Plasma Arginine and Leucine Kinetics and Urea Production Rates in Burn Patients

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We measured plasma arginine and leucine kinetics and rates of urea production (appearance) in 12 severely burned patients (mean body surface burn area, 48%) during a basal state (low-dose intravenous glucose) and while receiving routine, total parenteral nutrition ([TPN] fed state) including an L-amino acid mixture, supplying a generous level of nitrogen (mean, 0.36 g $N \cdot kg^{-1} \cdot d^{-1}$). The two nutritional states were studied in random order using a primed 4-hour constant intravenous tracer infusion protocol. Stable-nuclide–labeled tracers were L-[guanidino-^{13}C]arginine, L-[1-^{13}C]leucine, [^{18}O]urea, and $NaH^{13}CO_3$ (prime only), with blood and expired air samples drawn at intervals to determine isotopic abundance of arginine, citrulline, ornithine, α-ketoisocaproate ([KIC] for leucine), and urea in plasma and $^{13}CO_2$ in breath. Results are compared with data obtained in these laboratories in healthy adults. Leucine kinetics (flux and disappearance into protein synthesis) indicated the expected higher turnover in burn patients than in healthy controls. Mean leucine oxidation rates are also higher and compared well with values predicted from urea production rates, provided that urea nitrogen recycling via intestinal hydrolysis is taken into account. The plasma urea flux was also higher than for normal subjects. Arginine fluxes as measured in the systemic whole body, via the plasma pool, were correspondingly higher in burned patients than in healthy controls and were in good agreement with values predicted from leucine-KIC kinetics. However, systemic whole-body arginine flux measured via the plasma pool was only 20% of the arginine flux estimated from the urea flux plus the rate of protein synthesis. This finding is strong evidence that there is a significant synthesis of arginine (probably via recycling of ornithine) occurring in a sequestered pool (presumably in the hepatocyte) that is not in close communication with the plasma pool. Further, these data for plasma arginine flux suggest that the net rate of arginine degradation is increased in burn trauma, while there also appears to be a continued low and possibly unchanged net rate of de novo arginine synthesis contributing arginine to the plasma pool. This interpretation leads to the conclusion that there are at least two significant metabolic pools of arginine, one highly sequestered related specifically to urea synthesis and the other a systemic pool in equilibrium with the plasma. Because the hepatic urea-arginine cycle does not contribute substantially to making arginine available for protein synthesis, we propose from these findings that arginine is a conditionally essential (indispensable) amino acid in the nutrition of severely burned patients and that an exogenous arginine source is needed to maintain arginine balance.

THE PROFOUND INFLUENCE of severe burn injury and sepsis on the nitrogen economy of the burn patient is now well documented.[1-7] Nevertheless, alterations in the metabolism of specific amino acids and the interactions among them, which account for and/or determine the pattern and extent of tissue and whole-body N losses, and the consequent increase in requirements for amino acids and total nitrogen remain to be better identified and understood. The importance of this knowledge is to help establish quantitative levels of amino acid intake and the ideal proportions among amino acids that would most effectively meet the metabolic/nutritional requirements of the burn patient for a prompt and vigorous recovery and complete tissue repair. Currently, amino acid mixtures used routinely in the parenteral feeding of burn patients have been formulated largely on empirical grounds and none with particular reference to burn-related changes in substrate metabolism. Hence, it can be reasonably questioned whether commercially available formulations might be modified to enhance their capacity to support and maintain tissue/organ protein content and function in these patients. We have therefore explored the kinetics of plasma arginine metabolism, particularly in view of (1) the L-arginine–nitric oxide pathway,[8] which is of importance in the metabolic etiology of toxic shock,[9,10] and (2) the modulation by arginine of nitrogen metabolism and immune function in disease, including sepsis, trauma, and cancer.[11,12] Furthermore, it has been suggested[13] that by pharmacologically modifying the transmembrane passage of plasma arginine, it might be possible to affect the biosynthesis of nitric oxide and thereby attenuate the septic response. Hence, we report here measurements of plasma arginine kinetics and their relations to leucine kinetics. We[14] and others[15] have used the latter as an index[16] of the dynamic status of whole-body protein turnover in thermal injury; leucine kinetics and nitrogen turnover have been shown previously to be altered in burn pediatric[17,18] and adult patients.[14,15,19] Additionally, rates of urea production were determined in the present study to provide an assessment of the overall status of the N economy of our patients. Our findings suggest that the metabolism of arginine, particularly the rate of degradation, is stimulated in thermal injury, and on this basis, it is probably a conditionally indispensable amino acid in the nutrition of burned patients. These are the first studies, to our knowledge, that have been concerned with the quantitative aspects of urea metabolism in vivo, with particular reference to the urea cycle intermediate, arginine, in such a profoundly stressful

