Reduced citrulline production in sepsis is related to diminished de novo arginine and nitric oxide production$^{1-3}$

Yvette C Luiking, Martijn Poeze, Graham Ramsay, and Nicolaas EP Deutz

ABSTRACT

**Background:** L-Arginine is an important precursor of nitric oxide (NO) and protein synthesis. Arginine is produced in the body (mainly kidney) by de novo production from citrulline and by protein breakdown. Arginine availability appears to be limited in sepsis.

**Objective:** The objective was to compare arginine and citrulline metabolism in septic patients and nonseptic control patients in an intensive care unit (ICU) and in healthy control subjects.

**Design:** Ten patients with septic shock, 7 critically ill control patients, and 16 healthy elderly subjects were studied. Metabolism was measured by using a primed continuous (2 h) stable-isotope infusion protocol. NO production was calculated as the conversion rate of arginine to citrulline; de novo arginine production was calculated as the conversion rate of citrulline to arginine. Arterial blood (arterialized venous blood in healthy subjects) was collected for the measurement of amino acid enrichment and concentrations.

Data are reported as means ± SDs.

**Results:** Whole-body citrulline production was significantly lower in septic patients (4.5 ± 2.1 μmol · kg$^{-1}$ · h$^{-1}$) than in ICU control patients (10.1 ± 2.9 μmol · kg$^{-1}$ · h$^{-1}$; $P < 0.01$) and in healthy control subjects (13.7 ± 4.1 μmol · kg$^{-1}$ · h$^{-1}$; $P < 0.001$). Accordingly, de novo arginine production was lower in patients with sepsis (3.3 ± 3.7 μmol · kg$^{-1}$ · h$^{-1}$) than in healthy controls (11.9 ± 6.6 μmol · kg$^{-1}$ · h$^{-1}$; $P < 0.01$) and tended to be lower in septic patients than in ICU control patients (10.9 ± 9.4 μmol · kg$^{-1}$ · h$^{-1}$; $P = 0.05$). NO production was lower in septic patients than in healthy control subjects ($P < 0.01$), whereas a larger part of arginine was converted to urea in sepsis.

**Conclusions:** Citrulline production is severely low in patients with sepsis and is related to diminished de novo arginine and NO production. These metabolic alterations contribute to reduced citrulline and arginine availability, and these findings warrant further studies of therapeutic nutritional interventions to restore arginine metabolism in sepsis. *Am J Clin Nutr* 2009;89:1–11.

INTRODUCTION

Arginine is an important amino acid in the biosynthetic pathways of proteins, nitric oxide (NO), agmatine, creatine, urea, and polyamines; as such, it has an important role in cellular regeneration, wound healing, immune function, and protein turnover (1, 2). Citrulline, which is produced in the intestine from glutamine, is the endogenous source of de novo arginine synthesis, which mainly occurs in the kidney (3–5). Glutamine depletion diminishes plasma citrulline, whereas citrulline supplementation increases plasma citrulline and arginine in healthy subjects (6, 7).

In addition to being produced during citrulline conversion, arginine is also produced through protein breakdown.

Under normal conditions, low amounts of NO are produced by the constitutively expressed NO synthase isoforms NOS1 (neuronal NOS) and NOS3 (endothelial NOS) (8, 9) to regulate blood perfusion and to act as a neurotransmitter. It has been suggested that, during inflammation, inflammatory mediators such as cytokines greatly increase NO synthesis through induction of the NOS2 (inducible) isoenzyme (10).

Sepsis is defined as a systemic response to an infection (11). It is a major health problem because of its significant morbidity and overall mortality rate of ~30% and generally requires intensive care treatment (12). In septic patients, arginine concentrations were shown to be markedly lower than those in healthy control subjects or control hospital patients (13–15). One study in patients with septic shock attributed this reduced availability of arginine to a slower rate of arginine production (15). Another metabolic alteration that might contribute to reduced arginine availability is the increased arginine clearance that has been shown in septic patients (14). However, the specific sources of reduced arginine production (ie, protein or citrulline) as well as the specific products of arginine clearance (eg, protein, NO, or urea as major products) have not been quantified in vivo in human sepsis.

NO synthesis is believed to be elevated in sepsis on the basis of elevated plasma concentrations of the elimination products nitrate and nitrite (NOx) (16–19). However, discrepancies between the degree of plasma NOx elevation and actual in vivo NO production were described previously (15, 20, 21). Only one study measured in vivo NO production in adult patients with sepsis by measuring the conversion rate of arginine to NOx; this study showed a slower fractional synthesis rate and equal absolute

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synthesis rate of NOx in septic patients compared with healthy control subjects (15).

In summary, arginine availability is reduced in patients with sepsis because of lower plasma arginine concentrations and a slower arginine production rate. However, the underlying metabolic alterations in arginine production and use have not been fully elucidated in vivo in septic patients. In addition, data on NO production in severe sepsis are not uniform. Therefore, this study was aimed at determining the alterations in arginine and citrulline metabolism during sepsis. Total arginine and citrulline production, production of arginine from citrulline, protein kinetics, and arginase activity were quantified with the use of stable-isotope techniques. Knowledge of the changes in arginine and citrulline metabolism in sepsis is the first step toward the development of therapeutic nutritional interventions to restore arginine metabolism in sepsis.

SUBJECTS AND METHODS

Subjects

Ten critically ill patients with a diagnosis of septic shock (intensive care unit (ICU) patients with sepsis), 7 critically ill patients without infection (ICU control patients), and 16 healthy volunteers (healthy control subjects) were studied. Diagnosis of septic shock was defined according to the International Guidelines for Diagnosis of Sepsis (11). We excluded the following patients: 1) those who were highly hemodynamically unstable or had a life expectancy of <24 h, 2) those who were metabolically affected by prolonged or high-dose use of corticosteroids or had insulin-dependent diabetes mellitus, 3) those who had diagnosed metastasis or hematologic malignancies or who had undergone chemotherapy, 4) those who had preexisting liver or renal failure, or 5) those who were receiving hemodialysis or continuous veno-venous hemofiltration treatment. All septic shock patients were examined ≤48 h after their diagnosis.

