Changes in Arginine Metabolism during Sepsis and Critical Illness in Children

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Abstract

Arginine is an important amino acid during disease and healing because of functions in the immune system and as precursor of nitric oxide (NO). In critically ill adults and children, plasma arginine and citrulline concentrations are substantially decreased, indicating an arginine-deficient state. Arginine availability is reduced because of increased arginine disposal in combination with reduced de novo arginine synthesis. The latter is most likely caused by reduced citrulline availability. As a result, NO synthesis may be impaired, which might compromise microcirculation. These metabolic changes seem to be dependent on the severity of inflammation. Arginine or citrulline supplementation in severe inflammation might therefore be beneficial. Possibly, the use of protein-energy-enriched formulas may be a first step to improve arginine availability and NO synthesis. In critically ill children, arginine metabolism and supplementation is however a virtually unexplored field. Since pediatric sepsis is a significant health problem, which differs in epidemiology and pathophysiology from sepsis in adults, and because of the scarcity of data in this population, studies focused on pathophysiological mechanisms and possible interventions in arginine metabolism in pediatric critical illness are warranted.

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Critical Illness and Sepsis in Children

Critical illness is a life-threatening condition which usually requires intensive care unit level care. It may result from, among others, trauma, surgery, severe burns, sepsis and shock. These conditions trigger a systemic activation of the innate immune response, resulting in a nonspecific clinical syndrome of inflammation termed the systemic inflammatory response syndrome (SIRS). To classify SIRS, specific (age-dependent) pediatric criteria exist [1]. Sepsis is defined as SIRS associated with a suspected or proven infection. When sepsis is complicated by organ dys-
function, it is referred to as severe sepsis [1]. Sepsis may lead to cardiovascular dysfunction, characterized by signs of hypoperfusion (in children in the early state not necessarily involving arterial hypotension), which is then called septic shock [1].

Sepsis in children is a significant health problem with high morbidity and mortality and a substantial consumption of health care resources [2, 3]. In 1995, the incidence of severe sepsis in children in the United States was >42,000 cases (0.56 cases per 1,000 population per year) with a ten times higher risk in infants than in older children and with an underlying disease in 49% of cases. The hospital mortality rate was 10.3% [2]. Worldwide, sepsis is the most common cause of death in infants and children [3]. Pediatric sepsis is different from adult sepsis in epidemiology, pathophysiology and management strategy due to different comorbidities, different sites of infection, different pathogens and different physiologic characteristics of children versus adults [3].

In adults, the amino acid arginine has received much attention in the context of critical illness and sepsis, due to its involvement in immune function and as precursor of the signaling molecule nitric oxide (NO), which is involved in vascular tension. In the following section, an overview of arginine metabolism will be given. Then, changes in plasma arginine concentrations and arginine kinetics during sepsis and critical illness will be described. Unfortunately, limited data are available in critically ill children on changes in arginine metabolism and nutritional interventions in this context. Therefore, some data in critically ill adults will be discussed as well.

**Arginine Metabolism**

Arginine is a nonessential amino acid under healthy conditions. Besides being needed for protein synthesis, it has many other important functions. Arginine metabolism is highly compartmentalized, because its enzymes are expressed to different extent in varying tissues. Arginine is metabolized into urea and ornithine via isoforms of the enzyme arginase [4]. Arginase I is highly expressed in the liver, where it detoxifies ammonia via urea synthesis in the urea cycle. It is limitedly expressed in other tissues. Arginase II is mostly expressed in extrahepatic tissues, e.g. kidney, brain, small intestine and macrophages, and directs arginine towards the synthesis of ornithine and subsequently polyamines and proline [4]. The latter products are important for cell growth and cell differentiation and for connective tissue formation, respectively. A second catabolic pathway of arginine is the pathway of NO synthesis via the enzyme NO synthase (NOS). Three isoforms of NOS exist: neuronal NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2) and endothelial NOS (eNOS or NOS3) [4]. NOS1 and NOS3
are constitutive enzymes and necessary for the function of NO as neurotransmitter and for vascular tension, respectively. NOS2, which is found in macrophages, is normally not expressed, but induced to a great extent by cytokines during inflammation and hence plays a role in immune function. NO produced by NOS2 contributes to cytotoxic actions in macrophages, modulates cytokine production and T-helper cell development and is a free radical scavenger [5]. In addition, it is alleged to lead to (excessive) vasodilatation during sepsis [6]. It has been postulated that during induction of NOS2, NOS3 activity is diminished because of modulation of arginine transport systems by inflammatory cytokines. Hence, cationic amino acid transporter (CAT)-1, which is colocalized with NOS3, is downregulated, whereas CAT-2 transporters are upregulated. As a result, arginine is directed towards NOS2 and NOS3 activity is diminished, which may lead to compromised microcirculation during inflammatory states [5, 6].

