Arginine and ornithine kinetics in severely burned patients: increased rate of arginine disposal

YONG-MING YU, COLLEEN M. RYAN, LETICIA CASTILLO, XIAO-MING LU, LOUIS BEAUMIER, RONALD G. TOMPKINS, AND VERNON R. YOUNG

Shriners Burns Hospital and Trauma Service, Massachusetts General Hospital, Boston 02114; and Laboratory of Human Nutrition and Clinical Research Center, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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IN EARLIER STUDIES in severely burned patients, we have used arginine and citrulline tracers to explore the dynamic, metabolic interrelationships between these amino acids (39–41). We observed that the net rate of de novo arginine synthesis was not increased in burn patients receiving parenteral nutrition compared with the rate found in healthy adults. However, there was an increased plasma arginine flux that paralleled the higher leucine fluxes and increased rates of leucine oxidation, also when compared with estimates for healthy subjects. On this basis, we have proposed that there are relatively higher rates of arginine loss after burn injury and that an exogenous intake of preformed arginine would be needed to balance this putative increased rate of arginine oxidation and to maintain body arginine homeostasis. To further evaluate this hypothesis, we have now determined the rate of conversion of arginine to ornithine and the rate of ornithine oxidation in a group of severely burned patients. Compared with data obtained in our laboratories in healthy adults, it is concluded that net arginine loss is increased and that a parenteral supply of arginine would be necessary to maintain optimum amino acid balance and homeostasis in burn patients who are fed by this route.

MATERIALS AND METHODS

Materials. L-[15N2-guanidino-5,5-2H2]arginine [99% atom percent excess (APE)], L-[5,5-2H2]proline, and L-[5,13C]ornithine (>98% 13C) were purchased from MassTrace (Woburn, MA). L-[5,5,5-2H3]leucine (2H 98%) was acquired from Cambridge Isotope Laboratories (Woburn, MA); NaH13CO3 (99% APE) was obtained from Prochem (Summit, NJ). The isotopically labeled tracers were used to make stock solutions at the Pharmacy of the Massachusetts General Hospital (MGH). Before use, they were confirmed to be sterile and pyrogen free. The total parenteral nutrition (TPN) solutions were prepared in the Nutritional Support Unit, Department of Surgery, MGH. Novamine (KabiVitrum, Alameda, CA) 11.4% was used as the amino acid source; its composition is shown in Table 1.

Burn patients. The study included nine severely burned adults (5 males, 4 females). The general condition of the patients is shown in Table 2. The age (mean ± SE) was 51 ± 6 yr (range 32–85 yr). On the admission physical examination, percent total body surface area burned was 61 ± 6% (SE) (range 35–90%). Smoke inhalation injury, as diagnosed by admission bronchoscopy, was present in eight of nine patients (89%). Mortality was 33%, consistent with the predicted mortality for this group with severe burns and smoke inhalation (30). All patients were treated with standard burn resuscitation and critical care measures, including serial excision and grafting procedures beginning early in the hospital course (mean of 2 days, range 1–4 days after admission) (31). Enteral feedings were administered as early as possible;

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Table 1. Composition of L-amino acid mixture used for TPN support in burn patients

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoleucine</td>
<td>570</td>
</tr>
<tr>
<td>Leucine</td>
<td>790</td>
</tr>
<tr>
<td>Lyssine</td>
<td>900</td>
</tr>
<tr>
<td>Methionine</td>
<td>570</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>790</td>
</tr>
<tr>
<td>Threonine</td>
<td>570</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>190</td>
</tr>
<tr>
<td>Valine</td>
<td>730</td>
</tr>
<tr>
<td>Alanine</td>
<td>1,650</td>
</tr>
<tr>
<td>Arginine</td>
<td>1,120</td>
</tr>
<tr>
<td>Histidine</td>
<td>680</td>
</tr>
<tr>
<td>Proline</td>
<td>680</td>
</tr>
<tr>
<td>Serine</td>
<td>450</td>
</tr>
<tr>
<td>Glycine</td>
<td>780</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>30</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>570</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>330</td>
</tr>
</tbody>
</table>

TPN, total parenteral nutrition. The amino acids isoleucine through valine are the conventionally defined “essential amino acids.” Note: analysis was provided by manufacturer (Novamine; KabiVitrum, Alameda, CA).

However, TPN was administered when tube feedings were poorly tolerated.

The experimental protocol was approved by the Subcommitte for Human Studies, Committee of Research, MGH and the Partners Health Care System. Written consent was obtained, either from the patient after being informed of the purpose, design, and possible hazards of the experiment or, alternatively, from the family members.

