Plasma arginine and citrulline concentrations in critically ill children: strong relation with inflammation\textsuperscript{1,2}

Dick A van Waardenburg, Carlijn T de Betue, Yvette C Luiking, Monique Engel, and Nicolaas E Deutz

ABSTRACT

Background: The amino acid arginine plays a key role in many metabolic processes in health and disease. Low arginine concentrations are found in various illnesses in children.

Objective: The objective was to investigate the relation between plasma concentrations of arginine (and precursor amino acids) and severity of inflammation in critically ill children.

Design: This was an observational cohort study in children with viral respiratory disease (n = 21; control group), accidental or surgical trauma (n = 19), or sepsis (n = 19) who were admitted to a pediatric intensive care unit.

Results: Plasma arginine and citrulline concentrations were lower in subjects with sepsis and trauma than in those with viral disease (arginine: 33 ± 4, 37 ± 4, and 69 ± 8 μmol/L, respectively, P < 0.01 for both; citrulline: 10 ± 1, 14 ± 1, and 23 ± 2 μmol/L, respectively, P < 0.01 for both) and correlated strongly and inversely with severity of inflammation as indicated by plasma CRP concentration (r = −0.645 and r = −0.660, respectively; P < 0.001 for both). During recovery, plasma arginine and citrulline concentrations increased and were strongly related to the reduction in inflammation as shown by the inverse correlation between arginine and citrulline concentrations and the CRP concentration on days 3 (r = −0.832 and r = −0.756, P < 0.001 for both) and 7 (r = −0.784 and r = −0.694, P < 0.001 for both).

Conclusions: Plasma concentrations of arginine and citrulline are low during the acute phase of critical illness in children and normalize again during recovery. Plasma arginine and citrulline are strongly related to the severity of inflammation indicated by plasma CRP concentrations. Am J Clin Nutr 2007;86:1438–44.

KEY WORDS Plasma amino acid concentrations, arginine, citrulline, children, critical illness, inflammation, C-reactive protein, CRP

INTRODUCTION

The amino acid arginine plays a key role in many metabolic processes in health and disease (1), such as detoxification of ammonia (urea cycle), synthesis of polyamines and creatine, modulation of immune function, release of anabolic hormones, and synthesis of nitric oxide (NO) (2). Arginine is considered a conditionally essential amino acid because endogenous arginine synthesis may not be sufficient to meet metabolic needs, especially during growth (infants and children) (3) and during highly catabolic conditions such as sepsis (4) and burns (5, 6). Children with critical illness may therefore be at particular risk of developing arginine deficiency.

Low plasma arginine concentrations have been described in children with various pathological conditions, such as in premature infants with necrotizing enterocolitis (NEC) (7) and children with asthma (8), cerebral malaria (9), or sickle cell crisis (10). Arginine supplementation can increase arginine concentrations and has shown beneficial effects, such as the prevention of NEC in premature infants and lowering of pulmonary pressure during sickle cell crisis (10, 11). However, data on plasma arginine concentrations during critical illness in children are lacking. Critical illness is often associated with a strong inflammatory response that is related to increased NO production, increased arginine activity (4), and decreased renal de novo arginine synthesis (12). Inflammation could therefore play a key role in arginine depletion and the anticipated need for arginine supplementation in these conditions.

We therefore studied arterial plasma concentrations of arginine and amino acids involved in arginine metabolism (citrulline, glutamine, and ornithine) in critically ill children with different diagnoses associated with mild (viral infection), moderate (accidental or surgical trauma), or severe inflammation (sepsis) and evaluated the relation between plasma concentrations of these amino acids and inflammatory variables.

SUBJECTS AND METHODS

Subjects

Children admitted to the pediatric intensive care unit (PICU) of the University Hospital Maastricht between January 2004 and December 2005 were enrolled in the study if they fulfilled the following criteria: 1) age between 1 mo and 16 y; 2) diagnosis of viral respiratory disease, accidental or surgical trauma, or sepsis; 3) and indwelling arterial catheter. Sepsis was defined according to the criteria of the Society of Critical Care Medicine and the American College of Chest Physicians, adapted to infants and children (13). Exclusion criteria were known metabolic or endocrine disease. The Medical Ethical Committee of the University...
by mixing with sulfosalicylic acid (8 mg/100 g) and immediately put on into precooled heparin-containing tubes (BD Microtainer, Becton-Dickinson, Franklin Lakes, NJ). Blood analyses