In addition, arginine is metabolized into creatine (precursor of creatinine) and agmatine.

Arginine is derived from dietary intake and released from body protein through body protein breakdown. It is de novo synthesized from its only precursor citrulline, primarily in the proximal tubules of the kidney [4]. Citrulline is a non-protein amino acid which itself is synthesized in the gut from glutamine and proline [4]. Also, citrulline is formed as byproduct during NO synthesis.

In healthy adults, de novo arginine synthesis contributes sufficiently to arginine production to make it a nonessential amino acid. However, in conditions with increased metabolic needs, such as during growth in infants and children and during conditions of catabolic stress, de novo arginine synthesis cannot fully meet the needs and therefore is considered a conditionally essential amino acid [4]. Among these conditions are critical illness and sepsis.

**Plasma Arginine Concentrations in Sepsis and Critical Illness**

In adults, sepsis is associated with reduced plasma amino acid concentrations [7, 8] and increased amino acid clearance [8]. Plasma arginine concentrations, in healthy individuals in the range of ~80–120 μmol/l, may be reduced to as low as 40 μmol/l during sepsis [7, 9, 10]. A meta-analysis of studies on plasma arginine concentrations in sepsis showed that sepsis not associated with trauma or surgery is indeed a profoundly hypoargininemic state, with reductions of 33.9 μmol/l as compared with nonseptic controls [11]. Importantly, in patients who died from sepsis plasma arginine concentrations were lower than in surviving patients [7]. Also during critical illness other than sepsis (e.g. pancreatitis, ruptured aortic aneurysm, trauma), most plasma amino acid concentrations are
Plasma citrulline concentrations are reduced in septic patients as compared to healthy controls and to a lesser extent in intensive care unit controls (pulmonary insufficiency, subarachnoidal bleeding, neurotrauma) [9]. In critically ill adults with different diagnoses, low plasma citrulline concentrations were even an independent factor of mortality [14].

In critically ill children, fewer studies on plasma arginine and citrulline concentrations have been conducted. We found reduced plasma arginine and citrulline concentrations in children with sepsis and trauma as compared to children with viral infection, with the lowest plasma concentrations in the septic group [15]. In addition, we found a strong inverse correlation of arginine and citrulline concentrations with C-reactive protein (CRP) as marker of inflammation. The association between arginine and CRP persisted during recovery, and CRP was a stronger predictor of plasma arginine concentrations than protein intake. We found reduced arginine/ornithine ratios in patients with sepsis, which may indicate increased arginase activity.

Only one other study has evaluated arginine and citrulline concentrations in pediatric sepsis, and indeed, arginine and citrulline concentrations were signifi-
significantly lower in the septic patients as compared to healthy controls, but not different from febrile controls [16]. Also in this study, reduced arginine/ornithine ratios were found in septic patients. It should be noted that arginine concentrations in the latter study were already very low in control subjects, possibly due to a different method to determine arginine concentrations. Therefore, quantitative comparisons of this study with other studies should be made with caution.

### Changes in Arginine Kinetics during Sepsis and Critical Illness

Reduced plasma amino acid concentrations alone do not provide insight into the underlying changes in metabolic pathways. With stable isotope methodology, changes in arginine anabolism and catabolism can be quantified. Few studies using this method in critically ill patients have been conducted though; results are summarized in table 1 and explained below.