The patients were studied from 7 to 25 days after the burn, with two of the studies at 25 days after injury, when patients had ~23% of unhealed wound. Because patients are frequently still hypermetabolic many weeks after the initial injury, we have included these two patients in our database. For the present group of patients, energy expenditure, measured via indirect calorimetry and based on O2 consumption (see Tracer studies), was equivalent to 38 ± 2 (SE) kcal·kg⁻¹·day⁻¹ for the “basal” state and 42 ± 4 kcal·kg⁻¹·day⁻¹ for the TPN phase. The general condition of this group of patients is comparable to that of the burned patients studied earlier (39, 41).

Experimental design. Tracer studies were performed when the patients were in a relatively stable condition, as assessed by blood pressure, heart rate and cardiac function, respiration rate, body temperature, and liver and kidney functions.

As in previous studies (39–41), each patient was studied twice: first during a basal or “fast” phase, when a low glucose infusion rate was maintained to prevent hypoglycemia, and later while they were in a “fed” (TPN) phase. The order of the two phases was randomized (Table 2) and conducted within 1 or 2 days of each other. During the TPN condition, patients received nutritional support that had begun ≥2 days before the tracer study. Average intakes were 0.36 ± 0.02 g N·kg⁻¹·day⁻¹, with nonprotein calories equivalent to 32 ± 1 kcal·kg⁻¹·day⁻¹ being supplied by the glucose. The basal condition was created by terminating the TPN about 10 h before the labeled tracer studies were begun. It was not possible to completely fast these very sick patients, and because it was necessary to prevent hypoglycemia, they received an infusion of 5% dextrose at the average rate of glucose intake of 0.06 ± 0.01 g·kg⁻¹·h⁻¹ during this basal state. After the tracer infusions were completed, TPN feedings were either resumed or replaced by enteral feeding in accordance with orders written by the attending clinicians.

Tracer studies. Primed constant intravenous infusions of L-[¹⁵N₂-guanidino-5,5-²H₂]arginine (M+4 arginine), L-[⁵-¹³C]ornithine (M+1 ornithine), [⁵,⁵-²H₂]proline (M+2 proline), and L-[⁵,⁵,⁵-²H₃]leucine (M+3 leucine) were used to determine the plasma kinetics of arginine, ornithine, proline, and leucine, respectively. The tracer studies were generally started between 0600 and 0700 and lasted for 330 min. In our previous studies on citrulline and arginine kinetics (39), we observed a slight increment in the enrichment of M+1 ornithine (~0.5 APE) after the infusion of L-[¹⁵N₂-guanidino-5,5-²H₂]arginine (M+4) at the rate of 0.2 μmol·kg⁻¹·min⁻¹; a plateau was reached between 90 and 120 min after the start of the tracer infusion. Therefore, in the present study, for a more accurate estimate of the plasma [⁵-¹³C]ornithine enrichment arising exclusively from the infusion of [⁵-¹³C]ornithine (M+1) tracer, the L-[¹⁵N₂-guanidino-5,5-²H₂]arginine (M+4) and [⁵,⁵-²H₂]proline (M+2) tracers were infused for a total of 330 min, and then the primed constant infusion of L-[⁵-¹³C]ornithine and L-[⁵,⁵,⁵-²H₃]leucine was given during the last 180 min of this 5.5-h period. Blood samples were taken at 120 and 150 min before the administration of L-[⁵-¹³C]ornithine. They served to estimate the baseline enrichments of L-[⁵-¹³C]ornithine infused between 150 and 330 min. Before the isotope infusion was started, arterial blood and expired air samples were taken for measurements of background isotopic levels in plasma arginine, ornithine, proline, and α-ketoisocaproate, which serves as a surrogate of intracellular leucine enrichment (24), and of the ¹³CO₂ in expired air. Blood samples (~3 ml) were taken at 120, 150, 285, 300, 315, and 330 min after the commencement of the tracer infusion. Four sets of expired air samples were also taken at intervals of 15 min between 285 and 330 min, concomitant with the time points for blood sampling, for determination of the isotopic steady-state level of ¹³CO₂ enrichment in the expired air. Timed expired air samples also were collected for determination of total O₂ consumption and CO₂ production, as described previously (39, 41). The targeted, but known, infusion rates of labeled arginine, ornithine, proline, and leucine were, respectively, 0.15, 0.07, 0.09, and 0.07 μmol·kg⁻¹·min⁻¹. Priming doses of these tracer
Analytic methods. Samples for isotopic abundance of arginine, ornithine, proline, and α-ketoisocaproatate were measured in duplicate and determined against calibration standards. The methods have been described in detail, previously (10, 39, 41). In brief, for determination of the isotopic enrichment of arginine, ornithine, and citrulline, 200 μL of plasma were passed through an ion exchange resin (H+ form, AG 50W, 100–200 mesh, Bio-Rad, Hercules, CA), and a methyl ester trifluoroacetyl derivative was formed, first by esterification with acetyl chloride and methanol and then by acylation with trifluoroacetic acid and dichloroethane. Analysis of the derivatives was performed with the aid of an HP 5890 series II gas chromatograph, using on-column injection, coupled to an HP 5988 A mass spectrometer (Hewlett-Packard). We used negative ion chemical ionization, with methane as reagent gas. Ornithine, citrulline, and arginine eluted at 5.2, 7.2, and 7.7 min, respectively. Selective ion monitoring (SIM) was carried out at m/z 456 (M – 20), m/z 457, m/z 458, and m/z 460 for natural arginine (M + 0), [5-15C]- or [5-2H]ornithine (M + 1), and [5,5-2H2]ornithine (M + 2), respectively. For the arginine isotopologs, SIM was carried out at m/z 338, m/z 339, and m/z 340 for natural ornithine (M + 0), [15C]- or [2H]ornithine (M + 1), and [5,5,5-2H3]ornithine (M + 2), respectively. The ion clusters of all tracers determined in the negative ion chemical ionization mode overlapped with the labeled products of the other tracers. Thus a multiple regression approach was used to calculate the isotopic abundance of the amino acids from the mass spectrometry data, as we have described in detail for isotopologs of tyrosine and citrulline (37).