C-reactive protein (CRP), and leukocyte concentrations were measured on the AutoDelfia (Perkin-Elmer Life and Analytic Sciences, Wellesley, MA) with an immunofluorimetric monoclonal antibody assay and cortisol by using a monoclonal antibody assay on an Immulite 2000 system (DPC, Los Angeles, CA). Amino acid concentrations were measured on a Synchron LX20 PRO (Beckman Coulter Inc, Fullerton, CA) by using the glucose oxidase, bromcresol purple, and immunoturbidimetric methods, respectively. Plasma insulin concentrations were measured on the AutoDelfia (Perkin-Elmer Life and Analytic Sciences, Wellesley, MA) with an immunofluorimetric monoclonal antibody assay and cortisol by using a counting immunoassay on an Immulite 2000 system (DPC, Los Angeles, CA).

Clinical variables

Severity of illness was assessed on the basis of the pediatric risk of mortality score (PRISM) (15). Other clinical variables recorded included the use of mechanical ventilation, inotropic and vasopressor therapy, and corticosteroids.

Blood sampling

Arterial blood samples were collected from an indwelling arterial catheter. Day 1 samples were collected within 24 h after admission in the acute phase of the disease and after a fasting period of ≥6 h. In a subgroup of 10 patients with either viral disease or sepsis, additional blood samples were collected during recovery on days 3 and 7 during enteral feeding. Patients with accidental or surgical trauma were not included in this follow-up analysis because of their predominantly short stay in the PICU. Plasma amino acid, glucose, insulin, cortisol, albumin, C-reactive protein (CRP), and leukocyte concentrations were measured in all blood samples collected.

Blood analyses

Arterial blood (500 μL) for amino acid analysis was collected into precooled heparin-containing tubes (BD Microtainer, Becton-Dickinson, Franklin Lakes, NJ) and immediately put on ice. Within 30 min the samples were centrifuged for 10 min at 3500 × g (4000 rpm) and 4°C, and the plasma was deproteinized by mixing with sulfosalicylic acid (8 mg/100 μL plasma). Samples were subsequently frozen in liquid nitrogen and stored at −80°C until analyzed. Amino acid concentrations were measured in plasma by HPLC as described by van Eijk et al (14) with variation coefficients <3%. Glucose, albumin, and CRP were measured on a Synchron LX20 PRO (Beckman Coulter Inc, Fullerton, CA) by using the glucose oxidase, bromcresol purple, and immunoturbidimetric methods, respectively. Plasma insulin concentrations were measured on the AutoDelfia (Perkin-Elmer Life and Analytic Sciences, Wellesley, MA) with an immunofluorimetric monoclonal antibody assay and cortisol by using a counting immunoassay on an Immulite 2000 system (DPC, Los Angeles, CA). Blood cell counts were made with a Beckman Coulter LH 750 (Beckman Coulter, Fullerton, CA).

Statistical analysis

All data were analyzed by using the statistical program SPSS (12.0) for WINDOWS (SPSS Inc, Chicago, IL). Normally distributed data were evaluated by using the Kolmogorov-Smirnov test. Variables with a normal distribution were compared between the groups with a one-factor analysis of variance with post hoc Bonferroni analysis. When a variable was not normally distributed, Kruskal-Wallis and post hoc Mann-Whitney U tests with adjustment for multiple comparisons were applied. Correlations between amino acid concentrations, protein intake, and clinical and laboratory variables were tested by using Spearman’s ρ. The influence of CRP and protein intake on amino acid concentrations was further analyzed by using (stepwise multiple) linear regression analysis. Significance was defined as P < 0.05. Data are presented as means ± SEMs.

RESULTS

Patient characteristics

During the study period 59 patients were included: 21 patients with viral respiratory disease, 19 patients with accidental or surgical trauma, and 19 patients with sepsis. Patient characteristics are shown in Table 1. Mean age, body weight, and total fasting time did not differ between patients with viral respiratory disease and those with sepsis. Trauma patients, however, were significantly older, had a higher body weight, and had a more prolonged fasting time than did both other groups. Interestingly, disease severity expressed by the PRISM score did not differ between groups. Patients with sepsis were more often treated with inotropic support (dopamine, norepinephrine, or both) than were patients with viral respiratory disease and accidental or surgical trauma.

Hospital Maastricht approved the study. Written informed consent was obtained from all patients or caregivers.