The Medical Ethical Committee of the University Hospital Maastricht approved the study protocol (MEC 01-124). Written informed consent was obtained from the patients’ relatives and from the healthy volunteers.

Study design

All subjects were examined in the postabsorptive state. One septic patient had already received total parenteral nutrition, which was stopped 2 h before the start of the protocol; 4 control patients had already received enteral feeding, which was stopped 8 h before the start of the protocol; and healthy subjects fasted from 10 h before the start of the protocol. When patients received intravenous low-rate 5% glucose infusion (nonprotein calories), this infusion continued during the study. Before the start of stable-isotope infusion, background blood samples were collected to measure natural tracer enrichment. The 2-h primed, constant intravenous infusion of stable isotopes of phenylalanine, tyrosine, arginine, citrulline, and urea was then started for simultaneous measurement of protein and arginine metabolism. Arterial blood was collected with a systemic arterial catheter from ICU patients, and arterialized blood was collected from healthy control subjects with a venous catheter placed retrograde in a dorsal vein of the left hand by using the heated box technique

(22). Blood was collected at 0, 60, 75, 90, 105, and 120 min during the tracer study to confirm steady state tracer conditions during the study.

In the ICU patients, temperature, heart rate, arterial pressure, hemodynamic variables, and routine clinical blood variables were measured. APACHE II (Acute Physiology and Chronic Health Evaluation) (23) and TISS (Therapeutic Intervention Scoring System) (24) scores were calculated as measures of severity of disease in ICU patients.

Stable isotopes for measurement of protein and arginine metabolism

For protein metabolism, subjects were infused with L-[ring-2H2]phenylalanine ([2H2]Phe; prime: 2.19 μmol·kg⁻¹·h⁻¹; infusion: 2.26 μmol·kg⁻¹·h⁻¹), L-[ring-2H2]tyrosine ([2H2]Tyr; prime: 0.31 μmol·kg⁻¹, and L-[ring-2H2]tyrosine ([2H2]Tyr; prime: 0.95 μmol·kg⁻¹; infusion: 0.77 μmol·kg⁻¹·h⁻¹) (Cambridge Isotope Laboratories Inc, Andover, MA). For measurement of arginine metabolism in ICU patients, we used a combination of L-[guanidino-15N2-5,5-2H2]arginine ([15N2-2H2]Arg; prime: 3.65 μmol·kg⁻¹; infusion: 3.51 μmol·kg⁻¹·h⁻¹) (Mass Trace, Woburn, MA), L-[ureido-13C]citrulline ([13C]Cit; prime: 0.58 μmol·kg⁻¹; infusion: 0.27 μmol·kg⁻¹·h⁻¹), and [13C]urea (prime: 36.5 μmol·kg⁻¹; infusion: 7.42 μmol·kg⁻¹·h⁻¹) (Cambridge Isotope Laboratories Inc). In healthy subjects, arginine metabolism was measured by using a different combination of stable isotopes because the [15N2-2H2]Arg isotope was no longer available; this also affected the choice of citrulline isotope. Therefore, L-[guanidino-15N2]arginine ([15N2]Arg; prime: 5.48 μmol·kg⁻¹; infusion: 5.27 μmol·kg⁻¹·h⁻¹) (Cambridge Isotope Laboratories Inc), L-[ureido-13C-3,3,4,2H4]citrulline ([13C-2H3]Cit; prime: 0.58 μmol·kg⁻¹·h⁻¹) (ARC, Apeldoorn, Netherlands), and [13C]urea (prime: 36.5 μmol·kg⁻¹; infusion: 7.42 μmol·kg⁻¹·h⁻¹) (Cambridge Isotope Laboratories Inc) were chosen after verifying their suitability in a pilot mouse experiment (see Calculations of whole-body metabolism below). All stable-isotope solutions were processed for intravenous use by the hospital pharmacy. A sample of the tracer solution was analyzed for amino acid content.

Sample processing

Blood was collected and then immediately distributed into prechilled tubes on ice containing either heparin (Hep; 4 mL evacuated tubes, 68 IU sodium-heparin; Becton Dickinson, Franklin Lakes, NJ) or EDTA (Becton Dickinson). Blood was centrifuged immediately (10 min, 4000 rpm, 4°C), and the plasma was collected and kept on ice. For the glucose and lactate concentration analysis, 500 μL Hep-plasma was added to 50 μL 50% trichloroacetic acid solution to ensure stability of the substances. For analysis of amino acid concentrations and tracer enrichments, Hep-plasma was deproteinized by mixing 500 μL with 20 mg dry sulfosalicylic acid. The remaining Hep-plasma and EDTA-plasma were stored without further addition of substances for analysis of insulin and nitrate concentrations. All samples were frozen in liquid nitrogen and stored at −80°C until further analysis.

