis Kinetics

Parameter	Protein Intake		
	Normal	High	Very High
Plasma urea concentration (mmol/L)	1.86 ± 0.24	2.85 ± 0.30	3.96 ± 0.70
Urea Ra (μmol/kg/min)	3.17 ± 0.51*	4.75 ± 0.41	6.48 ± 0.70*
Urea excretion (μmol/kg/min)	3.25 ± 0.64	4.79 ± 0.58	7.11 ± 1.38
Urea recycling (%)	−1.5 ± 4.3	−0.4 ± 8.7	−7.2 ± 10.6
Endogenous phenylalanine Ra (μmol/kg/min)	1.18 ± 0.12	1.28 ± 0.16	1.31 ± 0.14
Muscle protein FSR (%/d)	NA	2.15 ± 0.39	2.50 ± 0.35
Skin protein FSR (%/d)	NA	7.37 ± 2.34	7.54 ± 3.13

Abbreviation: NA, not available.
*$P < .05$, normal *v* very high.

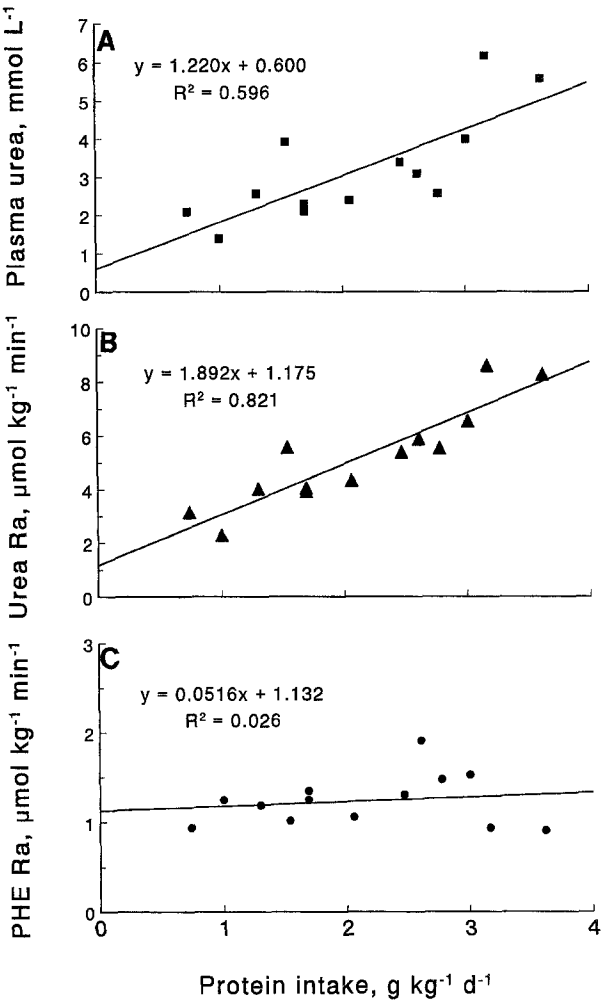

Fig 1. Urea and phenylalanine kinetic parameters as a function of dietary protein intake. Linear regression lines and coefficients are shown for all plots. (A) Plasma urea concentration; (B) plasma urea Ra; (C) endogenous plasma phenylalanine Ra.

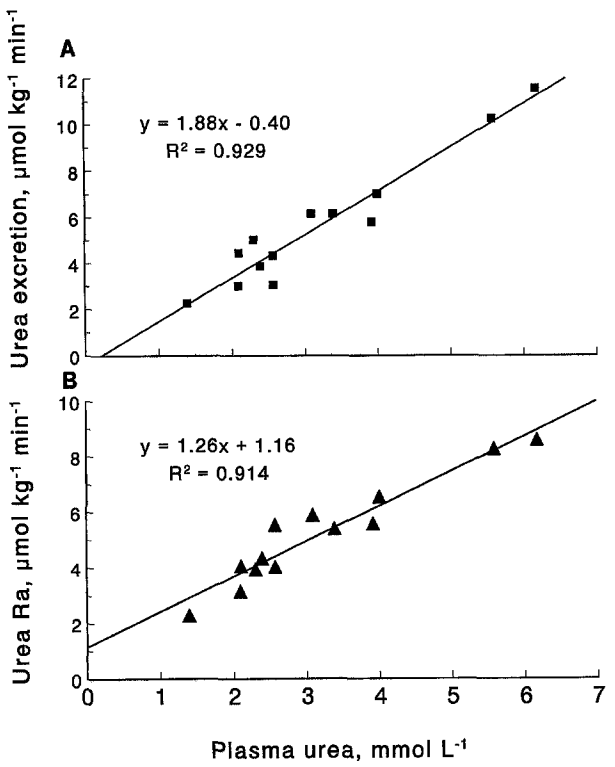

Fig 2. Urea excretion and production rates as a function of plasma urea concentration. Linear regression lines and coefficients are shown for all plots. (A) Rate of urea urinary excretion; (B) plasma urea Ra.

constant proportion of the plasma concentration. However, the urea Ra intercept was significantly greater than zero.