Table 1

Baseline characteristics of the study groups

<table>
<thead>
<tr>
<th></th>
<th>Viral respiratory disease (n = 21)</th>
<th>Accidental or surgical trauma (n = 19)</th>
<th>Sepsis (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>2.2 ± 0.7a</td>
<td>10.3 ± 1.4b</td>
<td>2.7 ± 0.6a</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>12.5 ± 2.4a</td>
<td>35.2 ± 4.0b</td>
<td>13.7 ± 1.7a</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>3/18</td>
<td>10/9</td>
<td>4/15</td>
</tr>
<tr>
<td>PRISM</td>
<td>16.0 ± 2.8a</td>
<td>12.3 ± 2.0a</td>
<td>20.2 ± 2.2a</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>37.1 ± 0.2a</td>
<td>37.2 ± 0.2a</td>
<td>37.0 ± 0.1a</td>
</tr>
<tr>
<td>Fasting time (h)</td>
<td>13.3 ± 2.2a</td>
<td>34.5 ± 3.7a</td>
<td>19.3 ± 3.7a</td>
</tr>
<tr>
<td>Mechanical ventilation (yes/no)</td>
<td>17/4</td>
<td>8/11</td>
<td>16/3</td>
</tr>
<tr>
<td>Inotropic support (yes/no)</td>
<td>0/21</td>
<td>2/17</td>
<td>11/8</td>
</tr>
<tr>
<td>Corticosteroids (yes/no)</td>
<td>7/14</td>
<td>4/15</td>
<td>5/14</td>
</tr>
</tbody>
</table>

1 PRISM, pediatric risk of mortality. Means within a row with different superscript letters are significantly different, P < 0.01 (ANOVA or Kruskal-Wallis test with adjustment for multiple comparisons).

2 x ± SEM (all such values).

3 Measured at the time of blood sampling.
both other groups, whereas corticosteroid treatment was not different between the groups.

The subgroup of patients in whom blood was also analyzed on days 3 and 7 consisted of 5 patients with viral disease (median age: 0.4 y; range: 0.3–0.8 y; median body weight: 4.6 kg; range: 4.3–7.3 kg) and 5 patients with sepsis (median age: 0.74 y; range: 0.1–6.3 y; median body weight: 6.0 kg; range: 4–24 kg). Patients with sepsis in this subgroup were older and had higher body weights than did patients with viral disease \((P < 0.05\) for both). Mean protein intake was 0, 1.0, 0.6, and 1.8 g \(\cdot\) kg\(^{-1}\) \(\cdot\) d\(^{-1}\) on days 1, 3, and 7, respectively.

Arterial plasma amino acid concentrations

Plasma arginine concentrations were significantly lower in patients with severe inflammation (sepsis and trauma) than in patients with mild inflammation (viral disease) (Figure 1). This was also true for plasma citrulline and glutamine concentrations, precursors of de novo arginine synthesis (Figure 1). Plasma concentrations of ornithine, a product of arginine catabolism via the enzyme arginase, were only decreased in the patients with accidental or surgical trauma (Figure 1). The ratio of arginine to ornithine was significantly lower in septic patients than in the other diagnostic groups, which may indicate increased arginase activity in septic patients (Table 2). The sum of the concentration of all measured amino acids was also lower in the patients with sepsis and trauma than in children with viral disease. Concentrations of tyrosine, glycine, alanine, serine, tryptophan, and lysine were also lower, whereas plasma histidine concentrations were higher in septic patients than in patients with viral disease (Table 2).

Inflammatory and metabolic variables

The results of the inflammatory and metabolic evaluations in the different study groups are shown in Table 3. The plasma concentration of CRP, a marker of the acute phase response during inflammatory states, was significantly higher in patients with sepsis than in patients with either trauma or viral respiratory disease, whereas the CRP concentration in trauma patients was significantly higher than in patients with viral disease. The plasma concentration of albumin was lower in the septic patients than in both other groups. The leukocyte count was significantly higher in the septic patients than in patients with viral disease. The plasma insulin concentration was lower in the septic patients than in both other groups, whereas glucose and cortisol concentrations were not significantly different between the groups.

Correlation between plasma amino acids and inflammatory and metabolic variables

To better understand the relation between inflammation and arginine metabolism, we investigated the association between plasma amino acid concentrations on the one hand and metabolic and inflammatory variables on the other hand. The most striking finding was a strong but inverse correlation between plasma arginine, citrulline, and glutamine concentrations on the one hand and severity of inflammation as expressed by the CRP concentration on the other hand \((r = -0.645, r = -0.660, \text{ and } r = -0.525, \text{ respectively}; P < 0.001\) for all). CRP was also significantly correlated with the ratio of arginine to ornithine \((r = -0.518, P < 0.01)\).