Sample analysis

Plasma glucose, lactate, and urea concentrations were measured by using commercially available kits on a Cobas Mira S (Hoffman
La Roche, Basel, Switzerland), as described previously (25). Plasma amino acid concentrations were determined by HPLC analyses (26). Enrichments, calculated as tracer:tracee ratios (TTRs), of amino acids were determined with a fully automated liquid chromatography–mass spectrometry system (Thermoquest LCQ, Veenendaal, Netherlands) after precolumn derivatization with 9-fluorenylmethylchloroformate (27). For urea enrichment, a newly developed method that combines reversed phase liquid chromatography with an ion-trap spectrometer was applied (HM van Eijk, unpublished observations, 2002). Details of the isotope enrichment analysis for amino acids and system precision were described by van Eijk et al (27); for urea, the mass m+1/m+0 TTR of an unlabeled urea standard was analyzed 14-fold in the present study, which resulted in a mean enrichment of 2.07% (for comparison, natural enrichment is 1.92%), an SD of 0.09%, and a variation coefficient of 4.4%. For measurement of plasma nitrate, 50 µl plasma were added to 100 µl acetonitrile (Biosolve LTD, Valkenswaard, Netherlands). Nitrite and nitrate were separated on an IC-PackHR column (75 × 4.6 mm; Waters, Etten-Leur, Netherlands). The column effluent was monitored at 205 nm with an Ultraviolet-975 detector (Jasco, Wassenaar, Netherlands). Anions were isocratically eluted from the column by using a 6-mmol/L sodium chloride solution containing 1 mmol/L potassium dihydrogenphosphate (pH: 6). Nitrate concentrations in healthy subjects were derived from a historic control group of healthy subjects (MPKJ Engelen, unpublished observations, 2004).

Calculations of whole-body metabolism

Plasma arginine, citrulline, phenylalanine, tyrosine, and urea production rates [flux (Q) or whole-body production] were calculated from the arterial isotopic enrichment values of the following, respectively: [15N2-2H2]Arg, [13C]Cit, [15N-2H2]Phe, and [13C]urea by using the standard steady state isotope dilution equation (28):

\[ Q = \frac{I}{TTR} \]

where I is the rate of infusion of the tracer. The units for Q are expressed as µmol · kg⁻¹ · h⁻¹. TTR was corrected for background enrichment, and, whenever multiple masses of one amino acid were enriched, the contribution of isotopomers from lower masses to the measured TTR was accounted for as described by Vogt et al (29).

Isotopic steady state conditions were reached after 60–90 min of amino acid and urea tracer infusion, as shown in Figure 1, and reached mean enrichment levels for infused tracers and their derivates as listed in Table 1. The moving average of the measures of protein and arginine metabolism obtained from the time points of 90, 105, and 120 min was used for between-group comparison.

Whole-body arginine and citrulline metabolism

In a pilot experiment in mice (YC Luiking et al, unpublished observations, 2004), we compared the measurement of arginine metabolism and NO production by simultaneously infusing the isotopes [15N2-2H2]Arg and [15N2]Arg, as were infused in ICU patients and healthy subjects, respectively, in the present study. Arginine and NO metabolism appeared to be comparable with both isotopes, but the variance in NO production with the [15N2]Arg tracer was larger, which can be explained by the larger background enrichment of the formed [15N]Cit than of [15N2]Cit. Analysis of [15N2]Cit as performed in the ICU patients is therefore more precise and the variance is less. For readability, the formulas are described only for [15N2-2H2]Arg and [13C]Cit tracers (used in ICU patients), but these are comparable for the [15N2]Arg and [13C]Cit tracers (used in healthy subjects).

The rate of NO production was measured as arginine-to-citrulline flux (30) and was derived by using the following equation:

\[ Q_{\text{Arg} \rightarrow \text{Cit}} = Q_{\text{Cit}} \times \frac{TTR_{15N2-2H2}^{\text{Cit}}}{TTR_{15N2-2H2}^{\text{Arg}}} \]

where \( Q_{\text{Cit}} \) is plasma citrulline production measured by infusion of [15C]Cit.

The rate of de novo arginine production was measured as citrulline-to-arginine flux (31), and was derived by using the following equation:

\[ Q_{\text{Cit} \rightarrow \text{Arg}} = Q_{\text{Arg}} \times \frac{TTR_{13C}^{\text{Arg}}}{TTR_{13C}^{\text{Cit}}} \]

where \( Q_{\text{Arg}} \) is plasma arginine production measured by infusion of [15N2-2H2]Arg tracer.

Arginase activity, which is defined as the absolute synthesis rate (ASR) of urea from its precursor arginine, was measured by first calculating the fractional synthetic rate (FSR) according to the precursor-product equations for ICU patients (Equation 4) and for healthy subjects (Equation 5), which is similar to calculating FSR for protein or glutathione (28, 32).

![FIGURE 1](image-url) Mean (± SD) tracer:tracee ratios (cTTR) of arginine, citrulline, phenylalanine, and urea stable isotopes during the 2-h tracer infusion in healthy control subjects (n = 16), control patients in an intensive care unit (ICU) (n = 7), and ICU patients with sepsis (n = 10), corrected for baseline (T0) enrichment. cTTR, TTR corrected for background enrichment.
TABLE 1
Isotopic enrichments (m+1), calculated as tracer:trace ratios (cTTRs) of infused isotopes and derived substrates at steady state (mean at 90–120 min) during the infusion protocol in each group.

<table>
<thead>
<tr>
<th>cTTR</th>
<th>Healthy control subjects (n = 16)</th>
<th>ICU control patients (n = 7)</th>
<th>ICU patients with sepsis (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cit(m+1)</td>
<td>1.42 ± 0.72</td>
<td>3.12 ± 0.81$^2$</td>
<td>8.36 ± 3.00$^2$</td>
</tr>
<tr>
<td>Cit(m+3)</td>
<td>—</td>
<td>0.79 ± 0.83</td>
<td>0.93 ± 0.81</td>
</tr>
<tr>
<td>Cit(m+4)</td>
<td>1.79 ± 0.92$^2$</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Arg(m+1)</td>
<td>—</td>
<td>0.53 ± 0.32</td>
<td>0.53 ± 0.55</td>
</tr>
<tr>
<td>Arg(m+2)</td>
<td>7.20 ± 2.11$^2$</td>
<td>4.43 ± 1.33$^2$</td>
<td>5.49 ± 2.00$^2$</td>
</tr>
<tr>
<td>Phen(m+5)</td>
<td>5.63 ± 1.52$^2$</td>
<td>3.92 ± 0.93$^2$</td>
<td>3.92 ± 1.66$^2$</td>
</tr>
<tr>
<td>Tyr(m+2)</td>
<td>2.02 ± 0.56$^2$</td>
<td>1.69 ± 0.52$^2$</td>
<td>2.05 ± 0.75$^2$</td>
</tr>
<tr>
<td>Tyr(m+4)</td>
<td>0.62 ± 0.25</td>
<td>0.26 ± 0.16</td>
<td>0.45 ± 0.43</td>
</tr>
<tr>
<td>Urea(m+1)</td>
<td>1.36 ± 0.55$^2$</td>
<td>0.47 ± 0.30$^2$</td>
<td>0.46 ± 0.23$^2$</td>
</tr>
<tr>
<td>Urea(m+2)</td>
<td>0.048 ± 0.025</td>
<td>0.030 ± 0.012</td>
<td>0.056 ± 0.064</td>
</tr>
</tbody>
</table>