The relationship between urinary urea excretion rate and plasma urea Ra for each individual study is shown in Fig 3. The line on this figure represents the line of identity (ie, excretion = Ra). With the exception of the two highest values, all values are essentially on the line of identity. The two highest values were the repeated studies of the same subject on the very–high-protein diet. The close similarity of these values from repeated studies demonstrates the reproducibility of the tracer protocol and long-term stability of the subject. This subject had 34% and 24% higher urinary excretion than plasma Ra on days 8 and 22 postburn, respectively.

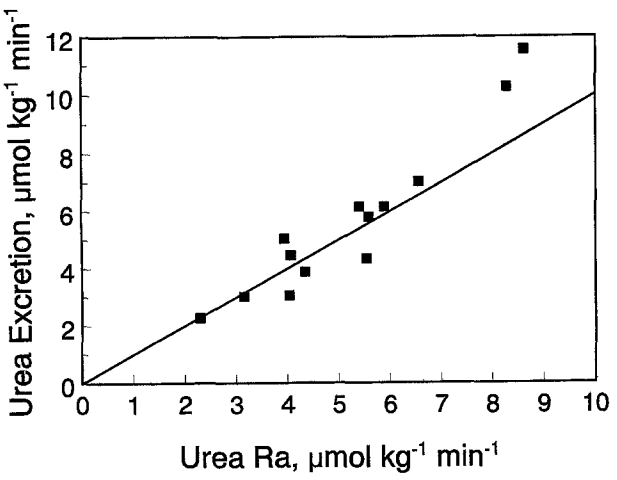

Fig 3. Correlation between urinary urea excretion rate and plasma Ra. The line illustrates identity, where urinary excretion = plasma Ra.

Muscle and skin protein synthesis rates are plotted against dietary protein intakes in Fig 4. Muscle protein synthesis was independent of protein intake (slope not significantly different from zero, $P = .35$, $R^2 = .12$), whereas skin protein synthesis was positively correlated with protein intake (slope different from zero, $P = .026$, $R^2 = .48$). Thus, although skin protein FSR was not significantly different between discrete dietary groups (Table 2), there was a significant increase in skin FSR with protein intake (Fig 4).

One subject was studied with a crossover design, first with the high-protein diet (6 days postburn) followed by the very–high-protein diet (10 days postburn). (It was not possible to perform crossover studies on other patients, since they requested to receive solid food after the initial study period.) Urea Ra and excretion rate increased by approximately 40% on the very–high-protein diet (Ra, 4.03 *v* 5.54 μmol/kg/min; excretion, 3.06 *v* 4.34 μmol/kg/min). This subject was the only subject in the study who exhibited a significant proportion of urea recycling, with a urea Ra 24% and 22% higher than urinary excretion on the high-protein and very–high-protein diets, respectively. The endogenous phenylalanine Ra in this subject increased slightly on the very–high-protein diet (1.19 *v* 1.48 μmol/kg/min), and muscle protein FSR was virtually unchanged (0.0203 *v* 0.0237 pools/d). In contrast, skin protein FSR doubled on the very–high-protein diet (0.040 *v* 0.080 pools/d).

DISCUSSION

Burn patients are particularly vulnerable to protein-calorie malnutrition; therefore, it is customary to place burn patients on high-protein/high-calorie diets during recovery,[6] although there is no consensus regarding actual dietary protein requirements for such individuals. Alteration or manipulation of protein metabolism may not affect survival in subjects with small- to moderate-sized burns. However, we have previously demon-