Regression analysis showed that the slopes and intercepts of the regression lines of the relation between CRP and arginine and
between CRP and citrulline in the 3 diagnostic groups were not significantly different. Therefore, one regression equation could be calculated for the relation between arginine and CRP (plasma arginine = 120.1 – 41.6 × logCRP) and between citrulline and CRP (plasma citrulline = 31.1 – 8.9 × logCRP).

Plasma citrulline and arginine concentrations were positively correlated with the plasma albumin concentration (r = 0.400 and r = 0.436 and r = 0.402, respectively), but no correlation was found between plasma amino acid concentrations and the leukocyte count. Contrary to expectations, the plasma concentration of insulin, an anabolic hormone that suppresses amino acid uptake, was positively correlated with low concentrations of several amino acids, such as glutamine and arginine (r = 0.400 and r = 0.315, respectively; P < 0.01 for both), whereas concentrations of cortisol, a catabolic hormone that promotes net protein breakdown and amino acid output from muscle, showed an inverse correlation with glutamine and arginine concentrations (r = −0.377 and r = −0.402; P < 0.01 for both).

Amino acid concentrations during recovery

To investigate plasma arginine, citrulline, glutamine, and ornithine concentrations during recovery, we measured amino acid and plasma CRP concentrations on postadmission days 3 and 7 in a subgroup of patients with either sepsis or viral disease while they were receiving continuous enteral feeding. In septic patients, a significant increase was observed in plasma concentrations of arginine (from 27 ± 7 to 68 ± 15 μmol/L), citrulline (from 11 ± 3 to 20 ± 3 μmol/L), glutamine (from 410 ± 70 to 807 ± 180 μmol/L), and ornithine (from 59 ± 8 to 109 ± 30 μmol/L) (P < 0.05 for all) between days 1 and 7 after admission, whereas the mean plasma CRP concentration decreased (from

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**TABLE 2**

Arterial amino acid concentrations in the different study groups

<table>
<thead>
<tr>
<th></th>
<th>Viral respiratory disease (n = 21)</th>
<th>Accidental or surgical trauma (n = 19)</th>
<th>Sepsis (n = 19)</th>
<th>µmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosine</td>
<td>48 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Taurine</td>
<td>36 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>372 ± 9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>178 ± 11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>172 ± 20&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Glutamate</td>
<td>104 ± 9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60 ± 5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>52 ± 5&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Asparagine</td>
<td>47 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34 ± 5&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>188 ± 15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>154 ± 13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>130 ± 13&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td>139 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86 ± 7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>82 ± 10&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>20 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>52 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td>31 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>48 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>84 ± 7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83 ± 7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>133 ± 10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>137 ± 10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>115 ± 10&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>66 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73 ± 7&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>139 ± 14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89 ± 8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88 ± 10&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>107 ± 11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69 ± 9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68 ± 14&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Arginine:ornithine</td>
<td>1.4 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>BCAAs</td>
<td>267 ± 22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>263 ± 21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>216 ± 22&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Sum AAs</td>
<td>2328 ± 123&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1610 ± 104&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1538 ± 137&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> All values are x ± SEM. BCAAs, branched-chain amino acids; Sum AAs, sum of all measured amino acids (including arginine, citrulline, glutamine, and ornithine). Means within a row with different superscript letters are significantly different, <sup>b</sup>P < 0.05, <sup>c</sup>P < 0.01 (ANOVA or Kruskal-Wallis test with adjustment for multiple comparisons).

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**TABLE 3**

Inflammatory and metabolic variables in the different study groups

<table>
<thead>
<tr>
<th></th>
<th>Viral respiratory disease (n = 21)</th>
<th>Accidental or surgical trauma (n = 19)</th>
<th>Sepsis (n = 19)</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/L)</td>
<td>21 ± 5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>117 ± 22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>242 ± 24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Leukocytes (× 10&lt;sup&gt;9&lt;/sup&gt;/L)</td>
<td>15 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8–14</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.7 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.2 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.5 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.1–5.6</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>12.0 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.4 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.8 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4–25</td>
</tr>
<tr>
<td>Cortisol (nmol/L)</td>
<td>433 ± 79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>553 ± 119&lt;sup&gt;a&lt;/sup&gt;</td>
<td>818 ± 213&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100–700</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>29 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35–50</td>
</tr>
</tbody>
</table>

<sup>a</sup> CRP, C-reactive protein. Means within a row with different superscript letters are significantly different, <sup>b</sup><sup>c</sup>P < 0.01 (ANOVA or Kruskal-Wallis test with adjustment for multiple comparisons).