$^1$ All values are means ± SDs. ICU, intensive care unit.

$^2$ Infused isotopes.

Additional calculations

The homeostasis model assessment (HOMA) index for insulin resistance was calculated as [fasting blood glucose concentration (mmol · L$^{-1}$) × serum insulin concentration (mU · L$^{-1}$)]/22.5 (34).

Statistical analyses

Results are expressed as means ± SDs, and group sizes are indicated. Normal distributions of the data were evaluated with the Shapiro-Wilk test on standardized residuals, and equal variances of the groups were evaluated with the Levene test. Variables with a normal distribution and equal variance were compared between the groups with a one-factor analysis of variance (ANOVA) with Bonferroni adjustment for subgroup analyses; for those variables that were only measured in ICU patients (ie, not in healthy subjects), an independent-sample $t$ test was used for comparison. When a variable failed the normality or equal variance tests, Kruskal-Wallis and Mann-Whitney analysis with Bonferroni adjustment was applied; for those variables that were only measured in ICU patients (ie, not in healthy subjects), the Mann-Whitney test was used for comparison. The use of ANOVA or the Kruskal-Wallis with Mann-Whitney tests is specified for each variable in the tables and figures or in the Results. Spearman’s $ρ$ was used to correlate variables. Statistical significance was defined as a 2-tailed $P < 0.05$. Analysis was performed with the SPSS statistical software package for Windows (version 13; SPSS Inc, Chicago, IL).

RESULTS

The characteristics of the patients with sepsis are listed in Table 2, and the main hemodynamic and blood variables in ICU patients are listed in Table 3. All ICU patients were mechanically ventilated (except for one control patient) and treated with antibiotics. The underlying causes of sepsis were pneumonia ($n = 2$), acute (biliary) pancreatitis ($n = 2$), postoperative septic hemorrhage ($n = 1$), cervical abscess ($n = 1$), catheter-related sepsis ($n = 1$), infected hip prosthesis ($n = 1$), necrotizing fascitis ($n = 1$), and urosepsis ($n = 1$). The ICU control patients were diagnosed with pulmonary insufficiency ($n = 2$), exacerbation of chronic obstructive pulmonary disease ($n = 2$), neurotrauma ($n = 1$), and subarachnoidal bleeding ($n = 2$). During
the study protocol, 9 of 10 septic patients received an infusion of 5% glucose (average: 277 mL/h, 13.85 g glucose/2 h) compared with 3 of 7 ICU control patients (average: 104 mL/h, 5.2 g glucose/2 h). C-reactive protein concentrations in ICU patients were elevated compared with those in healthy control subjects (P < 0.001) and tended to be higher in septic patients than in ICU control patients (P = 0.08), which indicated severe inflammation in patients with sepsis and moderate inflammation in control patients. Although the septic patients had no preexisting renal insufficiency, their elevated plasma creatinine concentration indicated renal insufficiency during sepsis. The HOMA index for insulin resistance (34) was higher in the ICU control patients (5.3 ± 4.2) than in healthy control subjects (1.2 ± 0.7; P < 0.05) and in 9 of the patients with sepsis (3.6 ± 2.5) than in healthy control subjects (P < 0.01; Kruskal-Wallis and Mann-Whitney tests).

### TABLE 2
Characteristics of intensive care unit (ICU) patients and healthy control subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Healthy control subjects (n = 16)</th>
<th>ICU control patients (n = 7)</th>
<th>ICU patients with sepsis (n = 10)</th>
<th>P²</th>
<th>P³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>61 ± 6¹</td>
<td>58 ± 11</td>
<td>NS</td>
<td>56 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.4 ± 2.7</td>
<td>25.2 ± 2.8</td>
<td>NS</td>
<td>24.0 ± 4.1</td>
<td>NS</td>
</tr>
<tr>
<td>APACHE II</td>
<td>NA</td>
<td>23 ± 7</td>
<td>—</td>
<td>22 ± 7</td>
<td>NS</td>
</tr>
<tr>
<td>TISS</td>
<td>NA</td>
<td>28 ± 8</td>
<td>—</td>
<td>49 ± 7</td>
<td>—</td>
</tr>
<tr>
<td>28-d Survival (n)</td>
<td>16/16</td>
<td>7/7</td>
<td>—</td>
<td>5/10</td>
<td>—</td>
</tr>
</tbody>
</table>

¹ All patients with sepsis have septic shock. APACHE, Acute Physiology and Chronic Health Evaluation score; TISS, Therapeutic Intervention Scoring System; NA, not applicable; NS, not significant (P > 0.05). For comparison between groups, statistics were performed using Kruskal-Wallis with Mann-Whitney analyses (for age and BMI) or independent samples t test (for APACHE II and TISS scores).

² Compared with healthy control subjects.

³ Compared with ICU control patients.

⁴ Means ± SD (all such values).