Plasma proline enrichment was measured using a heptafluorobutyric acid derivative and was monitored at nominal mass m/z ratios of 335/333 and 334/333 for [5,5-2H2]proline (M + 2) and [5-2H]proline (M + 1).

Measurement of plasma α-[5,5,5-2H3]ketoisocaproatate (KIC) was conducted as previously described for analysis of [1-13C]KIC (30, 32, 33). The natural KIC and [5,5,5-2H3]KIC were monitored, using electron impact mass spectrometry, at m/z 259 and m/z 262, respectively.

Plasma free amino acid levels and concentrations of arginine, citrulline, proline, and leucine in the infusates were measured by an automated high-performance liquid chromatograph (Beckman System Gold model 126, with model 506A Autosampler, controlled by System Gold Chromatography Software, Beckman Instrument, San Ramon, CA), using a post column derivatization reaction with ninhydrin (Trizone) and quantitation with the aid of a programmable detector model 168.

Estimation of amino acid kinetics. The metabolic flux (Q) of arginine, ornithine, proline, and leucine was estimated using steady-state dilution equations (38). Briefly, the metabolic fluxes (represented by Q, in μmol·kg⁻¹·h⁻¹) for arginine, ornithine, proline, or leucine were calculated from the equation

\[ Q = \frac{I}{E_i} \left( \frac{E_i}{E_p} - 1 \right) \]  

where I is the rate of tracer infusion, Ei is the isotopic abundance of the tracer infused, and Ep is the plateau isotopic abundance of the tracer in plasma. In the case of leucine, the plasma enrichment of α-[5,5,5-2H3]KIC was used as the index of the intracellular L-[5,5,5-2H3]leucine enrichment (24).

The rate of ornithine oxidation (COra) was calculated as follows

\[ CO_{ra} = V_{13CO2} \cdot \left( \frac{1}{E_p} - \frac{1}{E_i} \right)/k \]

