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ORIGINAL COMMUNICATION

High plasma arginine concentrations in critically ill patients suffering from hepatic failure

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Objective: In physiological conditions, the liver plays an important role in the regulation of plasma arginine concentrations by taking up large amounts of arginine from the hepatic circulation. When hepatic failure is present, arginine metabolism may be disturbed. Therefore, we hypothesized high arginine plasma concentrations in critically ill patients suffering from hepatic failure.
Design: We prospectively collected blood samples from a cross-section of intensive care unit patients.
Setting: Surgical intensive care unit of a Dutch university medical center.
Subjects: A total of 52 critically ill patients with clinical evidence of dysfunction of more than two organs were recruited.
Measurements: Plasma arginine concentrations were determined by HPLC. We identified correlations of arginine concentrations with organ failure scores and laboratory variables by univariate and multiple regression analyses.
Results: High plasma arginine concentrations were found in critically ill patients developing organ failure. Patients who were in the highest quartile of plasma arginine concentrations had significantly lower fibrinogen concentrations, higher lactic acid concentrations, and longer prothrombin time. Stepwise multiple regression analysis showed that concentrations of arginine were independently associated with the presence of hepatic failure ($P=0.03$) and renal failure ($P=0.048$). In addition, lactic acid proved to be an independent determinant of plasma arginine concentration ($P=0.014$).
Conclusions: Critically ill patients who suffer from hepatic failure have elevated plasma arginine concentrations. Additional arginine in the treatment of these patients can be harmful, and therefore should not be used as a standard nutritional regimen until further evaluation.
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Keywords: arginine; critically ill patients; hepatic failure; renal failure

Introduction

In unstressed animals and humans, arginine might be classified as a dispensable amino acid, but when the degradation and/or utilization is increased, such as in wound healing, trauma, and sepsis, arginine becomes an indispensable amino acid. Therefore, arginine is considered as a conditionally essential amino acid.

Arginine has multiple biological functions: it enhances wound healing (Barbul *et al*, 1990), improves immune function (Reynolds *et al*, 1988) and T-cell antitumor immunity (Barbul *et al*, 1985), has anticatabolic effects (Barbul *et al*, 1984), plays an important role in the urea cycle by eliminating nonessential nitrogen-containing compounds from the body (Brusilow & Horwich, 1989), and is the sole precursor of nitric oxide (NO) (Palmer *et al*, 1988). NO is an important vasodilator and plasma concentrations of arginine seem to be rate limiting in NO synthesis (Nakaki *et al*, 1990; Creager *et al*, 1992; Lorente *et al*, 1999), thereby playing a major role in the regulation of systemic and splanchnic circulation in both normal and pathological

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situations. In a study in rats, we observed a reduced blood flow to the small intestine when plasma concentrations of arginine were low in the presence of low-grade endotoxemia (Prins *et al*, 2000a). Circulating arginine is reduced in a number of clinical conditions, for example, after thoracoabdominal aortic surgery (Nijveldt *et al*, 2000), after surgery for esophageal and lung cancer (Naini *et al*, 1988), after trauma (Houdijk *et al*, 1998), during sepsis (Freund *et al*, 1978), and in severe burns (Yu *et al*, 1995). Because of the many physiological functions of arginine, low levels of this amino acid may be related to the high morbidity and mortality rates in these patient groups. In order to study the effect of arginine on postoperative morbidity and mortality, arginine-enriched nutrition has been tried in patients undergoing major elective surgery. A recent meta-analysis proved that immune-enhancing diets containing arginine reduce the number of infectious complications and duration of hospital stay in surgical patients (Heyland *et al*, 2001). However, immunonutrition containing arginine in critically ill patients was not associated with any apparent clinical benefit. Moreover, it has recently been shown that intensive care unit (ICU) mortality in septic patients was significantly higher in patients receiving enteral arginine-enriched nutrition compared to patients receiving parenteral nutrition (Bertolini *et al*, 2003). Nevertheless, not much is known about the underlying mechanism by which arginine-enriched nutrition may possibly be harmful. It has been shown that dietary arginine supplementation decreases the mRNA expression of inflammatory cytokines in burned rats (Cui *et al*, 2000), and that pharmacological doses of arginine are immunosuppressive by inhibiting lymphocyte proliferation *in vitro* (Wiebke *et al*, 1997). Furthermore, arginine is known to be a stimulator of growth hormone synthesis, and growth hormone therapy in critically ill patients results in adverse outcome (Takala *et al*, 1999). In patients with renal insufficiency, arginine supplementation can lead to cardiotoxic plasma potassium concentrations (Hertz & Richardson, 1972). Another negative effect of arginine can be overproduction of NO in organs that already are challenged by enormous amounts of NO formed by the enzyme-inducible NO synthase. High concentrations of NO may further aggravate tissue damage by inhibiting enzymes of oxidative substrate utilization and nuclear DNA synthesis, but also by increased formation of peroxynitrite which has many deleterious actions on cell function.