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condition. Our findings emphasize the importance of now performing investigations aimed at obtaining direct estimates of rates of arginine synthesis and degradation in burn injury, and determining how the balance between these rates might be manipulated to the clinical benefit of the patient.

SUBJECTS AND METHODS

Materials

L-[guanidino-^{13}C]arginine (99% atom percent excess [APE]) was purchased from Tracer Technologies, (Somerville, MA). L-[1-^{13}C]leucine (^{13}C, 99%) and [^{18}O]urea (^{18}O, 96%) were purchased from Cambridge Isotope Laboratory (Woburn, MA). $NaH^{13}CO_3$ (99% APE) was obtained from Prochem (Summit, NJ). The isotopically labeled tracers were made into stock solutions by the pharmacy of Massachusetts General Hospital (MGH). Before use, they were confirmed to be sterile and pyrogen-free. Total parenteral nutrition (TPN) solutions were prepared in the Nutritional Support Unit, Department of Surgery, MGH, using 11.4% Novamine (KabiVitrum, Alameda, CA) as the amino acid source. Its composition is listed in Table 1.

Burn Patients

This study was conducted in 12 severely burned patients who had been admitted to the Trauma Service at MGH. Clinical details are listed in Table 2. The patient group included seven men and five women. Average total body surface burn area was 48%. All were studied between 6 and 13 days postburn, except for subject no. 2, who was studied 28 days after being injured. Because patients are frequently still in a hypermetabolic state at this time, data for this patient have been included in the present summary. On average, 26% uncovered wound existed at the time of tracer studies. The experimental protocol was approved by the Subcommittee for Human Studies, Committee of Research, MGH. Written consent was obtained from each patient after being informed of the purpose, design, and possible hazards of the experiment.

Table 1. Composition of L-Amino Acid Mixture Used for TPN Support in Burn Patients

Amino Acid	mg/100 mL
Isoleucine	570
Leucine	790
Lysine	900
Methionine	570
Phenylalanine	790
Threonine	570
Tryptophan	190
Valine	730
Alanine	1,650
Arginine	1,120
Histidine	680
Proline	680
Serine	450
Glycine	790
Tyrosine	30
Glutamic acid	570
Aspartic acid	330

NOTE. Analysis provided by manufacturer (Novamine; KabiVitrum, Alameda, CA).

Table 2. General Characteristics of the Burn Patients

Patient No.	Order of Study	Age (yr)/ Gender	Ideal Body Weight (kg)	% Burn Body Surface Area	% Open Wound at Study
1	TPN, B	26/M	73	90	54
2	TPN, B	37/M	65	90	40
3	TPN, B	22/M	73	35	15
4	TPN, B	84/F	51	20	10
5	TPN, B	48/F	58	83	40
6	B, TPN	63/F	53	42	20
7	B, TPN	22/M	65	40	30
8	B, TPN	66/M	56	30	16
9	B, TPN	37/M	37	64	50
10	B, TPN	63/F	63	25	9
11	TPN, B	27/M	27	25	15
12	B, TPN	76/F	67	35	15
Mean ± SEM		48 ± 7/—	61 ± 3	48 ± 8	26 ± 5

Abbreviation: B, basal phase.

Experimental Design

Tracer studies were performed when patients were in a relatively stable condition, as demonstrated by vital signs including blood pressure, heart rate, cardiac function, respiration rate, oral temperature, normal liver and kidney functions, and absence of clinical signs of general infection and septicemia.