<sup>b</sup> x ± SEM (all such values).
229 ± 51 to 57 ± 12; *P* < 0.05). In the children with viral disease, the concentrations of arginine, citrulline, glutamine, and ornithine also increased and CRP decreased, but the differences were not significant. Similar to the observations on day 1, strong inverse correlations were found between plasma arginine and citrulline concentrations and the plasma CRP concentration on day 3 (r = −0.832 and r = −0.756 for arginine and citrulline, respectively; *P* < 0.01 for both) and day 7 (r = −0.784 and r = −0.694 for arginine and citrulline, respectively; *P* < 0.001 for both) (data not shown). Regression analysis showed that the slopes and intercepts of the regression lines of the relation between CRP and arginine and between CRP and citrulline in the 3 diagnostic groups were not significantly different on each separate day. Therefore, one regression equation could be calculated for the relation between arginine and CRP and between citrulline and CRP in both groups on days 1, 3, and 7 (for day 3 and 7, see Figure 2).

Protein intake was positively correlated with arginine (r = 0.502, *P* < 0.001) and citrulline (r = 0.302, *P* < 0.05) in the total group of patients. Stepwise linear regression analysis using either the plasma arginine or citrulline concentration as the dependent variable and dietary protein intake and CRP concentrations as independent variables showed that CRP was the only significant factor of plasma arginine and citrulline concentrations, explaining 66% of the variation in arginine and 76% of citrulline concentrations.

**DISCUSSION**

In the present study we investigated arterial plasma concentrations of arginine and other amino acids involved in arginine metabolism (citrulline, glutamine, and ornithine) in different diagnostic groups of critically ill children, both in the acute phase and during recovery. Our results clearly show that plasma arginine concentrations are decreased in the acute phase of critical illness, whereas a gradual increase in plasma concentrations is observed during recovery. This may indicate that arginine depletion develops in the early course of acute illness. Similar results were found for citrulline, glutamine, and ornithine. Moreover, an interesting and new observation is that the reduction in arginine and citrulline concentrations in critical illness is predominantly related to the severity of systemic inflammation, as indicated by the plasma CRP concentration—a very sensitive nonspecific biochemical marker of inflammation and tissue damage.

Reference values for plasma amino acid concentrations in healthy children are only available in venous blood (16), often collected in the postprandial state and under less uniform and optimal conditions. This may explain the large variation in reference values reported (16–18) and makes the comparison of these values with arterial plasma amino acid concentrations, as obtained in our study under optimal conditions (19), difficult. For obvious ethical reasons it was impossible to obtain arterial reference values from a control group of healthy children. However, children with respiratory insufficiency due to viral upper or lower airway disease are admitted to the PICU primarily because of airway obstruction. Although these children need intensive care treatment, they only had mild inflammatory disease (CRP < 50 mg/L) and were therefore chosen as the control group. Indeed, their plasma amino acid concentrations were close to reported reference values (16–18). Still, in this group of patients, a comparable relation was found between plasma arginine and citrulline concentrations and the CRP concentration, both in the acute phase and during recovery. Although there are limited data on plasma amino acid concentrations in critically ill children, our results are supported by studies that have reported low arginine...
concentrations in other childhood diseases associated with inflammation, such as necrotizing enterocolitis in premature infants (7), cerebral malaria (9), sickle cell disease during vaso-occlusive crises (20), and asthma during acute exacerbations (8). In the latter 2 studies, normalization was also seen during recovery, as in the present study. A direct relation between plasma arginine concentration and level of inflammation, however, was not described in these studies. Only in a single study in adult patients with chronic kidney disease was a relation between chronic inflammation expressed by plasma CRP concentration and reduced plasma concentrations of specific plasma amino acids, including arginine, reported (21).