where \( V_{13CO2} \) is the rate of total 13CO2 expiration at isotopic steady state; \( E_i \) and \( E_p \) are the enrichments of [5-15C]ornithine in the infused and plasma at plateau, respectively; and k is the value used to correct the fraction of 13CO2 retention. This factor was estimated as follows. Six healthy young adults (4 males and 2 females), with mean (±SE) age 22 ± 1 yr and weight 73.9 ± 5.2 kg, who received an adequate L-amino acid diet (6), were admitted to the Clinical Research Center (CRC) of the Massachusetts Institute of Technology (MIT) at ~1500. At 1600, a constant intravenous infusion of L-[5-15C]glutamate (0.93 μmol·kg⁻¹·h⁻¹) was started that proceeded for 24 h. The percentages (means ± SE) of infused 15C recovered in expired air during the fast state (lasting from 270 to 380 min) and fed state (lasting from 990 to 1,050 min) were 40.2 ± 1.8 and 52.3 ± 0.6%, respectively. We have used these two correction factors to estimate the oxidation rate of ornithine, following the same rationale as estimating
the total enrichment of [5,5-2H2]arginine, the latter of which
expression
\[ Q = \frac{Q_{\text{Arg}}}{Q_{\text{Orn}}} \]
following equation
\[ \text{mol} \]
\[ z \]
arginine (\( z \text{guanidino-5,5-2H2} \)arginine. \( E_{2H2\text{Orn}} \) and \( E_{2H2\text{Arg}} \) are the respective plasma isotope abundance of [5,5-2H2]ornithine and
primed, constant infusions of [5-13C]ornithine and [15N2-guanidino-5,5-2H2]arginine, essentially according to the phenylalanine/tyrosine tracer models of Clarke and Bier (13) and Thompson et al. (36). Thus
\[ Q_{\text{Arg-Orn}} = \frac{E_{2H2\text{Orn}}}{E_{2H2\text{Arg}}} \frac{Q_{\text{Arg}}}{Q_{\text{Orn}}} \]
where \( Q_{\text{Orn}} \) and \( Q_{\text{Arg}} \) are the plasma fluxes of ornithine and arginine (\( \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \)), respectively, estimated from the primed, constant infusions of [5,5,5-2H3]KIC and [15N2-guanidino-5,5,5,5-2H4]arginine. \( E_{2H2\text{Orn}} \) and \( E_{2H2\text{Arg}} \) are the respective plasma isotopic abundance of [5,5-2H2]ornithine and the total enrichment of [5,5-2H2]arginine, the latter of which is the sum of plateau level plasma enrichments of [15N2-guanidino-5,5-2H2]arginine and [5,5-2H2]arginine. The expression \( Q_{\text{Arg-Orn}} \) corrects for the contribution of the arginine tracer infusion to arginine flux (36, 39).

The rate of arginine oxidation (\( C_{\text{Arg}} \)) was calculated by the following equation
\[ C_{\text{Arg}} = Q_{\text{Arg-Orn}} \frac{C_{\text{Orn}}}{Q_{\text{Orn}}} \]
where \( C_{\text{Orn}/Q_{\text{Orn}}} \) is the fraction of plasma ornithine oxidized.

It should be noted that the estimates of arginine and ornithine kinetics were based on use of plasma isotopic enrichments as a surrogate of the precursor enrichments in the pools other than the site of the intrahepatic urea cycle. Thus the validity of our interpretations and conclusions depends on this premise. We do offer some support for this in the discussion, particularly with respect to the estimates of arginine oxidation.

Evaluation of data. Statistical evaluation of the data was done using PROSTAT software (Poly Software International, Sandy, UT). All data were examined for normality of distribution and then for possible significant covariates, including age and open burn area. There were none. We then carried out paired \( t \)-tests to compare the metabolic measurements between the basal and the TPN states in burn patients. Values obtained from our recent investigations with healthy adults for plasma amino acid fluxes and ornithine oxidation were used here to help further evaluate the status of amino acid metabolism in these burn patients, compared by unpaired \( t \)-test.

RESULTS

Stable isotope abundance. The isotopic abundance of the infused arginine (Fig. 2), ornithine (Fig. 3), and proline (Fig. 4) tracers and that of \( \alpha-[5,5,5,5-2H4]\text{KIC} \) (Fig. 5) remained at relatively steady levels (slope, \( P > 0.05 \)) during the last 45 min of the tracer protocol, when blood samples were taken. Infusion of [15N2-guanidino-5,5-2H2]arginine and [5,5-2H2]proline resulted in a slight increment of \( M+1 \) ornithine during the 120- to 150-min period, reaching 0.5 \( \pm \) 0.2 (SE) APE. This value was subtracted from the plateau value for the [5-13C]ornithine enrichment measured for the
285- to 330-min period. Furthermore, after the infusion of [5,5-2H2]proline, there was a slight increment of M+1 proline enrichment between 120 and 150 min (Fig. 4), but this was not followed by a significant further rise during infusion of the [5-13C]ornithine tracer between 150 and 330 min.

**Kinetics of arginine, ornithine, and proline.** The estimates of plasma arginine, ornithine, and proline kinetics for the burn patients are shown in Table 3. For further interpretation and discussion (see DISCUSSION), these data are summarized here, together with those obtained in healthy young adults with the same tracers after similar study protocols (10) conducted at the MIT CRC and with the analyses carried out as for the burn patients in the same laboratory.

The plasma arginine flux (QArg) in this group of burn patients was 101.5 ± 10.3 µmol·kg⁻¹·h⁻¹ in the basal state, and it rose during the parenteral feeding. The change was essentially equivalent to the arginine provided by the TPN. The plasma ornithine flux (QOrn) in these burn patients during the basal state was 45.3 ± 4.2 µmol·kg⁻¹·h⁻¹, with the QOrn-to-QArg ratio being ~4.8%. The flux increased when patients were given parenteral feeding; the increase was in proportion to that observed for the plasma arginine flux (Table 3).