Each patient was studied twice: once during a basal phase and once during a fed phase. The order of the two phases was randomized (Table 2), and they were conducted within 1 to 2 days of each other. During the fed condition, patients received nutrients and support via TPN, which had been started at least 5 days before the tracer study. Average intakes during TPN were 0.36 g N $\cdot$ kg^{-1} $\cdot$ d^{-1}, with nonprotein calories equivalent to 31.3 ± 2.2 kcal $\cdot$ kg^{-1} $\cdot$ d^{-1} being supplied by glucose. The basal condition was created by terminating TPN approximately 10 hours before the arginine, urea, and leucine tracer studies were begun. However, these patients received an infusion of 5% dextrose (average infusion rate, 1.12 ± 0.22 mL $\cdot$ kg^{-1} $\cdot$ h^{-1}, or equivalent to 0.28 ± 0.06 kcal $\cdot$ kg^{-1} $\cdot$ h^{-1}) to prevent hypoglycemia. Immediately after completing the tracer infusion studies, TPN feedings were either resumed or replaced by enteral feeding, according to orders written by the responsible clinician.

Tracer Studies

Primed constant intravenous infusions of L-[^{13}C-guanidino]arginine, L-[1-^{13}C]leucine, and [^{18}O]urea were used to evaluate plasma fluxes of arginine, leucine, and urea. Tracer studies were started between 6 and 7 AM and lasted for 240 minutes. Before the isotope infusion was started, arterial blood and expired-air samples were taken for measurement of background isotopic levels in plasma arginine, urea, and leucine, and of the $^{13}CO_2$ level in expired air. The targeted infusion rates of labeled arginine, urea, and leucine were 0.2, 0.28, and 0.07 μmol $\cdot$ kg^{-1} $\cdot$ min^{-1}, respectively. Priming doses of the tracers were 6, 182, and 4.2 μmol $\cdot$ kg^{-1}, respectively. The blood samples (~3 mL) were taken at 20, 40, 60, 90, 120, and 150 minutes, and during the last hour seven sets of plateau blood samples were taken from the existing arterial line at 10-minute intervals. Four sets of expired-air samples at intervals of 20 minutes were also taken during the final hour of each tracer study for determination of the plateau level $^{13}CO_2$ enrichment. Isotopic abundances of L-[^{13}C-guanidino]arginine, L-[1-^{13}C]leucine, and [^{18}O]urea reached relatively steady levels within the first 180 minutes after commencing the infusions (Fig 1). Timed expired-air

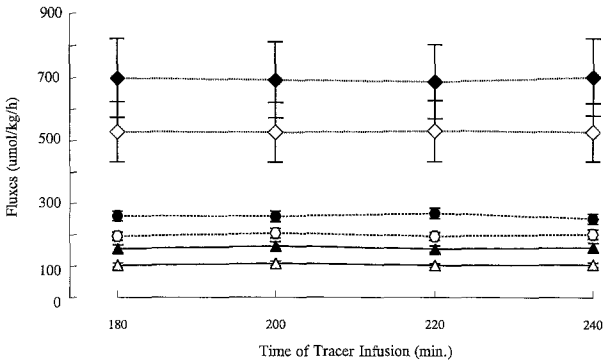

Fig 1. Metabolic fluxes (mean ± SEM, n = 12) of arginine, urea, and leucine (expressed as μmol/kg/h) measured at different time points of the isotopic plateau, basal, and TPN states. (△) Arg basal; (○) Leu basal; (◇) urea basal; (▲) Arg TPN; (●) Leu TPN; (◆) urea TPN.

samples were collected for determining total oxygen consumption as described previously.[14]

Calculation of Amino Acid and Urea Kinetics

With the aid of the L-[^{13}C-guanidino]arginine, [^{18}O]urea, and L-[1-^{13}C]leucine tracers, metabolic fluxes of arginine, urea, and leucine in the plasma pool of these burn patients were measured using steady-state dilution equations.[20] Briefly, therefore, the metabolic fluxes (micromoles per kilogram per hour) for arginine, leucine, and urea (Q) were calculated from the equation Q = i([Ei/Ep] − 1), where Ei is the isotopic abundance of the tracer infusate and Ep is the plateau enrichment of the tracer in plasma. In the case of L-[1-^{13}C]leucine, the plasma enrichment of ^{13}C-α-ketoisocaproate (KIC) was used as the surrogate for intracellular L-[^{13}C]leucine enrichment.[16,21] Rates of leucine oxidation, leucine incorporation into proteins, and leucine release from proteolysis were calculated as described previously.[14] We have assumed that the average leucine content (by weight) in whole-body proteins of adult humans is 8%, or 620 μmol/g protein.[22,23]