### TABLE 3
Principal hemodynamic and laboratory variables in intensive care unit (ICU) patients and healthy control subjects

<table>
<thead>
<tr>
<th>Marker</th>
<th>Normal reference values (range) or healthy control subjects (n = 16)²</th>
<th>ICU control patients (n = 7)</th>
<th>ICU patients with sepsis (n = 10)</th>
<th>P³</th>
<th>P⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)⁵</td>
<td>&gt;70</td>
<td>84 ± 14⁶</td>
<td>74 ± 15</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>60–90</td>
<td>74 ± 11</td>
<td>109 ± 23</td>
<td>—</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cardiac index (L · min⁻¹ · m⁻²)⁷</td>
<td>2.8–3.6</td>
<td>3.9 ± 0.1</td>
<td>4.3 ± 0.8</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Stroke volume (mL/beat)</td>
<td>60–100</td>
<td>96 ± 6</td>
<td>74 ± 15</td>
<td>0.09</td>
<td>—</td>
</tr>
<tr>
<td>SVRI²</td>
<td>770–1500</td>
<td>1697 ± 73</td>
<td>1084 ± 426</td>
<td>0.08</td>
<td>—</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>7.3–9.7</td>
<td>6.7 ± 1.1</td>
<td>5.6 ± 1.0</td>
<td>0.05</td>
<td>—</td>
</tr>
<tr>
<td>Leukocytes (×10⁹)</td>
<td>3.5–11.0</td>
<td>11.1 ± 2.5</td>
<td>13.9 ± 10.4</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Thrombocytes (×10⁹)</td>
<td>130–350</td>
<td>220 ± 59</td>
<td>122 ± 54</td>
<td>&lt;0.01</td>
<td>—</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>0.6–2.4</td>
<td>1.3 ± 0.3</td>
<td>2.5 ± 1.9</td>
<td>0.08</td>
<td>—</td>
</tr>
<tr>
<td>pH</td>
<td>7.35–7.45</td>
<td>7.42 ± 0.06</td>
<td>7.33 ± 0.05</td>
<td>&lt;0.01</td>
<td>—</td>
</tr>
<tr>
<td>Base excess (mmol/L)</td>
<td>−2.5–2.5</td>
<td>1.7 ± 5.3</td>
<td>−5.5 ± 4.5</td>
<td>&lt;0.01</td>
<td>—</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>53–110</td>
<td>103 ± 72</td>
<td>201 ± 121</td>
<td>—</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>34.0–45.0</td>
<td>20.2 ± 2.6</td>
<td>9.7 ± 3.5</td>
<td>&lt;0.001</td>
<td>—</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.1 ± 0.5</td>
<td>6.7 ± 1.0</td>
<td>7.2 ± 1.8</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Urea</td>
<td>3.7 ± 0.9</td>
<td>7.4 ± 6.9</td>
<td>9.7 ± 7.9</td>
<td>&lt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1.4 ± 1.8</td>
<td>85 ± 69</td>
<td>219 ± 123</td>
<td>&lt;0.001</td>
<td>0.08</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>5.1 ± 2.9</td>
<td>15.4 ± 13.9</td>
<td>10.8 ± 11.5</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

¹ When values for healthy control subjects were not available (and normal reference values are indicated), only ICU groups were compared by using an independent samples t test (for mean arterial pressure, heart rate, cardiac index, stroke volume, systemic vascular resistance index, hemoglobin, thrombocytes, pH, base excess, and albumin) or a Mann-Whitney test (for leukocytes, lactate, and creatinine). For comparison between the 3 groups, statistical analyses were performed by using Kruskal-Wallis and Mann-Whitney tests and Bonferroni adjustment (for glucose, urea, C-reactive protein, and insulin). SVRI, systemic vascular resistance index; CRP, C-reactive protein; NS, not significant (P > 0.05).

² In-hospital reference values for laboratory markers, except for glucose, urea, CRP, and insulin (measured in our healthy control subjects).

³ Compared with healthy control subjects.

⁴ Compared with ICU control patients.

⁵ Nine septic patients and 3 ICU control patients were treated with inotropic or pressor agents (dobutamine, dopamine, or norepinephrine).

⁶ Means ± SDs (all such values).

⁷ Measured with a pulmonary artery flotation catheter, which was in place in all septic patients and in 2 of 7 ICU control patients.

⁸ Those patients who received insulin treatment (3 septic patients and 1 ICU control patient) were excluded from calculations of the mean values; data were available in 6 ICU control patients and 6 patients with sepsis.
TABLE 4
Plasma amino acid concentrations in intensive care unit (ICU) patients and in healthy control subjects