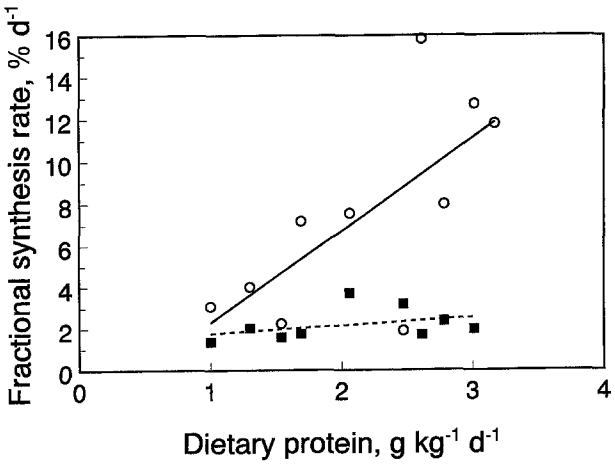

Fig 4. Muscle and skin protein FSRs as a function of dietary protein intake. Muscle and skin protein FSRs were measured between 1 and 4 hours into the tracer infusion protocol. Lines represent linear regression fits to the data. (■) Muscle protein; (○) skin protein.

strated that alterations in protein metabolism in burned children do not correlate with the hypermetabolic response to burn injury, and can persist long after the hypermetabolic response has abated.[2] Thus, alterations in protein metabolism following burn injury affect the long-term recovery and ultimate restoration of the patient to preburn physiologic condition.

Requirements are conventionally assessed in terms of some aspect of urea production (usually N excretion), since amino acids that are consumed in excess of the capacity for protein synthesis undergo oxidation coupled with a transfer of the nitrogen to urea.[22,23] In this study, we have directly assessed urea kinetics using an isotopic tracer to overcome the interpretative limitations of relying entirely on N excretion data.[24] We found that the Ra of plasma urea, plasma urea concentration, and rate of urinary urea excretion increase linearly with dietary protein intakes of 1 to 3.6 g/kg/d; there was no evidence in our studies that these values were approaching limiting plateau values at higher protein intakes. This suggests that the protein intakes in these studies exceeded requirements, such that excess amino acids were oxidized with a concomitant increase in urea production. There was no apparent beneficial effect of increased dietary protein intake either on whole-body protein breakdown, as reflected indirectly by phenylalanine Ra, or on muscle protein synthesis, as measured directly by labeled phenylalanine incorporation, again suggesting that the dietary protein intakes used in this study met metabolic requirements. If any amino acid had been limiting at the whole-body level on the lower protein intakes examined, there should have been an increase in muscle protein synthesis, which is the major contributor of whole-body protein synthesis, with higher levels of protein intake. In contrast, we observed that skin protein synthesis was stimulated by increasing protein intake, both in the intersubject comparisons (Fig 4) and in the one subject studied with a dietary crossover design. This suggests that there may be a positive effect of dietary protein on the wound healing process, although extrapolation of this point with respect to burn wound healing from the present study should be guarded, since these patients were not severely burned and the skin biopsy was not obtained from the wound or donor site.

The lack of a positive effect of an increasing protein intake at the whole-body level (as reflected by phenylalanine Ra) and in the muscle is consistent with an earlier study in severely burned adult patients given total parenteral nutrition.[8] In that study, the balance between whole-body protein synthesis and breakdown determined isotopically was not improved in patients receiving intravenous nutrition by increasing the rate of amino acid infusion from 0.21 g N/kg/d to 0.34 g N/kg/d (corresponding to 1.4 and 2.2 g protein/kg/d, respectively). Furthermore, in that study, increasing the amino acid intake significantly increased both urea concentration and urea production.[8] This is to be expected on the basis of studies by Marchini et al[22] and Young et al,[23] who have shown that dietary amino acids consumed in excess of requirements are predominately oxidized. As a consequence of this accelerated oxidation of extra amino acids, the N is ultimately incorporated into urea, thereby explaining the increased rate of urea production when protein intake is increased.

The results of the current study, as well as the previous study of protein kinetics in adult patients,[8] are in contrast to a number of studies in critically ill patients indicating a beneficial effect of high protein intake on N balance.[25] However, there appears to be a spurious apparent N retention at high protein intake. Hegsted[24] collated the results of a large number of studies in normal volunteers, and concluded that there is an "apparent retention" of approximately 20% of N intake above the maintenance needs that does not correspond to a detectable change in lean body mass over a prolonged period. This general point was confirmed in a study in which normal volunteers were given isocaloric diets that provided either 0.9 or 2.5 g protein/kg/d.[7] The nitrogen-balance data indicated an improvement of approximately 45% of the excess N intake above the normal value of 0.9 g protein/kg/d. However, whole-body protein turnover, as well as the measured synthetic rates of albumin, fibrinogen, fibronectin, and muscle protein, was not different during the two levels of protein intake.[7] Thus, in that study it appears that the results of N-balance data were spurious and did not correspond to changes in net protein synthesis. The basis for the apparent N retention at high protein intake according to the N-balance method is not clear, but it is likely that the accurate assessment of true N balance is further complicated in burn patients, who may lose N through wound exudate for which account is not taken. Thus, we conclude that excessively high protein intake is not useful in minimizing the muscle and whole-body protein catabolic response in burn patients.