To compare the estimate of leucine and arginine fluxes during the basal state, we have assumed that the arginine content in whole-body mixed proteins is 362 μmol/g protein,[23] or that the molar ratio of arginine to leucine in proteins is 0.57.

Analytical Methods

Plasma free amino acid levels and infusate arginine and leucine concentrations were measured by an automated high-performance liquid chromatograph (Beckman System, Gold Model 126, with Model 506A Autosampler; Beckman Instruments, Fullerton, CA) using a postcolumn derivatization reaction with ninhydrin (Trione, Beckman) and quantitation with the aid of a Programmable Detector Model 168 (Beckman Instruments). Isotopic enrichment of L-[^{13}C-guanidino]arginine was measured using 200 μL plasma. We extracted the amino acids and prepared the methyl ester trifluoroacetyl derivative using a procedure comparable to the one reported by Nissim et al,[24] which was analyzed using on-column injection with a HP5980 series II gas chromatograph coupled to a HP59988A mass spectrometer (Hewlett-Packard, Palo Alto, CA). Selective ion monitoring of arginine was conducted on the [M-20]$^-$ ion, using negative chemical ionization with methane as the reagent gas. This ion corresponds to a loss of HF from the molecular ion. Selective ion monitoring was conducted at *m/z* 456, *m/z 457* for natural, [^{13}C-guanidino]-labeled arginine. Measurement of plasma L-[^{13}C]KIC was performed using the method described previously.[25] The natural KIC and ^{13}C-KIC were monitored using electron-impact mass spectrometry monitoring mass ratios of *m/z* 259 and 260, respectively. Plasma urea isotope abundance was determined on the tetra-butyldimethylsilyl (t-BDMS) derivative,[26] using the electron-impact mode. Selective ion monitoring was performed at *m/z* 231 [M-57] for natural urea and at *m/z* 233 for [^{18}O]urea (M + 2).

Evaluation of Data

Statistical evaluation of the data was conducted using a SOLO Software package (BMDP Statistical Software, Los Angeles, CA). All data were examined for normal distribution, and then either paired or unpaired Student's *t* tests were used, as appropriate.

Values obtained in earlier investigations with healthy subjects[27,28] for plasma guanidino-arginine fluxes have been used here to help further evaluate the status of arginine and leucine metabolism in these burn patients. These control values were obtained using similar but not identical experimental conditions, and in the earlier case, plasma arginine fluxes were measured using either L-[^{13}C-guanidino]arginine or L-[$^{15}N_2$-guanidino,5,5, $^2H_2$]arginine. Our studies have indicated that these two tracers produce comparable estimates of plasma arginine fluxes when they are given via intravenous infusion.[28]

RESULTS AND DISCUSSION

Isotopic abundances of plasma arginine, (^{13}C-KIC), and urea, together with the production and ^{13}C-enrichment of expired carbon dioxide, are listed in Table 3. The values shown in Fig 1 are presented to indicate how enrichments of tracers varied during the fasted and fed phases of the tracer protocols. From these isotopic data, the parameters of leucine, arginine, and urea metabolism were determined.

