Arginine, citrulline and nitric oxide metabolism in sepsis

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ABSTRACT

Arginine has vasodilatory effects, via its conversion by NO synthase into NO, and immunomodulatory actions which play important roles in sepsis. Protein breakdown affects arginine availability and the release of asymmetric dimethylarginine, an inhibitor of NO synthase, may therefore affect NO synthesis in patients with sepsis. The objective of the present study was to investigate whole-body in vivo arginine and citrulline metabolism and NO synthesis rates, and their relationship to protein breakdown in patients with sepsis or septic shock and in healthy volunteers. Endogenous leucine flux, an index of whole-body protein breakdown rate, was measured in 13 critically ill patients with sepsis or septic shock and seven healthy controls using an intravenous infusion of [1-13C]leucine. Arginine flux, citrulline flux and the rate of conversion of arginine into citrulline (an index of NO synthesis) were measured with intravenous infusions of [15N2]guanidino-arginine and [5,5-2H2]citrulline. Plasma concentrations of nitrite plus nitrate, arginine, citrulline and asymmetric dimethylarginine were measured. Compared with controls, patients had a higher leucine flux and higher NO metabolites, but arginine flux, plasma asymmetric dimethylarginine concentration and the rate of NO synthesis were not different. Citrulline flux and plasma arginine and citrulline were lower in patients than in controls. Arginine production was positively correlated with the protein breakdown rate. Whole-body arginine production and NO synthesis were similar in patients with sepsis and septic shock and healthy controls. Despite increased proteolysis in sepsis, there is a decreased arginine plasma concentration, suggesting inadequate de novo synthesis secondary to decreased citrulline production.

INTRODUCTION

Arginine is a dispensable amino acid in normal health, but may be conditionally essential in stressed states such as sepsis [1]. In addition to acting as a substrate for protein synthesis, arginine serves important physiological roles, including ammonia detoxification to urea, cell growth and differentiation, wound healing and immune function. Arginine is also the only substrate for synthesis of NO by NOS (NO synthase), and the arginine–NO system is important in the regulation of vascular tone and blood pressure [2]. Low plasma arginine has been correlated with a worse prognosis in patients with sepsis [3], suggesting that there may be an overall increase in the requirement for arginine that is not met by endogenous production. At physiological concentrations of arginine, eNOS (endothelial NOS) should be saturated; however, supplementation of arginine in humans increases eNOS
activity, a discrepancy known as the l-arginine paradox [4]. Thus plasma arginine levels and arginine uptake can limit NO synthesis [5]. Decreased NO synthesis may underlie the poorer outcome seen in patients with sepsis with low plasma arginine.

Although increased NO synthesis has been implicated in the vasodilation and resistance to vasopressor drugs seen in septic shock [6], a multicentre trial of the NOS inhibitor N⁶-methyl-L-arginine in patients with septic shock was stopped early because of increased 28-day mortality in the drug-treated group [7]. On the other hand, in patients with acute lung injury, of whom 25 % had sepsis, higher urine NOx (NO metabolites; nitrites and nitrates), indicating increased NO synthesis, were associated with improved outcomes [8]. Taken together, these findings suggest that adequate NO synthesis, and therefore arginine availability, is critical for survival in sepsis. However, the actual picture is not clear because the limited studies of in vivo arginine and NO production in humans with sepsis have produced conflicting results. Although one study reported a faster NO synthesis but no difference in arginine flux between children with sepsis and healthy adults [9], another study reported a lower arginine flux but no difference in NO synthesis in hypotensive adults with sepsis compared with healthy controls [10].

Arginine is derived from the diet, de novo synthesis and protein breakdown. Citrulline, the precursor for arginine synthesis, is a non-protein amino acid produced from intestinal glutamine metabolism [11]. In humans, the major source of arginine is whole-body protein breakdown, since de novo arginine synthesis constitutes only 5–15 % of endogenous arginine flux [12]. Along with arginine availability, endogenous inhibitors of NOS, including ADMA (asymmetric dimethylarginine), may affect NO synthesis [13]. Dimethylarginines are synthesized by the methylation of arginine residues in proteins and are subsequently released by proteolysis [14]. We hypothesized that the rate of protein breakdown in patients with sepsis would affect both arginine availability and ADMA release and therefore the rate of NO synthesis. In the present study we tested this hypothesis by determining whole-body arginine and citrulline fluxes, the rate of NO synthesis and plasma ADMA concentration and their relationship to protein breakdown in patients with sepsis or septic shock and in healthy controls.