Three studies in septic adults [9, 10, 17] reported arginine kinetics in addition to reduced plasma arginine and citrulline concentrations. Arginine flux was not different between septic patients and controls in two studies [9, 17], but reduced in hypotensive septic patients as compared with healthy controls in the other

<table>
<thead>
<tr>
<th>Ra Cit, μmol/kg/h</th>
<th>De novo Arg synthesis, μmol/kg/h</th>
<th>NO synthesis, μmol/kg/h</th>
<th>Ra Leu/Ra Phe, μmol/kg/h</th>
<th>Plasma [Arg], μmol/l</th>
<th>Plasma [Cit], μmol/l</th>
</tr>
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<tbody>
<tr>
<td>25±7**</td>
<td>9.6±4.2</td>
<td>1.58±0.7*</td>
<td>153±13**/–</td>
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</tr>
<tr>
<td>15±5</td>
<td>9.2±1.4</td>
<td>0.96±0.1</td>
<td>96±21/–</td>
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<tr>
<td>4.4±0.5**</td>
<td>–</td>
<td>0.20±0.04</td>
<td>130.8±11.8/–*</td>
<td>40.2±3.8**</td>
<td>10.2±3.8**</td>
</tr>
<tr>
<td>10.6±0.8</td>
<td>–</td>
<td>0.15±0.04</td>
<td>89.7±3.0/–</td>
<td>85.5±3.3</td>
<td>21.4±2.5</td>
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<tr>
<td>4.5±2.1**,‡</td>
<td>3.3±3.7**</td>
<td>0.8±0.6**</td>
<td>–/62±21**</td>
<td>49±12**</td>
<td>18±6**</td>
</tr>
<tr>
<td>10.1±2.9</td>
<td>10.9±9.4</td>
<td>1.5±1.0</td>
<td>–/51±13*</td>
<td>69±37</td>
<td>21±10**</td>
</tr>
<tr>
<td>13.7±4.1</td>
<td>11.9±6.6</td>
<td>2.2±1.2</td>
<td>–/36±9</td>
<td>92±17</td>
<td>41±7</td>
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<td>40±11**</td>
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<td>–</td>
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<td>–</td>
<td>75±8</td>
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Citrulline flux was significantly lower [9, 17] and de novo arginine synthesis was severely decreased [9] in septic patients. Whole-body protein breakdown was increased in septic patients as compared to controls, and protein breakdown was positively associated with arginine production [17]. Regarding arginine catabolism, arginine clearance rates [17] and arginase activity [9] were increased in septic patients. Despite increased nitrate and nitrite (NOx) concentrations, NO synthesis was not different from healthy controls [17] or reduced in septic patients [9], and fractional NO synthesis rate was lower in the study of Villalpando et al. [10], leading to similar absolute NO synthesis rates as in healthy controls. In the latter study, NOx concentrations in septic patients were negatively correlated with glomerular filtration rate. Thus reduced renal function may contribute to increased NOx concentrations rather than that these are caused by increased NO production [10]. In addition, NOx concentrations were not (inversely) correlated with the mean arterial pressure.

Interestingly, in line with the relationship between plasma arginine and citrulline concentrations and CRP in critically ill children [15], in the study of Luiking et al. [9] there seemed to be an effect of inflammation on the extent of the metabolic changes. Moderate reductions in plasma arginine and citrulline concentrations and a moderate increase in protein breakdown were found in the ICU controls (CRP 85 mg/l) as compared to healthy controls (CRP 1.4 mg/l), and augmentation of these changes, in addition to decreased citrulline production and de novo arginine synthesis and increased arginase activity, was observed in the septic patients with severe inflammation (CRP 219 mg/l) [9].

In critically ill children, only one study on arginine kinetics with stable isotope methodology has been published [18]. Argaman et al. [18] compared septic children (6–16 years of age) with healthy young adults. They found similar arginine fluxes between both groups, but different than in septic adults, increased citrulline flux, similar de novo arginine synthesis, similar arginase activity rates and increased NO synthesis rates as in healthy adults.