Values for leucine kinetics are listed in Table 4. The mean plasma leucine flux during the basal condition was 199 μmol · kg^{-1} · h^{-1}, which is higher than values usually reported for well-nourished, healthy adults studied in the postabsorptive state.[29,30] In a recent study with eight healthy young adults,[27] the mean leucine-KIC flux for the postabsorptive state was 114 μmol · kg^{-1} · h^{-1}—significantly ($P < .001$) lower than the value found here with burn patients. This finding supports earlier studies showing a higher rate of whole-body protein turnover in trauma patients[5,14,15,17-19]; from this, we[7,31] would predict higher rates of oxidative losses for the nutritionally indispensable and conditionally indispensable amino acids. Accordingly, the mean rate of leucine oxidation was found to be 35 μmol · kg^{-1} · h^{-1} (Table 4), and this compares with rates of approximately 20 μmol · kg^{-1} · h^{-1} or less for nonstressed, postabsorptive healthy adult individuals.[26,29,30] Thus, assuming that whole-body mixed proteins contain 8% leucine,[22] the rate of leucine oxidation in basal burn patients is equivalent to a body protein (N · 6.25) loss of 9.2 mg N · kg^{-1} · h^{-1}, or 220 mg N · kg^{-1} · d^{-1}. This is higher than the rate of body protein oxidation in postabsorptive healthy subjects,[32] implying greater obligatory amino acid losses[31,33,34] and, in consequence, a greater dietary amino acid requirement in burned patients as compared with normal adults.

The higher basal rate of leucine oxidation in burned patients noted earlier, is paralleled by a higher mean urea production rate (Table 5), with the value being 526 μmol · kg^{-1} · h^{-1}. This rate is similar to that reported earlier from

Table 3. Isotopic Abundances in Various Metabolites During Plateau Periods of Basal and TPN Phases, and Carbon Dioxide Production Rate in Each Isotope Tracer Study

	L-[guanidino-^{13}C]arginine*		[^{18}O]urea*		[^{13}C]KIX*		$^{13}CO_2$ (APE × 10^3)		Total CO_2 Production (μ mol · kg^{-1} · min^{-1})	
Patient No.	Basal	TPN	Basal	TPN	Basal	TPN	Basal	TPN	Basal	TPN
1	7.50	5.69	2.46	1.74	1.87	1.48	5.0	4.9	182.8	227.9
2	7.19	5.43	1.83	1.52	1.32	1.33	3.9	4.2	253.6	277.9
3	8.16	5.84	4.81	3.93	2.63	1.92	4.7	4.7	298.2	222.6
4	12.0	7.67	4.24	3.13	2.48	2.10	2.7	4.7	342.5	309.5
5	11.0	6.00	3.64	2.71	1.86	1.46	2.9	3.9	210.1	235.9
6	10.1	6.39	3.07	3.25	1.79	1.57	6.3	5.9	140.0	198.5
7	11.5	5.74	4.78	3.29	1.90	1.48	2.2	5.0	339.7	291.6
8	9.57	5.78	2.32	1.52	1.51	1.46	4.7	5.5	273.7	220.9
9	9.20	6.41	2.00	1.68	1.92	1.34	5.6	4.5	206.5	247.1
10	9.42	6.27	1.97	1.78	2.16	2.03	5.3	6.4	158.3	180.4
11	8.42	4.66	6.15	3.31	2.30	2.17	4.0	6.5	178.9	183.8
12	9.37	7.05	1.39	1.17	2.05	1.99	4.6	6.5	146.4	140.1
Mean	9.45	6.08	3.22	2.41	1.98	1.69	4.3	5.2	227.6	228.0
SEM	0.48	0.39	0.47	0.44	0.12	0.10	0.4	0.3	20.9	14.2

NOTE. Each individual plasma value is the mean of 4 blood samples taken between 180 and 240 minutes of the tracer infusion protocol. $^{13}CO_2$ is a mean value for 4 breath samples taken at 180 and 240 minutes.

*Mol fraction % above baseline.

this laboratory for severely burned adult patients.[15] Rates of urea production in postabsorptive, healthy adults are generally in the range of 200 to 300 μmol · kg^{-1} · h^{-1}.[35,36] However, there was considerable variation in urea production rates among the 12 burn patients, with some showing a relatively normal value. This may be related, in part, to the different phases of recovery at which individual patients were studied, since Jahoor et al[18] found for burned children that urea production rates during the flow (catabolic) phase were approximately double those during the later convalescent period.