In normal volunteers receiving a normal diet, the rate of urea production exceeds the rate of urea excretion by as much as 50%.[15,26] This can be explained to at least some extent by a recycling of the urea N back into amino acids.[11-13] However, with the exception of one subject who exhibited urea recycling of approximately 20%, there was no evidence of significant urea nitrogen recycling in burned children in this study. For patients receiving either of the two clinical diets (high-protein and very–high-protein diets, approximately 1.8 and 2.9 g/kg/d, respectively) this was not unexpected, since high protein intake reduces the recycling and utilization of urea.[27,28] However, the lack of urea nitrogen recycling in subjects receiving the normal protein diet (1.15 g/kg/d) suggests that the capacity to recycle urea nitrogen may be compromised in these patients. We were not able to detect the formation of singly labeled $^{15}N_1$-urea during our infusion protocol. It is probable that recycling of urea N back into urea production did occur but was not detectable because of low enrichments of plasma $^{15}N_2$-urea during the infusion studies (approximately 5% TTR). Since the production of isotopically labeled urea was undetectable, our urea Ra determination includes production from all sources of nitrogen, including any that recycled from urea. However, our major observation was that there did not appear to be any recycling of urea N into products other than urea.

In contrast to the present results, in previous studies we demonstrated that urea production was approximately 40% greater than excretion in adult burn patients with a mean 70% burn surface area on dietary protein intakes of 1.4 or 2.2 g/kg/d intravenously.[8] Essentially identical results were obtained on both diets. It is difficult to explain the lack of recycling in the present studies compared with our previous results in adult burn patients[8] and in normal volunteers on dietary protein intakes of 1.5 g/kg/d.[26] The present study used a $^{15}N_2$-urea priming dose to infusion rate (P/I) ratio of 560 minutes, which has been

validated in normal subjects[29] in response to concerns over the validity of the $^{15}N_2$-urea infusion protocol.[30] Our previous studies in adult burn patients[8] and normal subjects[26] used P/I ratios of 285 and 388 minutes, respectively, such that perhaps a relative underprimed condition may have resulted in artificially lower plasma urea plateau enrichments and an overestimation of urea Ra, giving rise to the appearance of urea recycling in the previous studies. However, on the basis of the results of a study in which the effect of the priming dose on the ultimate plateau enrichment of urea was assessed, it is highly unlikely that with a P/I ratio of 300 to 400 minutes the true plateau enrichment was inaccurate by as much as 40%.[29] Regardless of the explanation for the discrepancy between current and previous results, it is unlikely that there is a methodological error in the current calculation of urea kinetics; in addition, results from the one subject studied twice under the same conditions were very reproducible. Thus, it is clear that urea N recycling does not occur to a significant extent in children with moderate burns, even when they are not given oral antibiotics. Furthermore, there seems to be essentially no benefit of very high protein intake at the whole-body level based on our observations of phenylalanine Ra and muscle protein synthesis. However, a possible beneficial effect on wound healing may justify a higher than normal protein intake, provided parameters of amino acid catabolism (plasma urea and ammonia concentration) are not markedly elevated, and suggests that additional specific organ systems (eg, gastrointestinal or immune systems) deserve further study.

ACKNOWLEDGMENT

The authors thank YongPing Chen and D.J. Doyle for excellent laboratory assistance, Allison Manning for assistance with dietary formulations, and the nursing staff of the Shriners Burns Institute in Galveston for assistance with the study protocol.

REFERENCES

1. Kinney JM: Metabolic responses to injuries, in Winters RW, Green HL (eds): Nutritional Support of the Seriously Ill Patient. New York, NY, Academic, 1983, pp 5-12

2. Jahoor F, Desai M, Herndon DN, et al: Dynamics of the protein metabolic response to burn injury. Metabolism 37:330-337, 1988

3. Shaw JH, Wildbore M, Wolfe RR: Whole body protein kinetics in severely septic patients. The response to glucose infusion and total parenteral nutrition. Ann Surg 205:288-294, 1987

4. Sakurai Y, Aarsland A, Herndon DN, et al: Stimulation of muscle protein synthesis by long-term insulin infusion in severely burned patients. Ann Surg 222:283-297, 1995

5. Gore DC, Honeycutt D, Jahoor F, et al: Effect of exogenous growth hormone on whole-body and isolated-limb protein kinetics in burned patients. Arch Surg 126:38-43, 1991