The strong association between plasma arginine and citrulline concentrations and systemic inflammation that we found in the children in our study may have several explanations. Concentrations of these amino acids are determined by different metabolic processes that are altered in inflammatory conditions. Arginine concentrations may be decreased by increased arginase I and 2 activity (with formation of ornithine and urea) (4, 22), increased NO synthesis by isoforms of the enzyme NO synthase (with formation of NO and citrulline) (4), or secondary to decreased endogenous de novo arginine synthesis from citrulline in the proximal renal tubule (1), which is a major pathway for the maintenance of plasma arginine concentrations (12, 23). Arginine concentrations may also be decreased by increased protein synthesis (formation of acute phase reactants) or decreased dietary protein intake. Citrulline, a non-protein amino acid, is predominantly produced by intestinal conversion of arterial and luminal glutamine, glutamate, and proline through the glutamate-to-ornithine pathway (1, 24), but also by NO synthesis (4) and by the breakdown of asymmetrical dimethyl arginine, an important nonselective nitric oxide synthase inhibitor (25) the plasma concentration of which increases during critical illness (26). Citrulline is metabolized by the conversion into arginine in the kidney (endogenous arginine synthesis) (1). Decreased citrulline concentrations may be caused by decreased substrate availability in the gut (glutamine, glutamate), but, because low citrulline concentrations have been associated with decreased gut function (27), we hypothesized that severe systemic inflammation may also have negative effects on gut function (for instance through splanchnic hypoperfusion) and thereby on citrulline production.

Argaman et al (4) and Yu et al (5, 28) studied whole-body arginine metabolism using stable isotope-methods in pediatric patients with sepsis and in pediatric patients with burn injury. In both studies, whole-body arginine breakdown increased, whereas de novo arginine synthesis did not change, which led to negative arginine balance. The increased arginine breakdown was associated with increased NO production and increased oxidation (by arginase) to urea and ornithine. Our results agree well with these studies but also show that the changes in arginine metabolism are directly related to the severity of inflammation. In our septic patients, low arginine concentrations were associated with increased arginase activity, as indicated by a low ratio of arginine to ornithine. However, this ratio too was inversely correlated with the severity of inflammation. Low arginine concentrations were also strongly associated with low citrulline concentrations, which suggests that decreased citrulline availability for arginine production in the kidney may play a role in the genesis of arginine depletion. However, to further investigate the mechanisms behind the effects of inflammation on plasma arginine and citrulline concentrations, an in-depth study of arginine and citrulline metabolism using stable-isotope methods is needed.

Analysis of the relation between plasma arginine concentrations and hormonal and metabolic variables showed a positive correlation between concentrations of insulin and those of arginine and glutamine, whereas an inverse correlation was found between these amino acids and cortisol concentrations. This is unexpected because, under normal conditions, insulin is known to lower plasma amino acid concentrations (by suppressing amino acid release from muscle tissue and by stimulation of amino acid output from muscle). Because severe inflammation is also strongly associated with high cortisol and low insulin concentrations (29), we suggest that the change in plasma amino acid concentrations is primarily the result of inflammation. However, the low arginine concentrations may also have limited the normal stimulatory effect of arginine on insulin secretion (30).

Dietary protein intake may modify amino acid profiles, but plasma amino acid concentrations in all children in our study were obtained in the fasting state on day 1. Fasting time, however, was more prolonged in the trauma patients but was similar in patients with viral disease and sepsis; therefore, it cannot explain the differences in amino acid concentrations that we found in this study. Moreover, in the subgroup of patients who were also studied during recovery while they received continuous enteral feeding, CRP was the only significant determinant of plasma arginine and citrulline concentrations, and dietary protein intake had no further effect, although our study may not have been adequately powered to detect an effect of diet.

Our results show that arginine and citrulline depletion develops early in the course of critical illness and is proportional to the severity of the inflammatory state. Standard pediatric enteral nutrition may not contain sufficient amounts of arginine or its precursors citrulline and glutamine to correct for this depletion, whereas the need for supplementation is probably also related to the severity of inflammation. Because the plasma CRP concentration is the most commonly used biochemical marker for inflammatory illness in most hospitals, it may be a good marker for metabolic alterations in inflammatory states and may even predict the need for supplementation of arginine and citrulline. Citrulline availability could be an important factor in arginine depletion in the critically ill and could also indicate that inflammation affects gut function, although the latter needs further proof.

In conclusion, plasma concentrations of arginine and citrulline are low during the acute phase of critical illness in children and normalize during recovery. Plasma arginine and citrulline are strongly related to the severity of inflammation indicated by plasma CRP concentrations.

The authors’ responsibilities were as follows—DAvW, YCL, and NED: initiated and designed the study; DAvW: responsible for and participated in all aspects of the study, including the planning and designing, data collection, analysis, and most of the manuscript writing; YCL and NED: assisted in analyzing the data and writing the report; and CTD-B and ME: responsible for data collection and participated in the manuscript writing. None of the authors had a conflict of interest.
REFERENCES