The mean urea production rate for the present group of patients amounts to a urea N appearance rate of approximately 14.7 mg N · kg^{-1} · h^{-1}. This exceeds the 9.2-mg · kg^{-1} · h^{-1} rate of loss of nitrogen as predicted from the leucine oxidation data. However, the rate of urea production exceeds that of urea excretion, due to urea hydrolysis and nitrogen recycling within the intestinal tract.[37,38] Therefore it is not surprising that there is a difference between the rate of total urea N appearance and the leucine-based estimate of the rate of urea N loss from the body. Indeed, assuming that urea N hydrolysis within the intestinal tract is approximately 25% of the total urea N appearance rate[37,38] and that the major proportion of N released from urea in the intestinal tract is recycled directly back into the urea pool,[39] this would bring the urea N kinetic data into good agreement with the leucine oxidation–N loss estimates. This therefore gives us further confidence that these stable-isotope tracer probes provide a reasonable picture of the quantitative status of amino acid and nitrogen metabolism in burn patients.

With parenteral feeding, there was a stimulation in the rate of nonoxidative disposal of leucine, which we take to be a measure of the response of whole-body protein synthesis

Table 4. Leucine Kinetics in Burned Patients During the Basal and TPN Phases

Parameter	Basal	TPN
L-[1-^{13}C]leucine infusion rate	3.9 ± 0.2	4.4 ± 0.2
Leucine		
Flux	199 ± 15	259 ± 17†
Intake	0	56 ± 5
Oxidation	35 ± 4	51 ± 4†
For protein synthesis (S)	165 ± 13	208 ± 15*
From protein breakdown (B)	199 ± 15	203 ± 16^{NS}
Balance (S − B)	−35 ± 4	5 ± 5†
Protein		
Synthesis (S_p)‡	6.6 ± 0.5	8.3 ± 0.6*
Breakdown (B_p)‡	8.0 ± 0.6	8.1 ± 0.7^{NS}
Balance ($S_p - B_p$)‡	−1.4 ± 0.2	0.2 ± 0.2†

NOTE. Data are presented as mean ± SEM and are expressed as μmol · kg^{-1} · h^{-1} except where specified.

*$P < .02$, †$P < .01$: v basal.

‡g · kg^{-1} · d^{-1}.

NS, not significantly different ($P > .05$) from basal.

Table 5. Urea and Arginine Kinetics and Flux Ratios in Burn Patients Studied During Basal and TPN Phases

	Basal	TPN
Urea kinetics		
^{18}O-urea infusion rate	73.8 ± 6.8	73.1 ± 6.6
Urea flux	526.1 ± 102.4	690.5 ± 117.2*
Arginine kinetics		
L-[guanidino]arginine infusion rate	10.8 ± 0.4	10.3 ± 0.5
Arginine-guanidino flux	104.5 ± 7.0	158.3 ± 13.3*
Arginine intake	0	49.8 ± 7.3
Flux ratios		
Arginine flux/leucine flux†	0.56 ± 0.07	0.54 ± 0.04
Arginine flux/urea flux	0.28 ± 0.05	0.33 ± 0.07

NOTE. Results are the mean ± SEM. Values are μmol · kg^{-1} · h^{-1} except for flux ratios.

*Significantly different ($P < .01$) from basal.

†Corrected for leucine and arginine intakes during TPN.

(Table 4). This stimulation following parenteral administration of the amino acid mixture is in accordance with data showing increased rates of muscle,[40] splanchnic,[41] and whole-body[42] protein synthesis when amino acids are given by vein. Also of possible additional interest is our finding that the whole-body rate of endogenous protein breakdown was not reduced with parenteral administration of amino acids. This agrees with a recent report by Goulet et al[42] for 8- to 16-year-old patients given each of three intake levels of amino acids via intravenous feedings. In contrast, with oral ingestion, the rate of whole-body proteolysis has been reported to decrease significantly,[29,30] but with variable findings for the effects of amino acid–containing meals on the rate of whole-body protein synthesis.[29] This raises the possibility that there may be major differences in the responses of whole-body protein synthesis and breakdown to an oral versus intravenous administration of amino acids, at least in burn patients. Although this requires additional research, our recent studies in an experimental animal model indicate differences in the processing and metabolic fate of leucine within the splanchnic region when amino acids are given by the enteral versus parenteral route.[43] Also, we have discussed the possibility that the intravenous leucine tracer model, as used here and widely applied by others,[5,16] may not permit an accurate picture of the acute response of body protein synthesis and breakdown rates to amino acid ingestion via the oral/intestinal routes, unless due regard is given to the first-pass uptake and disappearance of amino acids within the splanchnic region.