From the appearance of plasma [5,5-2H2]ornithine, which is derived from the infused [15N6]-guanidino-5,5,5-[2H3]arginine, and from the measured ornithine flux, we can estimate that the rate of arginine to ornithine conversion in burn patients under the basal condition is 38.6 ± 2.9 µmol·kg⁻¹·h⁻¹. This rate increased significantly when the patients received TPN.

The rate of ornithine oxidation in the burn patients during the basal state was 24.1 ± 4.4 µmol·kg⁻¹·h⁻¹ and did not show significant increase during parenteral feeding. Thus, in burn patients, ornithine oxidation rate accounts for 53.0 ± 5.8% of the ornithine flux under the basal condition, and this fraction was significantly reduced during the TPN feeding. Furthermore, from the rate of arginine-to-ornithine conversion and the fraction of the ornithine flux oxidized (Eq. 5), we can also estimate the irreversible rate of arginine disposal via ornithine oxidation (Table 3). This was 20.0 ± 2.5 µmol·kg⁻¹·h⁻¹ for the basal state, and it increased significantly during the TPN phase. It might be noted that this estimate of the irreversible loss of arginine was essentially equivalent to the rate of ornithine oxidation.

The basal plasma proline flux, measured with L-[5,5-2H2]proline in the patients, was 155.6 ± 8.8 (SE) µmol·kg⁻¹·h⁻¹. TPN feeding, supplying 58.9 ± 1.4 µmol proline·kg⁻¹·h⁻¹, was associated with an almost equivalent increase in the plasma proline flux (Table 3).

There was a detectable increment of M+1 proline during the infusion of L-[5,5-2H2]proline tracer (Fig. 4) that appeared before the L-[5-13C]ornithine infusion, with the enrichment of M+1 proline not further increasing significantly. This suggests, but does not prove, that the M+1 species arose as a result of the loss of a labeled proton through formation of Δ'-pyrroline-5-carboxylate (P5C) via proline oxidase and the return of P5C to proline via pyrroline carboxylate reductase with addition of an unlabeled proton.

**Table 3. Arginine, ornithine, and proline kinetics and plasma concentrations in burn patients**

<table>
<thead>
<tr>
<th>Metabolic Parameters</th>
<th>Burn Patients</th>
<th>Healthy Controls (Fasting)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine flux (QArg)</td>
<td>101.5 ± 10.3</td>
<td>162.1 ± 13.6† 56.0 ± 3.4</td>
</tr>
<tr>
<td>Ornithine flux (QOrn)</td>
<td>45.3 ± 4.2</td>
<td>74.5 ± 3.5† 28.4 ± 1.1‡</td>
</tr>
<tr>
<td>Ornithine oxidation (COrn)</td>
<td>24.1 ± 4.8</td>
<td>29.0 ± 3.0 13.1 ± 0.6‡</td>
</tr>
<tr>
<td>% of QOrn oxidized</td>
<td>53.0 ± 5.8</td>
<td>39.0 ± 3.7† 46.1</td>
</tr>
<tr>
<td>Arg intake</td>
<td>0</td>
<td>48.9 ± 6.2    0</td>
</tr>
<tr>
<td>QOrn/QArg ratio, %</td>
<td>48.3 ± 7.2</td>
<td>47.9 ± 4.1     50</td>
</tr>
<tr>
<td>Rate of Arg→Orn</td>
<td>38.6 ± 2.9</td>
<td>71.9 ± 3.4† 12.9 ± 1.1‡</td>
</tr>
<tr>
<td>Rate of CArg</td>
<td>20.0 ± 2.4</td>
<td>28.4 ± 3.3‡</td>
</tr>
<tr>
<td>Proline flux (QPro)</td>
<td>155.6 ± 8.8</td>
<td>232.3 ± 15.9 89 ± 33‡</td>
</tr>
<tr>
<td>Proline intake</td>
<td>0</td>
<td>58.9 ± 1.4</td>
</tr>
<tr>
<td>Plasma concentrations, µM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>93.3 ± 8.0</td>
<td>160.3 ± 7.7‡ 133 ± 5.4‡</td>
</tr>
<tr>
<td>Ornithine</td>
<td>78.3 ± 12.4</td>
<td>102.6 ± 15.1 46 ± 2.2‡</td>
</tr>
<tr>
<td>Proline</td>
<td>73.1 ± 11.3</td>
<td>166.3 ± 20.3* 135 ± 9.8‡</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in µmol·kg⁻¹·h⁻¹, except where indicated. CArg and COrn, arginine and ornithine oxidation, respectively, CArg was not measured or calculated in healthy adults. CArg was calculated from Arg→Orn conversion rate and the % of QOrn oxidized (see Eq. 5). QArg data are based on an average of reported values from Refs. 2, 6–11. Data from healthy controls for QOrn, COrn, % of QOrn oxidized, ratio of QOrn to QArg, and rate of Arg→Orn are recalculated from data in Ref. 10, using a fractional recovery of 0.402 for [15N6]arginine; for details see text. Data from healthy controls for QPro are taken from Ref. 18; those for Arg, Orn, and Pro plasma concentrations are from Ref. 10. *P < 0.05 by paired t-test vs. basal state; †P < 0.01 by paired t-test vs. basal state; ‡P < 0.05 by unpaired t-test vs. basal (fasted) burn patients.
**DISCUSSION**