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Healthy control subjects (n = 16)</th>
<th>ICU control patients (n = 7)</th>
<th>P²</th>
<th>ICU patients with sepsis (n = 10)</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/L</td>
<td>µmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamate</td>
<td>85 ± 20</td>
<td>61 ± 24</td>
<td>&lt;0.05</td>
<td>44 ± 15</td>
<td>&lt;0.001 NS</td>
</tr>
<tr>
<td>Glutamine</td>
<td>634 ± 70</td>
<td>509 ± 87</td>
<td>&lt;0.01</td>
<td>419 ± 89</td>
<td>&lt;0.001 NS</td>
</tr>
<tr>
<td>Serine</td>
<td>102 ± 21</td>
<td>91 ± 15</td>
<td>NS</td>
<td>77 ± 16</td>
<td>&lt;0.01 NS</td>
</tr>
<tr>
<td>Asparagine</td>
<td>49 ± 7</td>
<td>42 ± 20</td>
<td>NS</td>
<td>43 ± 15</td>
<td>NS</td>
</tr>
<tr>
<td>Histidine</td>
<td>80 ± 12</td>
<td>55 ± 7</td>
<td>&lt;0.001</td>
<td>89 ± 64</td>
<td>NS</td>
</tr>
<tr>
<td>Glycine</td>
<td>248 ± 51</td>
<td>238 ± 83</td>
<td>NS</td>
<td>265 ± 95</td>
<td>NS</td>
</tr>
<tr>
<td>Threonine</td>
<td>131 ± 24</td>
<td>102 ± 45</td>
<td>NS</td>
<td>81 ± 28</td>
<td>&lt;0.01 NS</td>
</tr>
<tr>
<td>Alanine</td>
<td>290 ± 88</td>
<td>208 ± 74</td>
<td>0.08</td>
<td>237 ± 86</td>
<td>NS</td>
</tr>
<tr>
<td>Taurine</td>
<td>50 ± 8</td>
<td>26 ± 16</td>
<td>&lt;0.05</td>
<td>32 ± 33</td>
<td>&lt;0.05 NS</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>55 ± 11</td>
<td>49 ± 14</td>
<td>NS</td>
<td>43 ± 17</td>
<td>&lt;0.05 NS</td>
</tr>
<tr>
<td>Valine</td>
<td>196 ± 43</td>
<td>193 ± 56</td>
<td>NS</td>
<td>141 ± 46</td>
<td>0.07 NS</td>
</tr>
<tr>
<td>Methionine</td>
<td>25 ± 4</td>
<td>24 ± 16</td>
<td>NS</td>
<td>25 ± 13</td>
<td>NS</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>63 ± 14</td>
<td>87 ± 37</td>
<td>NS</td>
<td>71 ± 30</td>
<td>NS</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>53 ± 5</td>
<td>79 ± 20</td>
<td>&lt;0.01</td>
<td>104 ± 31</td>
<td>&lt;0.001 NS</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>40 ± 7</td>
<td>60 ± 15</td>
<td>&lt;0.05</td>
<td>72 ± 40</td>
<td>&lt;0.05 NS</td>
</tr>
<tr>
<td>Leucine</td>
<td>113 ± 23</td>
<td>121 ± 45</td>
<td>NS</td>
<td>82 ± 35</td>
<td>NS</td>
</tr>
<tr>
<td>Ornithine</td>
<td>67 ± 14</td>
<td>60 ± 30</td>
<td>NS</td>
<td>48 ± 15</td>
<td>&lt;0.05 NS</td>
</tr>
<tr>
<td>Lysine</td>
<td>169 ± 30</td>
<td>143 ± 71</td>
<td>0.06</td>
<td>118 ± 35</td>
<td>&lt;0.01 NS</td>
</tr>
<tr>
<td>BCAAs</td>
<td>371 ± 79</td>
<td>424 ± 145</td>
<td>NS</td>
<td>318 ± 104</td>
<td>NS</td>
</tr>
<tr>
<td>Sum of AAs</td>
<td>2605 ± 291</td>
<td>2236 ± 443</td>
<td>0.06</td>
<td>2058 ± 397</td>
<td>&lt;0.01 NS</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs. BCAA, branched-chain amino acid; AA, amino acid; NS, not significant (P > 0.05). Statistical analyses were performed by using one-factor ANOVA with Bonferroni adjustment (for glutamate, glutamine, serine, and threonine) or Kruskal-Wallis and Mann-Whitney tests and Bonferroni adjustment (for asparagine, histidine, glycine, alanine, taurine, tyrosine, valine, methionine, isoleucine, phenylalanine, tryptophan, leucine, ornithine, lysine, BCAAs, and sum AAs).

2 Compared with healthy control subjects.

3 Compared with ICU control patients.

Plasma amino acid concentrations

Plasma amino acid concentrations are listed in Table 4 and Figure 2. Plasma glutamate, glutamine, citrulline, and taurine concentrations were significantly lower and phenylalanine and tryptophan concentrations were significantly higher in both septic and control ICU patients than in healthy control subjects. For most of these amino acids, the change was largest in patients with sepsis. Significantly lower concentrations of plasma arginine, serine, threonine, tyrosine, ornithine, lysine, and sum of amino acids were observed in patients with sepsis compared with healthy control subjects; these concentrations were not significant in ICU control patients. Plasma arginine concentrations were 69 ± 37 and 49 ± 12 µmol/L in ICU control patients and patients with sepsis, respectively; were significantly lower in patients with sepsis than in healthy control subjects (92 ± 17 µmol/L; P < 0.001); and tended to be lower in ICU control patients (P = 0.07; Figure 2). Plasma citrulline concentrations were 21 ± 10, 18 ± 6, and 41 ± 7 µmol/L in ICU control patients, patients with sepsis, and healthy control subjects, respectively (P < 0.001 for both groups of ICU patients compared with healthy control subjects; Figure 2).

Arginine and citrulline metabolism

Whole-body arginine production was not significantly different between healthy control subjects and septic and control ICU patients, with values of 72 ± 15, 59 ± 23, and 64 ± 19 µmol·kg⁻¹·h⁻¹, respectively (Figure 3). In contrast, a large reduction was observed in whole-body citrulline production (precursor for de

FIGURE 2. Mean (±SD) plasma concentrations of arginine and citrulline in healthy control subjects (n = 16), control patients in an intensive care unit (ICU) (n = 7), and ICU patients with sepsis (n = 10). ¹Significantly different from healthy control subjects, P < 0.01 (Kruskal-Wallis with Mann-Whitney and Bonferroni adjustment for arginine; one-factor ANOVA with Bonferroni adjustment for citrulline).
novo arginine production), which was significantly lower in patients with sepsis (4.5 ± 2.1 μmol · kg⁻¹ · h⁻¹) than in ICU control patients (10.1 ± 2.9 μmol · kg⁻¹ · h⁻¹; P < 0.01) and healthy control subjects (13.7 ± 4.1 μmol · kg⁻¹ · h⁻¹; P < 0.001). In ICU control patients, citrulline production tended to be lower than in healthy control subjects (P = 0.08; Figure 3). This difference in citrulline production was also indicated by a significantly lower de novo arginine production in septic patients (3.3 ± 3.7 μmol · kg⁻¹ · h⁻¹; P < 0.01) than in healthy control subjects (11.9 ± 6.6 μmol · kg⁻¹ · h⁻¹; P = 0.05; Figure 3).