There was an increased rate of leucine oxidation with administration of the parenteral amino acid mixture (Table 4), and this is to be anticipated in view of the higher plasma (and presumably tissue) leucine concentration that occurred with the parenteral feeding[26] (Table 6). The mean increase in oxidation was equivalent to 47% of the rate during fasting, and it was only slightly less than the level of leucine intake provided by TPN. Furthermore, this increase in leucine oxidation compares with a mean increase of 31% in the rate of urea production with feeding (Table 5). Hence, changes in leucine oxidation and urea production were in the same direction, but again there appears to be some discrepancy between the quantitative responses of leucine and urea metabolism to parenteral feeding. However, if the futile recycling of urea nitrogen[39] is taken to be approximately 25% of the total rate of urea production, as assumed earlier, there is again concordance between the leucine oxidation and urea output rates. For example, using the earlier assumptions, a mean leucine oxidation rate of 51.3 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ amounts to a protein loss of 13.4 mg $N \cdot kg^{-1} \cdot h^{-1}$, and the urea excretion rate (ie, 0.75 × urea production) would be predicted to be 14.5 mg $N \cdot kg^{-1} \cdot h^{-1}$. We have therefore used these kinetic data to help further evaluate the metabolic and nutritional significance of the arginine flux findings, as follows.

Table 6. Plasma Concentrations of Amino Acids During Basal and TPN Phases in Burn Patients

Amino Acid	Basal	TPN
Aspartic acid	14 ± 4	18 ± 4
Threonine	44 ± 12	59 ± 14
Proline	52 ± 22	99 ± 34
Glycine	149 ± 10	230 ± 14
Alanine	234 ± 31	427 ± 87
Citrulline	9.6 ± 1.2	12.7 ± 1.9
Valine	177 ± 17	275 ± 21
Methionine	28 ± 4	61 ± 5
Isoleucine	41 ± 9	59 ± 14
Leucine	107 ± 8	128 ± 9
Phenylalanine	105 ± 10	170 ± 10
Ornithine	75 ± 21	103 ± 24
Arginine	95 ± 15	131 ± 17

NOTE. Values are μmol/L (mean ± SE, n = 12). Fasting and TPN values are averages of baseline and final blood sample during infusion.

However, before turning to the results for arginine kinetics, it might be pointed out that the concentration of plasma free threonine for the basal state in these patients was 44 μmol/L, increasing to 59 μmol/L with parenteral feeding (Table 6). These levels are considerably lower than values in healthy, postabsorptive adults[44-46] and values reported by some investigators[47] for multiple trauma victims. Our finding is in agreement with those reported by Cynober et al[48,49] in burn patients, Jeevanandam et al[50] in multiple-trauma victims, and Vente et al[51] in patients with sepsis, and implies an increased catabolism of threonine and/or an inadequate/marginal intake of the amino acid.[44] Also, citrulline and arginine concentrations are lower than we[52] and others[45] have seen in healthy adults, possibly reflecting an increased loss of arginine and a reduced supply of its precursor, ornithine, and the availability of citrulline for subsequent arginine synthesis[52]; the level of proline was also less than that reported for healthy adults.[45] A reduced concentration of proline has been previously observed in burn patients.[48]

The mean plasma [^{13}C-guanidino]arginine flux was 104.5 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ for the basal condition, increasing to 158.3 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ during parenteral administration of the L-amino acid mixture (Table 5). The increase with feeding (50 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) was equivalent to the arginine supplied by the amino acid mixture (Table 5), and so the endogenous arginine flux for the fed state was 108.5 $\mu mol \cdot kg^{-1} \cdot h^{-1}$. This value is virtually identical to that for the fasting condition, and both values are significantly ($P < .001$) higher than the mean value of 69 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ that we[27] reported recently for healthy postabsorptive adults. Because the leucine-derived estimate of whole-body protein breakdown was unchanged with the parenteral feeding (Table 4), these arginine flux values indicate that there was no difference or change in the rate of de novo arginine synthesis between the fasting and fed states. It can be assumed further that the rate of appearance of arginine in plasma originating via body protein breakdown would occur in relation to the rate of leucine appearance, when adjusted for the molar concentration ratio of arginine to leucine in mixed body proteins. The measured mean ratio of the arginine to leucine flux in the fasted state was 0.56 (Table 5), and when corrected for intakes of arginine and leucine, during parenteral feeding it was 0.54. The predicted ratio of these fluxes, based on the amino acid composition of mixed body proteins, is 0.57. Hence, these