**Comparison with healthy controls.** To further help to interpret the metabolic and nutritional significance of these findings in severely burned patients, we compare our data with results from our published studies of leucine, ornithine, and arginine kinetics in healthy adult controls. The analytic methods and tracer paradigms were essentially the same for the present and earlier studies. In metabolic clinical investigations of the kind we have carried out here, it is necessary to depend on comparisons of this type, because burned patients, for quite obvious reasons, cannot be studied at the same time or in the same setting as a healthy control group. We believe the present comparison with “historical” data is appropriate, because 1) the arginine and leucine fluxes estimated here are comparable to those measured for the basal state in earlier studies in various groups of severely burned patients (39, 41), indicating that our observations are broadly reproducible among the various studies conducted at our hospital and in this type of patient, and 2) the fluxes of leucine (e.g., see Refs. 15, 16, 19), arginine (2, 6–11), and ornithine (10, 11) are similar among different studies that we have conducted in healthy controls.

A note might be made, however, about the fact that we could not completely fast our patients, whereas the data for healthy controls shown in Table 3 were generated in subjects after an overnight fast. Our patients received glucose, on average, at a rate of ~60 mg·kg⁻¹·h⁻¹ (or ~1 mg·kg⁻¹·min⁻¹) to prevent hypoglycemia. This level of input would not have any significant effect on basal insulin levels that are already elevated in burn patients (17) or impact on total energy expenditure over the tracer infusion period: the glucose provided 1.43 kcal/kg for the 330-min study period compared with a basal energy expenditure of ~119 kcal/kg for this same time frame. In addition, an infusion of glucose would, if anything, tend to lower the plasma fluxes of leucine, arginine, and proline and so reduce, rather than widen, differences between basal state burn patients and fasting healthy controls. Furthermore, our earlier study (29) revealed that, in healthy subjects, intravenous infusion of glucose at a rate of 2 mg·kg⁻¹·min⁻¹ did not significantly alter leucine kinetics.

Thus, from the summary in Table 3, we conclude that basal arginine and ornithine fluxes are distinctly higher in burn patients when compared with data for healthy controls, confirming an accelerated turnover of arginine after burn injury. The rate of ornithine oxidation is higher in burned patients than in healthy controls. Furthermore, when the values for leucine flux shown in Table 4 for burn patients are compared with those reported for healthy controls, which approximate 120 μmol·kg⁻¹·h⁻¹ (15, 19), a higher rate of whole body protein turnover is evident in the present group of burn patients. It might also be worth noting that, relative to the mean differences observed between the severely burned patients and healthy controls, age- and sex-related effects on amino acid kinetics are considered to be minimal and do not confound these comparisons (15, 16, 26).

**Increased loss of arginine.** Our previous intravenous tracer studies (39–41) demonstrated an increased arginine flux and a limited endogenous production rate of arginine in severe burn injury, a major purpose of this study was to determine the disposal rate of arginine via its conversion to and oxidation of the ornithine carbon skeleton in these patients. Furthermore, in addition to confirming a high rate of arginine turnover in response to the stress of burn injury, the present study has revealed that this increased arginine turnover correlates with its increased conversion to ornithine and subsequent oxidation. Hence, the irreversible disposal of arginine, via oxidative catabolism, is
significantly increased after burn injury. Our conclusion is based on the following observations: 1) the higher arginine flux is proportional to the higher ornithine flux in burn patients and is similar to the ornithine-to-arginine flux ratio in healthy subjects (Table 3; 2) the rate of ornithine oxidation is increased in burn patients, and this is in line with the increment of arginine-to-ornithine conversion and parallel to the increased rate of arginine oxidation. These results are also consistent with our conclusions, drawn from studies in adult healthy subjects, that arginine homeostasis is largely controlled by the conversion of arginine to ornithine and its subsequent oxidation and the relative rate of arginine intake (10). On the basis of these findings of an increased rate of arginine disposal in burn patients, taken together with our previous findings of a limited rate of arginine de novo synthesis after severe burn injury, it is reasonable to propose that arginine is a conditionally indispensable amino acid under these conditions of major stress.