Regarding the pathways of arginine catabolism, NO production was significantly lower in septic patients (0.8 ± 0.7 μmol · kg⁻¹ · h⁻¹) than in healthy control subjects (2.2 ± 1.2 μmol · kg⁻¹ · h⁻¹; P < 0.01) but did not differ significantly between ICU control patients (1.5 ± 1.0 μmol · kg⁻¹ · h⁻¹) and septic patients (Figure 3). In contrast, higher concentrations of plasma nitrate were observed in septic ICU patients (123 ± 85 μmol · L⁻¹) than in ICU control patients (40 ± 19 μmol · L⁻¹; P < 0.05) and healthy control subjects (37 ± 20 μmol · L⁻¹; P < 0.01; Figure 3). The conversion of arginine to urea (ie, arginase activity) was significantly higher in septic patients (40 ± 29 μmol · kg⁻¹ · h⁻¹) than in healthy control subjects (10 ± 5 μmol · kg⁻¹ · h⁻¹; P < 0.01; Figure 3). Arginase activity was 30 ± 32 μmol · kg⁻¹ · h⁻¹ in ICU control patients. As a consequence, the percentage of whole-body arginine production that is used by the arginase pathway was much larger in septic patients (46 ± 28%; n = 8) than in healthy control subjects (15 ± 8%; n = 16; P < 0.05) but was not significantly different from that in ICU control patients (26 ± 15%; n = 6; Kruskal-Wallis with Mann-Whitney analysis).

A significant correlation between whole-body citrulline production and de novo arginine production was found for all subjects combined (r = 0.8, P < 0.001) as well as for the different subgroups (ICU control patients: r = 0.8, P < 0.05; healthy control subjects: r = 0.7, P < 0.05). A significant

**FIGURE 3.** Parameters (mean ± SD) of arginine anabolism (left panels) and pathways involved in arginine catabolism (right panels) in healthy control subjects (n = 16), control patients in an intensive care unit (ICU) [n = 7, except for de novo arginine production and arginase activity (n = 6)], and ICU patients with sepsis (n = 9). For plasma nitrate, healthy control values were derived from a historic control group of elderly subjects (n = 9). Significantly different from healthy control subjects: *P < 0.01. Significantly different from ICU control patients: **P < 0.05, ***P < 0.01. One-factor ANOVA with Bonferroni adjustment was used for whole-body production (WB Ra) of citrulline and nitric oxide (NO) production, and Kruskal-Wallis with Mann-Whitney analysis and Bonferroni adjustment was used for WB Ra arginine, de novo arginine production, plasma nitrate, and arginase activity.
correlation was also observed between de novo arginine production and NO production \( r = 0.5, P < 0.05 \) when all subjects were combined.

**Protein metabolism**

Whole-body protein breakdown, which was measured as whole-body phenylalanine production, was higher in patients with sepsis \((62 \pm 21 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1})\) than in healthy control subjects \((36 \pm 9 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}; P < 0.01\) and was also higher in ICU control patients \((51 \pm 13 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1})\) than in healthy control subjects \((P < 0.05; \text{Figure 4})\). This was also indicated by the data on whole-body urea production, which was significantly higher in both ICU groups \((1667 \pm 908\) and \(2002 \pm 1184 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\) in ICU control and septic patients, respectively; \(P < 0.05\) and \(P < 0.01\), respectively) than in healthy control subjects \((509 \pm 184 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}; \text{Figure 5})\). Whole-body protein synthesis was also significantly higher in patients with sepsis \((60 \pm 24 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1})\) than in healthy control patients \((32 \pm 6 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}; P < 0.05\), whereas it tended to be higher in ICU control patients than in healthy control subjects \((46 \pm 14 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}; P = 0.08\).

Phenylalanine hydroxylation, which indicates net protein breakdown, did not differ between the groups \((4.3 \pm 2.7, 2.4 \pm 1.7, \text{ and } 4.6 \pm 2.9 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\) in healthy subjects, ICU control subjects, and ICU patients with sepsis, respectively; \text{Figure 4}). Infusion of 5% glucose intravenously did not affect whole-body protein breakdown in the 3 control patients who received a small glucose load throughout the study (data not shown).

**DISCUSSION**

Our data show that whole-body in vivo arginine and citrulline metabolism is greatly affected in patients with septic shock and severe inflammation. This is characterized by a large reduction in citrulline production, a reduction in de novo arginine production, a decrease in NO production, and enhanced arginase activity. Although a small reduction (tendency) in plasma arginine concentration and citrulline production also occurred in our ICU control patients with moderate inflammation, it was not associated with a significant change in de novo arginine production, NO production, or arginase activity. This suggests that, in this small-scale study, changes in de novo arginine production reduce arginine availability in sepsis and impair NO production. This impaired NO production might be related to diminished microcirculation and organ perfusion, which are prominent features of septic shock (35).

**Changes in plasma amino acid concentrations**

Of the changes in plasma amino acid concentrations in septic patients, citrulline (56%), glutamate (48%), and arginine (47%) showed the greatest decreases when compared with concentrations in healthy control subjects. Glutamine (33%), serine (25%), threonine (38%), taurine (36%), ornithine (28%), and lysine (30%) concentrations also decreased, and plasma total amino acid concentration decreased by 21%. In contrast, a 96% higher plasma phenylalanine concentration and an 80% higher tryptophan concentration were associated with sepsis. To a lesser extent, similar changes were also observed in the ICU control patients with moderate inflammation. This indicates that
inflammation induces changes in the amino acid profile, which are most pronounced when inflammation is severe. These data are largely in agreement with previous observations (13, 14, 37, 38). The reduction in plasma amino acid concentrations with inflammation is attributed, in large part, to an enhanced metabolic clearance of the released amino acids (14), probably facilitated by the liver for hepatic protein synthesis, gluconeogenesis, and urea synthesis (39, 40). Urea production was indeed elevated in both ICU patient groups.