relatively close agreements further indicate that the net rate of arginine de novo synthesis, presumably taking place largely via conversion of citrulline to arginine in the kidney,[53] is likely to be low. If this was not the case, the plasma arginine to leucine flux ratio should be higher than we have found, and there would not be as good a match between the flux ratio and the arginine-leucine concentration ratio in mixed body proteins. A significant mismatch is clearly the case for glutamine, glycine, and alanine, since there is a quantitatively significant rate of endogenous synthesis of each of these amino acids.[54] It would be highly desirable now to attempt to estimate directly the de novo rate of arginine synthesis in these patients, possibly using combinations of simultaneously infused citrulline and arginine stable-isotope–labeled tracers.[52]

The relatively close result between the value for measured plasma arginine flux and the value predicted from the leucine flux (protein turnover) data further emphasizes the tight compartmentation of arginine metabolism in vovo. Thus, plasma [^{13}C-guanidino]arginine flux was considerably less than the rate of urea production, indicating that the plasma arginine pool was not in extensive communication with the arginine assembled via the hepatic urea cycle. We have pointed out this distinction between plasma arginine and hepatic urea cycle arginine pools previously.[27,28,52] It is now important to determine whether this compartmentation is in any way compromised as a consequence of burn injury.

The foregoing results lead to the suggestion that the higher turnover of body proteins (Table 4) and associated catabolic losses of indispensable amino acids, such as leucine (Table 4), are accompanied by higher rates of arginine degradation, probably without any simultaneous and compensatory increases in the de novo rate of net arginine synthesis. Hence, body arginine balance could only be achieved by increasing the exogenous supply of arginine to balance the increased loss of arginine. In this case, the dietary content of arginine becomes important and is then termed a conditionally indispensable amino acid[55-57] in reference to the nutritional support of burn trauma victims. This hypothesis might be tested by modifying the arginine content, and possibly also that of proline, which can be a precursor of arginine via its close metabolic association with ornithine,[58] of parenteral L-amino acid mixtures with evaluation of the effect of these changes on the dynamics of body arginine/amino acid/nitrogen metabolism and balance. The present findings may also be relevant to the mild orotic aciduria that has been found in trauma patients with multiple fractures and extensive soft-tissue damage.[59] Arginine deficiency in animals causes increased orotic acid excretion,[60] although human subjects may differ from other species to some extent in that a diet devoid of arginine given for 5 days to well-nourished, nonstressed adult humans was not found to cause an orotic aciduria.[61]

In conclusion, we have studied whole-body kinetic parameters of leucine, arginine, and urea metabolism in burn patients during a basal state and during the parenterally nourished state, and compared the findings with published data for healthy controls. Arginine kinetics show increases following thermal injury as compared with values reported for healthy adults and parallel the changes seen in leucine kinetics and urea metabolism. From the observed metabolic relationships, we interpret these findings to suggest that the rate of de novo arginine synthesis is probably low and unaffected by burn injury, whereas arginine loss via catabolism is increased, leading to its conditional indispensability in the nutrition of burn individuals. This hypothesis now should be explored, especially because arginine supplementation and/or administration might be of clinical benefit in these and/or other highly catabolic patients.[62,63] On the other hand, supplementation actually may be contraindicated in cancer patients[12,64] and during septic shock.[65] Further, arginine restriction may be a useful therapeutic strategy, since increased production of NO, for example, can lead to a suppression of hepatic drug metabolism[66] and cause hepatocellular dysfunction.[67] The uptake of arginine by the hepatocyte is restricted under normal circumstances but stimulated in stress via the cytokine, tumor necrosis factor.[68] Given these various clinically relevant issues, the physiology and quantitative aspects of arginine metabolism in vivo now deserve further intensive investigation directly on human subjects.

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