We should caution the reader that our interpretations are based on data generated via an intravenous tracer paradigm in burn patients, where providing nutrients via an oral route was not feasible. If the isotope tracer and amino acid substrates had been given by the oral route, the interrelationships and quantitative estimates could have been different. This remains for new research to resolve, but we believe that our findings do lead to a coherent picture, as we will discuss further.

**Estimates of arginine oxidation.** The validity of the conclusions drawn above are also dependent, of course, on the extent to which we have been able to estimate the actual rate of arginine catabolism. We can arrive at assessment of this problem as follows. First, the estimate of the arginine flux during the basal state appears to be reasonable, because the ratio of arginine to leucine flux was 0.55 in the basal state, or close to the expectation, when we assume an approximate concentration ratio of arginine to leucine in whole body mixed proteins to be 0.58 (34); second, if the rate of leucine oxidation was 35 μmol·kg⁻¹·h⁻¹ in burn patients (41), then the rate of incorporation of arginine into proteins would be 85 [=(189–35) × 0.55] μmol·kg⁻¹·h⁻¹. Therefore, since the flux of arginine is 102, and the rate of disappearance of arginine into protein is 85, then arginine must be removed by conversion to ornithine and its subsequent oxidation at a rate of 102 – 85 = 17 μmol·kg⁻¹·h⁻¹. The estimated rate of arginine oxidation was 20 ± 2 μmol·kg⁻¹·h⁻¹, or essentially the same as the rate of ornithine oxidation (24 ± 4 μmol·kg⁻¹·h⁻¹). Thus whole body arginine oxidation in the basal state appears to have been estimated with a reasonable degree of accuracy, provided that the foregoing assumptions are valid. Furthermore, during this basal state, body arginine balance would be negative by an amount equal to the difference between arginine oxidation and net de novo arginine synthesis. We (39) have estimated the latter value to be 4 μmol·kg⁻¹·h⁻¹, so arginine balance would be −16 μmol·kg⁻¹·h⁻¹ (= 4–20 μmol·kg⁻¹·h⁻¹). On this basis, the negative leucine balance would be expected to be about −29 μmol·kg⁻¹·h⁻¹, which compares favorably with the value of −30 to −35 μmol·kg⁻¹·h⁻¹ reported by us previously (39).

For the TPN phase, we estimated arginine oxidation to be 28 μmol·kg⁻¹·h⁻¹ when the rate of exogenous arginine intake is 49 μmol·kg⁻¹·h⁻¹. Hence, TPN improved arginine balance by this difference plus that due to additional net de novo arginine synthesis. This would be a higher balance (>20 μmol·kg⁻¹·h⁻¹) than would be expected from our earlier study, showing a leucine balance of +8 μmol·kg⁻¹·h⁻¹ or equivalent to about +5 μmol·kg⁻¹·h⁻¹ for arginine balance. It might be, therefore, that either we have underestimated the rate of irreversible arginine loss in the TPN state and/or the present subjects were in a greater anabolic state than for our previous group of burned patients (39, 41). An underestimation of the irreversible rate of arginine loss would arise in the TPN condition if arginine were converted to and retained as proline, which our tracer technique has not probed. Thus the present arginine balance estimates are likely to be maximal values and actually less positive than we have stated.

**Relationship between arginine and ornithine and compartmentation of the urea cycle.** The acceleration of urea production/excretion, and therefore of an increased urea cycle activity, is a metabolic feature of the response to burn injury. Our studies on arginine, ornithine, and proline metabolism have further suggested that the urea cycle is compartmentalized (10, 39, 41). Thus we and others (41) have estimated the rate of urea production to be −540 μmol·kg⁻¹·h⁻¹ in severely burned patients. Here, however, the plasma arginine-guanidino flux (99.7 μmol·kg⁻¹·h⁻¹) appears to be about one-fifth of the rate of urea production. Furthermore, the rate of conversion of plasma arginine to ornithine is ~39 μmol·kg⁻¹·h⁻¹, indicating that the plasma arginine compartment contributes only ~7% of the total urea production, with the remainder due to the turnover of arginine in a segregated site of urea cycle activity in the liver. In support of these in vivo findings, studies by Cheung et al. (12) demonstrated that the extramitochondrial enzymes catalyzing the cytosol portion of the urea cycle are spatially organized in such a way that the urea cycle intermediates are synthesized and metabolized in situ within the cells, exclusively for the purpose of the urea cycle. The physiological significance of this is presumably to maintain the availability of arginine in the peripheral circulation for supporting visceral and muscle protein synthesis, as well as in other metabolic pathways, including nitric oxide formation (5). This compartmentation of arginine metabolism may be of particular importance under conditions of an elevated urea cycle activity, as in stressed conditions or after an ingestion of protein-rich meals in healthy subjects (11).