6. Alexander JW, MacMillan BC, Stinnett JD, et al: Beneficial effects of aggressive protein feeding in severely burned children. Ann Surg 192:505-517, 1980

7. Carraro F, Hartl WH, Stuart CA, et al: Whole body and plasma protein synthesis in exercise and recovery in human subjects. Am J Physiol 258:E821-E831, 1990

8. Wolfe RR, Goodenough RD, Burke JF, et al: Response of protein and urea kinetics in burn patients to different levels of protein intake. Ann Surg 197:163-171, 1983

9. Yu YM, Wagner DA, Walesreswski JC, et al: A kinetic study of leucine metabolism in severely burned patients. Comparison between a conventional and branched-chain amino acid–enriched nutritional therapy. Ann Surg 207:421-429, 1988

10. Jahoor F, Shangraw RE, Miyoshi H, et al: Role of insulin and glucose oxidation in mediating the protein catabolism of burns and sepsis. Am J Physiol 257:E323-E331, 1989

11. Tanaka N, Kubo K, Shiraki K, et al: A pilot study on protein metabolism in the Papua New Guinea highlanders. J Nutr Sci Vitaminol 26:247-259, 1980

12. Richards P, Metcalfe-Gibson A, Ward EE, et al: Utilisation of ammonia nitrogen for protein synthesis in man, and the effect of protein restriction and uremia. Lancet 2:845-849, 1967

13. Giordano C, de Pascale C, Balestrieri C, et al: Incorporation of urea ^{15}N in amino acids of patients with chronic renal failure on low nitrogen diet. Am J Clin Nutr 21:394-404, 1968

14. Patterson BW, Carraro F, Klein S, et al: Quantification of incorporation of [^{15}N]ammonia into plasma amino acids and urea. Am J Physiol 269:E508-E515, 1995

15. Danielsen M, Jackson AA: Limits of adaptation to a diet low in protein in normal man: Urea kinetics. Clin Sci 83:103-108, 1992

16. Biolo G, Fleming RYD, Wolfe RR: Physiologic hyperinsulinemia stimulates protein synthesis and enhances transport of selected amino acids in human skeletal muscle. J Clin Invest 95:811-819, 1995

17. Biolo G, Fleming RYD, Maggi SP, et al: Transmembrane transport and intracellular kinetics of amino acids in human skeletal muscle. Am J Physiol 268:E75-E84, 1995

18. Wolfe RR: Radioactive and Stable Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis. New York, NY, Wiley-Liss, 1992

19. Patterson BW, Carraro F, Wolfe RR: Measurement of ^{15}N enrichment in multiple amino acids and urea in a single analysis by gas chromatography/mass spectrometry. Biol Mass Spectrom 22:518-523, 1993

20. Patterson BW, Zhang X-J, Chen Y, et al: Measurement of very low stable isotope enrichments by gas chromatography/mass spectrometry: Application to measurement of muscle protein synthesis. Metabolism (in press)

21. Wolfe RR: Measurement of urea kinetics in vivo by means of a constant tracer infusion of di-^{15}N-urea. Am J Physiol 240:E428-E434, 1981

22. Marchini JS, Cortiella J, Hiramatsu T, et al: Requirements for indispensable amino acids in adult humans: Longer-term amino acid kinetic study with support for the adequacy of the Massachusetts Institute of Technology amino acid requirement pattern. Am J Clin Nutr 58:670-683, 1993

23. Young VR, Marchini JS, Cortiella J: Assessment of protein nutritional status. J Nutr 120:1496-1502, 1990 (suppl 11)

24. Hegsted DM: Assessment of nitrogen requirements. Am J Clin Nutr 31:1669-1677, 1978

25. Dominioni L, Trocki O, Fang CH: Enteral feeding in burn hypermetabolism: Nutritional and metabolic effects of different levels of calorie and protein intake. J PEN 9:269-279, 1985

26. Carraro F, Kimbrough TD, Wolfe RR: Urea kinetics in humans at two levels of exercise intensity. J Appl Physiol 75:1180-1185, 1993

27. Picou D, Phillips M: Urea metabolism in malnourished and recovered children receiving a high or low protein diet. Am J Clin Nutr 25:1261-1266, 1972

28. Tripathy K, Klahr S, Lotero H: Utilization of exogenous urea nitrogen in malnourished adults. Metabolism 19:253-262, 1970

29. Jahoor F, Wolfe RR: Reassessment of primed constant-infusion tracer method to measure urea kinetics. Am J Physiol 252:E557-E564, 1987

30. Matthews DE, Downey RS: Measurement of urea kinetics in humans: A validation of stable isotope tracer methods. Am J Physiol 246:E519-E527, 1984