**MATERIALS AND METHODS**

**Subjects**

The study was reviewed and approved by the Institutional Review Board at the Baylor College of Medicine in Houston, TX, U.S.A. Thirteen adult patients admitted with sepsis to the MICU [medical ICU (intensive care unit)] of Ben Taub General Hospital in Houston, TX, U.S.A. were enrolled in the study. Sepsis was defined according to the International Sepsis Definitions Conference [15]. All patients had clinical evidence of infection and met the definition of sepsis. Five of the 13 patients also satisfied the criteria for septic shock. All MICU patients had been diagnosed with sepsis in the 48-h period prior to the study, and nine of the patients were studied within 24 h of admission. None of the patients were receiving systemic corticosteroids or had a diagnosis of malignancy or end-stage renal disease.

Seven healthy adult volunteers participated in the study as control subjects. All control subjects were in good health as established by medical history, physical examination and blood chemistry measurements. They were selected to be similar in age, gender and BMI (body mass index) to the patients. All patients and controls were enrolled after written, informed consent was obtained. The present study was part of a larger investigation of metabolic changes in patients with sepsis.

**Isotope tracer infusion**

Tracer infusions were performed in healthy subjects in the adult GCRC (General Clinical Research Center) of Baylor College of Medicine, and the patients with sepsis were studied in the MICU of Ben Taub General Hospital.

Endogenous leucine flux (an index of whole-body protein breakdown), arginine flux and citrulline flux were measured with the tracers [1-¹³C]leucine, [¹⁵N₂]guanidino-arginine and [5,5-²H₂]citrulline respectively. The conversion of [¹⁵N₂]guanidino-arginine into [¹⁵N]citrulline, an index of NO synthesis, was determined. Sterile solutions of [¹³C]leucine, [¹⁵N₂]arginine, [²H₂]citrulline and [¹⁵N]citrulline (Cambridge Isotope Laboratories) were prepared in 9 g/l of saline.

After an overnight fast, the healthy subjects were admitted to the GCRC and an intravenous catheter was placed in an antecubital vein for isotope infusions and in a hand vein of the contralateral arm for blood sampling. The hand was heated to arterialize blood samples. After a baseline blood sample was obtained and primed constant infusions of [¹⁵N₂]arginine (prime = 8 μmol/kg of body weight, infusion = 8 μmol·kg⁻¹·h⁻¹ of body weight·h⁻¹) and [²H₂]citrulline (prime = 1 μmol/kg of body weight, infusion=1 μmol·kg⁻¹ of body weight·h⁻¹) were administered for 6 h. The citrulline pool was also primed with [¹⁵N]citrulline (prime = 0.16 μmol/kg of body weight). At 2 h, a primed constant infusion of [1-¹³C]leucine (prime = 6 μmol/kg of body weight, infusion = 6 μmol·kg⁻¹·h⁻¹ of body weight·h⁻¹) was started and maintained for 4 h. Additional blood samples were collected at 30 min intervals during the last 90 min of the infusions.

The same tracer infusions were performed in patients with sepsis who had been fasting for at least 8 h. The tracers were given through a pre-existing central venous catheter or through an intravenous catheter placed in the antecubital fossa. Blood samples were obtained
from pre-existing arterial or central venous catheters. Although blood samples were drawn from arterial and central venous sites in patients with sepsis and heated dorsal hand veins in healthy controls, the use of heated dorsal hand vein sampling as a surrogate for direct arterial sampling has previously been validated [16,17].