We have studied arginine kinetics in 10 critically ill children (1–10 years of age) with different rates of inflammation (septic shock, meningitis, pleural empyema, pneumonia and bacterial laryngitis) [de Betue et al., unpubl. data]. We found a strong inverse correlation between CRP and plasma arginine concentrations, and CRP and citrulline flux. Citrulline flux was strongly positively correlated with de novo arginine synthesis. Arginine clearance tended to be positively correlated with CRP. Arginine flux and protein breakdown were not correlated with CRP. These results were in line with results in adults [9] and again underline that changes in arginine metabolism seem to be proportional to the severity of inflammation.

One study reported NOx concentrations in children, which were increased in septic patients at admission as compared to patients undergoing elective car-
diac surgery, and higher in septic children with organ dysfunction at 48 h than in those without organ dysfunction [19]. No relationship with markers of tissue perfusion or survival was found. It remains unclear though what the value of measuring NOx is as a proxy for NO production [20].

Arginine Deficiency in Critical Illness and Sepsis

Altogether, the data obtained with plasma amino acid concentrations and stable isotope technology in adults and children suggest that arginine availability is reduced during sepsis and critical illness and that it thus becomes an essential amino acid in these conditions. Plasma arginine concentrations are reduced because of increased arginine utilization, in part due to increased arginase activity and increased protein synthesis, which despite increased protein breakdown is not met by the arginine production rate. Of importance is that de novo arginine synthesis is reduced, probably due to reduced citrulline availability. The resulting arginine deficiency may lead to impaired NO synthesis, possibly impairing microcirculation through reduced NOS3 activity. NOx concentrations as indicator of NO synthesis may be misleading in this context. Results suggest that NO synthesis is not responsible for the hypotension in sepsis and that impaired renal function is a contributor to increased NOx concentrations.

The extent of these metabolic changes is likely influenced by the severity of inflammation, resulting in greater derangements with increasing severity. This hypothesis is depicted in figure 1.

Reduced citrulline availability may result from reduced citrulline production in the gut. This can be caused by reduced precursor availability, which is supported by reduced plasma glutamine concentrations [9, 15], or due to reduced (functional) intestinal mass [21], which may result from inflammation and/or splanchnic hypoperfusion during sepsis.

Because of differing results between the stable isotope studies in children and adults and the different pathophysiology of pediatric and adult sepsis, more studies on arginine kinetics in critically ill children are needed.

Other Factors Contributing to Decreased Nitric Oxide Synthesis

Reduced arginine availability may result in limited NO synthesis because extracellular arginine can regulate NO synthesis despite high intracellular arginine concentrations, which is called the arginine paradox [5, 6]. Also, arginine competes with lysine and ornithine for arginine transporters; hence, especially in
Fig. 1. Hypothesis of changes in arginine (Arg) metabolism in moderate inflammation and severe inflammation (or sepsis). Plasma arginine is slightly reduced during moderate inflammation. This is due to a slight reduction in arginine synthesis, because of moderately reduced citrulline availability, and a moderate increase in arginine catabolism. These changes are augmented during severe inflammation, leading to a severe reduction in de novo arginine synthesis and extensively enhanced arginine utilization for protein synthesis and by arginase. As a result, plasma arginine concentrations are further decreased, leading to reduced availability for NO synthesis, possibly compromising microcirculation via NOS-isoform NOS3. Reproduced with permission from American Society for Nutrition [9].
low-arginine conditions, arginine transport into the cell and towards its catabolic enzymes may be reduced. Other factors that may reduce NO synthesis are competition of arginase (of which the activity is increased during sepsis) and NOS for arginine as substrate and increased plasma concentrations of endogenous NOS inhibitors, e.g. asymmetric dimethylarginine (ADMA) [5, 6]. In critically ill adults, increased ADMA levels have been associated with increased mortality. Especially the ratio between arginine and asymmetrical DMA or asymmetrical plus symmetrical DMA seems of importance [22]. Reduced ratios, primarily caused by reduced arginine, were associated with severe sepsis, severity of illness and increased hospital mortality [22]. Therefore, the imbalance between arginine as substrate for NOS and DMA as NOS inhibitor, resulting in reduced NO synthesis, seems an important feature in the pathophysiology of sepsis. Restoration of the balance, primarily by restoring the reduced arginine availability, may be a therapeutic target [22].