The HOMA index was elevated in both ICU patient groups, as it was in patients with type 2 diabetes (41). This finding indicates that insulin sensitivity is impaired in ICU patients, which is also the basis for the insulin treatment trials in ICU patients (42).

Changes in arginine and citrulline metabolism

In-depth studies of changes in arginine and citrulline metabolism in adult patients with sepsis are limited. One study measured arginine flux and the NOx production rate in hypotensive sepsis (15), and another study investigated changes in amino acid kinetics by measuring amino acid clearance from the circulation in adult patients with sepsis (14). In pediatric patients with sepsis, arginine metabolism was studied in detail by using stable isotopes (36). Our data showed that specific changes in the endogenous arginine synthesis pathway are present in sepsis, as indicated by a reduction in whole-body citrulline production and by a subsequent reduction in de novo arginine production. This reduction in de novo arginine production was not observed in septic children because they had an increase in whole-body citrulline production (36). This may indicate an age-related phenomenon. However, as in the present study, we also observed lower plasma citrulline and arginine concentrations in children with inflammation in a previous study (43). Because citrulline is produced from glutamine in the gut (3) and has been indicated as a marker of intestinal function (44), with glutamine depletion resulting in reduced plasma citrulline (6), impaired gut function or reduced glutamine availability could underlie the changes in de novo arginine production in adult sepsis. The importance of the gut is further supported by the fact that diminished renal function does not affect de novo arginine production, even though the kidney is the main site of arginine production from citrulline (3–5, 45). Protein breakdown (indicated by whole-body phenylalanine and urea production) was elevated to a greater extent in patients with sepsis than in ICU control patients, which is characteristic of the hypercatabolic state of sepsis (46). Our data showed no change in whole-body arginine production in ICU patients compared with healthy subjects, which agrees with the findings of a previous study in septic children (36); however, we did not confirm the reduced arginine flux that was shown by Villalpando et al (15) in patients with hyperdynamic septic shock.

When considering the main pathways that use arginine (ie, arginase, NOS, and protein synthesis), the increase in arginase activity and protein synthesis with inflammation was greater in patients with sepsis (severe inflammation). This finding seems in line with the 107% increase in arginine clearance that was previously described in septic adults (14). An increase in arginase activity, however, was not observed in septic children (36). This activation of arginase in sepsis may be arginase-isoform (arginase I and II) specific, time-specific, and tissue-specific, as was shown in lipopolysaccharide-induced sepsis in animal models (47, 48).

NO production, however, as measured with the use of stable isotopes, was lower in our patients with sepsis. This finding appears to contrast with the finding of enhanced NO production in animal models of sepsis (49, 50) and in critically ill children (36). However, our data seem in line with the finding of a slower fractional synthesis rate in adult patients with sepsis than in healthy control subjects and of equal absolute synthesis rates of NOx in both groups (15). Metabolic differences between animals and humans and age-dependent differences in the response to sepsis could play a role. In the present study, a discrepancy existed between stable isotope–measured NO production and NO production based on plasma nitrate; the latter, which was higher in the sepsis group, is in line with the findings of previous studies (16–19). This discrepancy may be explained by the fact that nitrate production from arginine is not elevated in septic patients (15), as was found for NO production in our study; impaired renal excretion of nitrate in this group (as indicated by elevated plasma creatinine concentrations in our study) contributes to the high plasma nitrate concentrations in these patients (15, 17). Another factor that might contribute to this discrepancy is time-specific changes in NOS enzyme activity in sepsis (47, 48, 50, 51). As a consequence, direct stable isotope measurement of the NO production rate is not completely synchronous with indirect plasma nitrate measurement. The latter is delayed by \(~20\) h through conversion of NO and urinary excretion (52). Potential exogenous sources of nitrate (eg, nutrition or NO medication) are considered of minor importance, because nutrition (total parenteral nutrition or tube feeding) was stopped before the measurement and none of the patients received NO medication. Therefore, impaired renal nitrate clearance and variability in the rate of NO production in the course of sepsis could contribute to the discrepancy between plasma nitrate and NO production rate and to the diversity in NO production rates as reported in the literature in human and animal sepsis models. However, this concept needs to be studied further.

On the basis of these data, we hypothesized that moderate inflammation initiates a slightly enhanced protein breakdown and a slightly (not significant) lower plasma arginine concentration (Figure 6). A state of severe inflammation, such as in septic shock, greatly increases protein breakdown and arginase activity and greatly decreases citrulline and de novo arginine production. These metabolic alterations greatly limit arginine availability, especially for NO production by the NOS3 enzyme (53). Activity of the NOS3 enzyme was recently linked to the enzymes for de novo arginine production (54), and a close relation between endothelial NO production and the citrulline-NO cycle has been shown (55). Regarding the role of NOS3 in endothelial NO production, which is important for the control of vascular dilatation and microcirculation (56), citrulline production as a source of de novo arginine production can be considered an important metabolic pathway. Citrulline supplementation can enhance systemic citrulline and arginine availability in healthy subjects (6); however, further investigation is required to determine whether citrulline can also support arginine metabolism in sepsis.

In conclusion, in this small-scale study we observed that citrulline production is severely reduced in patients with sepsis and is related to diminished de novo arginine and NO production.
These metabolic alterations contribute to reductions in citrulline and arginine availability and offer the potential for intervention to restore arginine metabolism in sepsis.

Nutritional supplementation with citrulline might be a way to achieve this goal, but this needs further study in larger patient groups before it can be recommended for the target population. Moreover, the relevance of the observed changes in arginine metabolism needs to be related to clinically relevant arginine-dependent processes that are abnormal in sepsis, such as decreases in microcirculation or in T cell function.

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