Sample analysis
The blood samples were drawn into prechilled tubes containing sodium fluoride and potassium oxalate. The tubes were centrifuged immediately at 4 °C, and the plasma was removed and stored immediately at −70 °C for later analysis.

The plasma isotopic enrichment of α-KICA (α-oxoisocaproic acid; α-ketoisocaproic acid), a surrogate of intracellular leucine, was measured by negative chemical ionization GC/MS of its pentafluorobenzyl derivative and monitoring of ions at m/z 129 and 130. The plasma arginine and citrulline isotopic enrichments were measured by tandem LC/MS. Plasma arginine and citrulline were converted into their DANS [5-(dimethylamino)-1-naphthalene sulfonamide] derivatives and analysed on a triple quadrupole mass spectrometer (TSQ Quantum Ultra; Thermo Fisher Scientific), equipped with an ESI (electrospray ionization) source, a Survey pump (Thermo Fisher Scientific) and a HTCL PAL autosampler (Leap Technologies). The ions were then analysed by SRM (selected reaction monitoring) mode. The transitions observed were precursor ion m/z 408 to product ion m/z 170 at 34 eV for arginine, and precursor ion m/z 409 to product ion m/z 392 at 14 eV for citrulline. Instrumental control, data acquisition and analysis were performed by the XCalibur (version 2.0) software package (Thermo Fisher Scientific).

Plasma arginine, citrulline and glutamine concentrations were measured by standard ion-exchange chromatography. Plasma concentrations of NOx were measured by in vitro isotope dilution as previously described [10]. Plasma concentrations of ADMA were also measured by in vitro isotope dilution. Briefly, 200 μl of the baseline plasma sample was spiked with a known quantity of [2H7]ADMA (Cambridge Isotope Laboratories) as an IS (internal standard). ADMA was then converted into its DANS derivative. The samples were analysed by LC/MS on an Atlantis T3 3 μm 2.1 mm × 150 mm column (Waters). ADMA and SDMA (symmetric dimethylarginine) were well separated by HPLC. Plasma ADMA and its IS were analysed by selected reaction monitoring at precursor ion m/z 436 to product ion m/z 170 for ADMA, and precursor ion m/z 443 to product ion m/z 170 for its [2H7]IS.

Calculations
The rate of appearance or total flux (Q) of leucine, arginine and citrulline were calculated from the steady-state equation (eqn 1):

\[
Q = \frac{(I_{E_{\text{inf}}}/I_{E_{\text{plateau}}}) \times i}{10^{-3} \text{kg}^{-1} \text{h}^{-1}}
\]

where \( I_{E_{\text{inf}}} \) is the isotopic enrichment (mole percentage excess) of leucine, arginine or citrulline in the infusate, \( I_{E_{\text{plateau}}} \) is the isotopic enrichment of α-KICA, arginine or citrulline in plasma at the isotopic steady-state, and \( i \) is the infusion rate of the tracer in μmol·kg⁻¹·h⁻¹. Endogenous leucine, arginine and citrulline fluxes (the rates of production of the given amino acids by the body) were determined by subtracting their infusion rate, \( i \), from \( Q \).

Under steady-state conditions, the rate of appearance of arginine equals the rate of disappearance. Therefore (eqn 2):

\[
Q_{\text{Arg}} = \frac{Q_{\text{Arg}}}{\text{plasma arginine concentration}}
\]

The NO synthesis rate was calculated from the rate of conversion of arginine into citrulline via the NOS reaction as previously described (eqn 3) [18]:

\[
\text{NO synthesis (} \mu\text{mol·kg}^{-1} \text{·body weight} \cdot \text{h}^{-1}) = \frac{Q_{\text{Arg}}}{\text{Cit}} \times IE_{\text{Cit}}/IE_{\text{Arg}} \times (Q_{\text{Arg}}/I_{\text{Arg}} + Q_{\text{Arg}})
\]

Where \( Q_{\text{Arg}} \) and \( Q_{\text{Cit}} \) are the fluxes of arginine and citrulline, \( IE_{\text{Cit}} \) is the plasma enrichment of the M+1 isotopomer of citrulline (that is, ureido-[15N]citrulline derived from [15N2]arginine), \( IE_{\text{Arg}} \) is the plasma enrichment of the M+2 isotopomer of arginine and \( I_{\text{Arg}} \) is the rate of infusion of [15N2]arginine.