One study in septic children investigated ADMA and arginine/ADMA concentrations [16]. In contrast with septic adults, ADMA concentrations were decreased in septic children, and arginine/ADMA ratios were not different from febrile and healthy controls, although there was a trend towards reduced ratios in septic patients. Also, there was a trend towards lower ratios in septic patients with a slow recovery compared with rapid recovery (based on need for inotropic therapy) [16]. More research in children is warranted to confirm these results.

**Arginine Supplementation**

Because of the presumed arginine deficiency, in part due to reduced citrulline availability, supplementation of either arginine or citrulline seems a logical next step. Arginine has been used as part of immunonutrition, in combination with nucleotides and omega-3 polyunsaturated fatty acids. Especially in postsurgical adult patients, immunonutrition is beneficial by reducing postoperative infections and length of hospital stay [23]. Reviews on immunonutrition in sepsis have not been uniform [24]. There is a concern for increased mortality, increased risk of hemodynamic instability and increased oxidative stress with arginine supplementation [5, 24]. Because of the different components of immunonutrition, it is not known what the exact effect of arginine is on outcome, though, and the methodological strength of studies reporting adverse outcome has been questioned [5, 24]. In children, Briassoulis et al. [25] studied immunonutrition containing arginine: it was less well tolerated than standard enteral nutrition in a mixed group of critically ill children, but there was a trend towards
reduced nosocomial infections and reduced gastric colonization; no effects on outcome were found in children with septic shock [26].

Supplementation of arginine or citrulline alone has not been studied in critically ill (septic) children. Continuous intravenous arginine monotherapy in critically ill adults increased plasma arginine concentrations up to four times baseline values and did not adversely affect hemodynamics [24]. Favorable effects of arginine supplementation have been reported in preterm infants to prevent necrotizing enterocolitis and in children and adults with sickle cell disease to reduce pulmonary hypertension. In children undergoing cardiac surgery with cardiopulmonary bypass, oral and intravenous citrulline was well tolerated and resulted in maintained arginine, citrulline and NOx concentrations, which may reduce the risk for pulmonary hypertension [27]. Arginine and citrulline supplementation therefore could be a valuable therapy to improve arginine availability in critically ill children as well. More research in these approaches is warranted in critically ill children.

Another approach may be to increase arginine intake as part of protein intake. We have previously shown that a protein-energy-enriched formula compared with a standard infant formula in critically ill infants with viral bronchiolitis, was safe, well tolerated [28] and led to increased whole-body protein turnover, resulting in a higher and positive protein balance at day 5 after admission [29]. Because of the higher protein intake with the protein-energy-enriched formula, arginine intake was significantly higher in this group as compared to the standard group. We hypothesized that the increased arginine intake would increase the rate of arginine appearance and would lead to increased availability for NO production. Indeed, with stable isotope methodology we found a higher rate of arginine appearance in the protein-energy-enriched formula-fed group [30]. NO production was significantly higher in the protein-energy-enriched fed group as well.

These results show that arginine metabolism and NO synthesis can be altered by simply providing more protein and energy in an enteral formula. This may be another beneficial approach in critically ill children to improve arginine availability and should be further studied.

**Conclusion**

In conclusion, pediatric critical illness and sepsis are presumably arginine-deficient states, similar to these conditions in adults. Most likely, arginine deficiency results from increased arginine utilization on the one hand, which is not matched by arginine production on the other hand. The latter is due to reduced
de novo arginine synthesis, which results from reduced citrulline availability. Since reduced arginine availability may result in impaired NO synthesis, possibly affecting microcirculation, supplementation of either arginine or citrulline may be beneficial. However, this has not been investigated in critically ill children. Possibly, the use of protein-energy-enriched formulas may be a first step to improve arginine availability and NO synthesis.

Since pediatric sepsis is a significant health problem, which differs in epidemiology and pathophysiology from sepsis in adults, and because of the scarcity of data in this population, studies focused on pathophysiological mechanisms and possible interventions in arginine metabolism in pediatric critical illness are warranted.

**Disclosure Statement**

None.

**References**