It is also evident that ornithine metabolism is similarly compartmentalized between the intrahepatic urea cycle and plasma or peripheral pools. The ornithine flux was about one-half of the plasma arginine flux. Although the ornithine flux was significantly ele-
vated above that reported for healthy subjects, the quantitative relationships between the ornithine and arginine fluxes appeared to be similar in both groups of individuals (Table 3). Therefore, the plasma ornithine flux reflects the status of non-urea-cycle arginine metabolism, and it appears that, when arginine turnover and oxidation are increased, ornithine flux is also increased. When the arginine flux is reduced below a usual, or normal rate, the ornithine flux and its oxidation rate are similarly reduced (10). This leads us to the perspective that the ornithine flux is a surrogate for arginine turnover and catabolism. This interpretation is supported by the studies of Alonso and Rubio (1) in mice, confirming the involvement of the reversible reaction catalyzed by ornithine amino transferase (OAT), whose apparent equilibrium favors glutamate-γ-semialdehyde synthesis with spontaneous conversion to glutamate. Our view is also consistent with the markedly elevated plasma concentration of ornithine in adult patients who have an OAT deficiency (32). However, we also recognize that proline turnover is increased in our burn patients, but the extent to which proline metabolism contributes to this arginine-ornithine relationship cannot be judged from our data.

Relationship among proline, ornithine, and glutamate. Proline, ornithine, and glutamate each occupies one corner of a metabolic triangle, which serves to connect these amino acids to the urea cycle, on the one hand, and via the action of glutamic acid dehydrogenase to the tricarboxylic acid cycle and energy metabolism. Our previous study (22) demonstrated that, in burn patients, there was an increased proline oxidation, accompanied by a compromised rate of proline de novo synthesis after burn injury, suggesting the possible importance of an exogenous supply of proline for the nourishment of burn patients. Studies by Cynober and colleagues (14, 23) have demonstrated beneficial nutritional and immunological effects of a combined supply of α-ketoglutarate and ornithine in burn patients. Thus the kinetic interrelationships among these three amino acids deserve further investigation.

The appearance of the M+1 proline species in plasma before the beginning of the infusion of L-[5-13C]ornithine, and then the lack of a perceptible change in the enrichment of M+1 proline after that, suggests that this phenomenon arose via the formation of P5C and its reconversion to proline via proline oxidase and Δ1-pyroline-5-carboxylate reductase, respectively. This does not mean that ornithine carbon is not incorporated into proline, because we have given the ornithine tracer via the gut, where extensive amino acid metabolism and interconversion occur during the first pass phase of amino acid utilization (28), the findings with [13C]ornithine and transfer of label to proline might have been different. In addition, however, these findings may also help to explain why the plasma proline flux measured with L-[5,5-2H2]proline (18) appears to be higher than that based on L-[1-13C]proline as a tracer in healthy subjects (21, 22) and also when the present data are compared with the somewhat lower fluxes obtained earlier in burn patients (21) with a [13C]proline tracer.

To conclude, we have confirmed an expected increased rate of arginine disposal in severely burned patients compared with healthy adults. This is reflected by equivalent increases in ornithine turnover and oxidation. Therefore, with the limited endogenous production rate and the increased irreversible disposal of arginine, it is further apparent that arginine is a conditionally indispensable amino acid in the support of parenterally fed, severely burned patients. This must now be confirmed through nutritional supplementation studies, as well as by additional investigations of the metabolic and kinetic relationships among proline, ornithine, and glutamate. When the feeding modality is parenteral nutrition, a preformed source of arginine appears to be obligatory; however, if the enteral route is used, proline and glutamate may serve as suitable precursors if given in high enough amounts. This point deserves study because Brunton et al. (4) have reported that proline ameliorates arginine deficiency during enteral but not parenteral feeding in neonatal pigs. It also is now quite clear that the route of nutrient administration can have an important impact on the ability of the organism to synthesize proline as well as arginine (3, 27, 35). We also speculate that the pathway of urea cycle anaplerosis may be a determinant of the availability and need for proline, glutamate, and/or glutamine in severe burn injury and other major stress conditions.

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Present address of L. Beaumier: Newborn Medicine Division, McGill University Health Center, Montreal, Quebec, Canada H3H 1P3.

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