Statistical analysis
Continuous variables were summarized by group as means ± S.E.M. unless otherwise indicated. Differences between groups of subjects were assessed by the unpaired Student’s t test. Tests were considered statistically significant if \( P < 0.05 \). Correlations were performed using Pearson’s correlation (r). Data analysis was performed with STATA software (version 9).

RESULTS

Subject characteristics
The characteristics of the patients and the controls are presented in Table 1. There were no significant differences in age, gender, ethnicity, weight or BMI between the two groups. The diagnoses of the 13 patients with sepsis were as follows: pneumonia in five patients, urosepsis in four patients, cellulitis in three patients and septic arthritis in one patient. Blood cultures drawn at admission from 11 of the 13 patients were positive. In eight patients,
blood cultures grew Gram-positive organisms. Of these, five were Streptococci, two were Staphylococci and one was Enterococcus. The remaining three patients had blood cultures that grew Gram-negative organisms. Five patients met the criteria for septic shock. Six patients had a previous diagnosis of Type 2 diabetes mellitus. The mean APACHE II score of the patients was 20 ± 2. There was no significant difference in APACHE II scores in patients with shock compared with those without shock. Four patients died during hospitalization.

### Leucine, arginine, citrulline and NO kinetics

Because leucine is an essential amino acid, in the fasted state its flux is derived only from whole-body protein breakdown. Therefore the endogenous flux of leucine is a reflection of the rate of whole-body proteolysis. Endogenous leucine flux was significantly higher in patients with sepsis than in the controls (P = 0.02; Table 2). There was no significant difference in arginine flux between patients and controls (P = 0.60), but the plasma concentration of arginine was significantly lower in patients with sepsis compared with controls (P < 0.001; Table 2). Arginine clearance was significantly higher in patients compared with controls (P = 0.001; Table 2). Citrulline flux was significantly lower in patients than in controls (P < 0.001), and plasma concentrations of citrulline and its precursor, glutamine, were significantly lower in the patients compared with controls (P < 0.001; Table 2). In patients with sepsis, arginine flux was positively correlated with endogenous leucine flux in healthy controls (r = 0.92, P = 0.003).

The rate of NO synthesis was not different in patients with sepsis than in controls (Table 2), or in patients with septic shock compared with patients without shock (0.19 ± 0.06 compared with 0.20 ± 0.06 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \) of

### Table 1  Demographic characteristics, vital signs and blood chemistry indices in controls (n = 7) compared with patients with sepsis (n = 13)

<table>
<thead>
<tr>
<th>Characteristic/measurement</th>
<th>Controls</th>
<th>Patients with sepsis</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female) (n)</td>
<td>4/3</td>
<td>8/5</td>
<td></td>
</tr>
<tr>
<td>Ethnicity (hispanic/non-hispanic) (n)</td>
<td>3/4</td>
<td>5/8</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>49 ± 6</td>
<td>54 ± 10</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72.7 ± 14.1</td>
<td>75.9 ± 23.9</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.4 ± 3.7</td>
<td>26.0 ± 7.2</td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>35.8 ± 0.7</td>
<td>37.6 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>88 ± 10</td>
<td>78 ± 16</td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>75 ± 14</td>
<td>112 ± 15</td>
<td></td>
</tr>
<tr>
<td>Respiration (breaths/min)</td>
<td>18 ± 2</td>
<td>23 ± 9</td>
<td></td>
</tr>
<tr>
<td>Leucocyte count (K/μl)</td>
<td>6.3 ± 1.6</td>
<td>15.3 ± 8.4†</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.9 ± 0.2</td>
<td>1.7 ± 0.8‡</td>
<td></td>
</tr>
<tr>
<td>AST (units/l)</td>
<td>18 ± 5</td>
<td>88 ± 99</td>
<td></td>
</tr>
<tr>
<td>ALT (units/l)</td>
<td>16 ± 6</td>
<td>47 ± 20*</td>
<td></td>
</tr>
</tbody>
</table>

(A) Whole-body endogenous leucine, arginine and citrulline kinetics, and plasma concentrations of arginine, citrulline and glutamine in controls with patients with sepsis

(B) Plasma concentrations of arginine, citrulline and glutamine in controls (n = 7) compared with patients with sepsis (n = 13). Values are means ± S.E.M. *P < 0.05, patients with sepsis compared with controls (measured using an unpaired Student’s t test).

### Table 2 Whole-body endogenous leucine, arginine and citrulline kinetics, the rate of NO synthesis, and plasma concentrations of arginine, citrulline and glutamine in controls with patients with sepsis

(A) Whole-body endogenous leucine, arginine and citrulline kinetics and the rate of NO synthesis from \( ^{15} \text{N} \)-labelled citrulline in controls (n = 13) compared with patients with sepsis (n = 13). Values are means ± S.E.M. *P < 0.05, patients with sepsis compared with controls (measured using an unpaired Student’s t test).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls</th>
<th>Patients with sepsis</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endogenous leucine flux (( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} ) of body weight)</td>
<td>89.7 ± 3.0</td>
<td>130.8 ± 11.8</td>
<td>0.02*</td>
</tr>
<tr>
<td>Arginine flux (( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} ) of body weight)</td>
<td>48.7 ± 2.8</td>
<td>53.0 ± 5.5</td>
<td>0.60</td>
</tr>
<tr>
<td>Arginine clearance (ml · kg⁻¹ · body weight · min⁻¹)</td>
<td>9.7 ± 0.8</td>
<td>23.6 ± 2.6</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Citrulline flux (( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} ) of body weight)</td>
<td>10.6 ± 0.8</td>
<td>4.4 ± 0.5</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>NO synthesis (( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} ) of body weight)</td>
<td>0.15 ± 0.04</td>
<td>0.20 ± 0.04</td>
<td>0.47</td>
</tr>
</tbody>
</table>

(B) Plasma concentration (\( \mu \text{mol/l} \))
Table 3 Correlations between different parameters in patients with sepsis (n = 13)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pearson’s correlation (r)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endogenous leucine flux compared with endogenous arginine flux</td>
<td>0.79</td>
<td>0.001*</td>
</tr>
<tr>
<td>Arginine flux compared with NO synthesis</td>
<td>0.52</td>
<td>0.07</td>
</tr>
<tr>
<td>Endogenous leucine flux compared with NO synthesis</td>
<td>0.17</td>
<td>0.57</td>
</tr>
<tr>
<td>NO synthesis compared with mean arterial pressure</td>
<td>0.0016</td>
<td>1.0</td>
</tr>
<tr>
<td>Plasma ADMA compared with NO synthesis</td>
<td>0.27</td>
<td>0.38</td>
</tr>
<tr>
<td>Plasma arginine compared with plasma citrulline</td>
<td>0.77</td>
<td>0.002*</td>
</tr>
<tr>
<td>Plasma citrulline compared with plasma glutamine</td>
<td>0.69</td>
<td>0.009*</td>
</tr>
<tr>
<td>Plasma ADMA compared with endogenous leucine flux</td>
<td>−0.09</td>
<td>0.78</td>
</tr>
<tr>
<td>Plasma ADMA compared with arginine flux</td>
<td>0.14</td>
<td>0.65</td>
</tr>
<tr>
<td>Plasma ADMA compared with creatinine</td>
<td>0.35</td>
<td>0.24</td>
</tr>
<tr>
<td>Plasma ADMA compared with ALT</td>
<td>0.27</td>
<td>0.38</td>
</tr>
<tr>
<td>Plasma ADMA compared with AST</td>
<td>−0.28</td>
<td>0.35</td>
</tr>
</tbody>
</table>

*P < 0.05. ALT, alanine aminotransferase; AST, aspartate aminotransferase.

There was no significant correlation between the rate of NO synthesis and mean arterial pressure. However, plasma NOx levels were significantly higher in patients with sepsis than in controls (P = 0.02; Table 2). There was a positive correlation between endogenous arginine flux and NO synthesis rate which just failed to reach statistical significance (P = 0.07; Table 3).

Although there was no difference in plasma concentrations of ADMA between patients with sepsis and controls, patients with sepsis had a wider range of ADMA concentrations (Figure 1). In the patient cohort, black-filled circles represent patients who survived and grey-filled circles represent patients who died.

**DISCUSSION**

The present study aimed to compare arginine and citrulline flux, plasma ADMA concentration and the rate of NO synthesis in patients with sepsis and septic shock and healthy volunteers, and to determine the relationship of these measurements to each other and to protein breakdown. The results show that, although leucine flux, an index of protein breakdown rate, was significantly higher in patients with sepsis than in healthy controls, there were no differences in arginine flux, plasma ADMA concentration and the rate of NO synthesis between patients and controls. However, higher arginine clearance was associated with a lower plasma arginine concentration, and both citrulline flux and its plasma concentration were significantly lower in patients than in controls. Furthermore, arginine production was positively correlated with the protein breakdown rate in patients with sepsis. These findings suggest that, although more arginine is being released from protein breakdown in patients with sepsis, its plasma concentration is decreased because of increased clearance. Decreased conversion of citrulline into arginine, leading to inadequate *de novo* arginine synthesis, may also be a contributing factor. Finally, synthesis of NO at the whole-body level does not seem to be altered by sepsis.

In the fasted state, all of leucine and the majority of arginine flux will be derived from the breakdown of body proteins [12]. The positive correlation between endogenous arginine and leucine flux was therefore expected, and parallel increases in both arginine and leucine fluxes have been shown in hypercatabolic states, such as burn injury [19,20]. In the present study, however, patients with sepsis had higher leucine flux, indicating faster protein breakdown, but similar arginine flux compared with controls. These results agree with those of Argaman et al. [9] who reported that children with sepsis had higher leucine flux, but similar arginine flux compared with healthy adults. One explanation may be that there is some compartmentalization of arginine produced; for instance, arginine present in the urea cycle may not be in equilibrium with the rest of the body pool [21]. Alternatively, impaired *de novo* arginine synthesis may explain the lack of increase in arginine flux despite its accelerated release from proteolysis. *De novo* arginine synthesis was not measured directly, because of the use of the [5,5-2H2]citrulline tracer. Castillo et al. [18] found
only a low level of arginine labelling with this tracer, which was attributed to recycling of the $^2\text{H}_2$ label [18].

De novo arginine synthesis in patients with sepsis may have been impaired because of decreased citrulline synthesis. In animal studies, it has been shown that citrulline is produced from intestinal glutamine and proline metabolism, and the majority is taken up by the kidney and converted into arginine [22,23]. A recent study in fasting non-cirrhotic patients confirmed in vivo the presence of the glutamine–citrulline–arginine pathway in humans and demonstrated a significant correlation between renal citrulline uptake and arginine release [24]. In a study in healthy humans, citrulline produced from glutamine contributed to 64% of de novo arginine synthesis [25]. In the present study, our finding that patients with sepsis had significantly lower citrulline flux and lower plasma concentration compared with healthy controls strongly suggests that decreased citrulline production led to decreased de novo arginine synthesis. This argument is further supported by the strong correlation between citrulline and arginine concentrations, a finding also seen in critically ill children [26]. Since gut citrulline synthesis is dependent on glutamine and proline availability, the decreased citrulline production seen in patients with sepsis probably reflects a decrease in glutamine and/or proline availability or metabolism. In support of this, we found that plasma glutamine concentrations were markedly lower in the patients with sepsis compared with the healthy controls, and glutamine concentrations were strongly correlated with citrulline concentrations. Furthermore, others have reported lower plasma proline concentrations in patients with sepsis [27]. Since glutamine is the primary precursor of de novo arginine synthesis, it has been suggested that glutamine supplementation may serve as a way of stimulating arginine synthesis in sepsis [28]. However, further information is needed on the metabolic interrelationships between glutamine, citrulline and arginine in sepsis.

Argaman et al. [9] previously found that, despite unchanged arginine flux, children with sepsis had a negative arginine balance owing to limited de novo synthesis and increased oxidative losses. The authors concluded that arginine is conditionally indispensable in sepsis. In the present study, there was no difference in endogenous arginine flux between the patients with sepsis and healthy controls, plasma arginine concentration was markedly lower in patients with sepsis, a finding which agrees with previous reports [10,27]. The lower plasma arginine concentration is secondary to increased clearance of arginine, which may be due to greater hepatic extraction or uptake by peripheral tissues [27]. Arginine supplementation may increase plasma arginine concentrations in sepsis and restore positive arginine balance.

In earlier studies, the plasma concentration of NOx was found to be markedly increased in patients with septic shock [29,30], which led to the generally accepted hypothesis that increased NO synthesis was responsible for the hypotension associated with sepsis [6]. However, in a large clinical trial, inhibition of NOS in sepsis improved blood pressure and vascular resistance, but increased mortality [7], suggesting that NO may be beneficial in sepsis. In the present study, we found increased plasma NOx concentrations in patients with sepsis compared with controls, but no difference in the rate of NO synthesis. Our findings are in accordance with a previous study by our group [10], which found increased NOx levels in hypotensive adults with sepsis compared with controls, but no difference in NO synthesis. The increase in NOx concentrations, without a concomitant increase in the rate of NO synthesis, may be due to altered renal function and extracellular volume changes in sepsis, which may affect the estimation of NO production using NOx concentrations [31]. Alternatively, measurement of NO production by stable isotope methods may not account for possible compartmentalization of arginine intracellularly and within organs or differential production of NO by different isoforms of NOS [32]. Although at the whole-body level NO synthesis is neither increased nor decreased in patients with sepsis, further understanding of the regulation of NO synthesis in sepsis, particularly at an organ-specific level, is needed. For example, maintaining adequate NO synthesis in sepsis may be necessary for protection of the kidney by causing local vasodilation and inhibiting platelet aggregation and leucocyte adhesion [33].

Another regulator of NO synthesis in sepsis is ADMA. In critically ill patients, increased plasma ADMA has been shown to be a risk factor for ICU mortality [34], death during ICU stay, duration of ICU stay, duration of inotropic and vasopressor treatment, mean APACHE II score and duration of ventilatory support [35]. The major mechanism for elimination of ADMA from the body is degradation by the enzyme DDAH (dimethylarginine dimethylaminohydrolase) [14]. Nijveldt et al. [36] hypothesized that ADMA concentration increases in critically ill patients because of increased release from proteolysis and decreased elimination secondary to renal and hepatic failure. In the present study, plasma concentrations of ADMA were not different between patients and controls, although patients had a much wider range of ADMA levels. There was no difference in ADMA concentrations in patients who died compared with those who survived; however, this was a small group of subjects and was probably underpowered to detect such a difference. Of clinical importance is the fact that, of the four patients with the highest ADMA levels, three of them died, which may support previous evidence that ADMA is a marker of mortality; however, there was no correlation between ADMA concentrations and the rate of NO synthesis or renal or hepatic function. It is possible that a static measurement of plasma ADMA concentration is insufficient to reveal...
the metabolic relationship between ADMA and NO synthesis.

In summary, whole-body arginine production and NO synthesis are similar in patients with sepsis and septic shock compared with healthy controls. Decreased de novo synthesis of arginine secondary to decreased citrulline production may be an important factor in arginine homeostasis in sepsis. However, the extent of contribution of the organ-specific or intracellular pool of arginine to NO formation has not yet been established. Further studies in sepsis are needed to determine the interrelationships between arginine, citrulline and glutamine metabolism and the potential utility of supplementation with arginine and/or glutamine. Finally, our limited data on ADMA concentrations suggest that it may be a marker of mortality in sepsis, and this should be investigated in a larger cohort of patients.

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