The liver plays a crucial role in the metabolism of arginine. In healthy subjects, large amounts of arginine are taken up from the portal venous and hepatic arterial blood supplies, probably to serve as a substrate in the urea cycle. The liver has therefore been called the major sink of arginine. The effect of liver injury on arginine concentrations is not yet fully understood, because both low (Roth *et al*, 1994; Houdijk *et al*, 1997) and high (Prins *et al*, 2000b) plasma arginine concentrations after liver surgery have been reported in human studies and animal models.

Critically ill patients may suffer from hepatic failure, and consequently arginine uptake capacity of the liver and subsequent degradation of arginine by the enzyme arginase may be reduced. We hypothesized high plasma arginine concentrations in these patients and, therefore, we determined arginine concentrations in critically ill patients who were admitted on the surgical ICU of a Dutch university medical center.

Patients and methods

Patients

In this prospective cross-sectional study, blood samples were drawn from 52 consecutive patients who were admitted to the surgical ICU of a Dutch university hospital. The protocol was approved by the institutional review boards, and informed consent was obtained from first-degree family members.

From April 2001 to December 2001, at weekly intervals, the senior intensivist of the surgical intensive care unit judged on the suitability for inclusion. Patients were included if they met both criteria: (1) clinical evidence of dysfunction of ≥ 2 organs, irrespective of the cause of organ dysfunction; (2) calculated total sequential organ failure assessment score ≥ 6 (SOFA score (Vincent *et al*, 1996), Table 1). Organ failure was defined as a SOFA score ≥ 3 for any system.

Blood sampling

After inclusion, a heparinized blood sample (0.5 ml) was drawn from an indwelling arterial line. Laboratory variables that indicate renal function (creatinine and urea), hepatic function (prothrombin time (PT), fibrinogen, and lactic acid), and hepatic enzyme abnormalities (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), and bilirubin) were determined. Plasma arginine concentrations were determined by HPLC as previously described (Teerlink *et al*, 2002).

Statistical analysis

Differences between two groups were tested using the nonparametric Mann–Whitney *U*-test. Results are presented as median and interquartile range (IQR). Multiple regression analysis was performed to determine the interdependent effects of variables on arginine concentrations. The regression equation was built step by step, in each step including the then most significant variable. Relations between variables were investigated using Pearson's correlation. Logarithmic transformation was performed when data were not normally distributed, as in the case of arginine, ALT, AST, bilirubin, AP, PT, lactic acid, creatinine, and urea. $P<0.05$ was considered statistically significant. Statistical analyses were performed using SPSS (SPSS 10.0 for Windows).

Table 1 SOFA score

SOFA score	*0*	*1*	*2*	*3*	*4*
Respiration					
PaO_2/FiO_2 (mmHg)	>400	301–400	201–300	101–200	≤100
(kPa)	>5.3	4.1–5.3	2.8–4.0	1.4–2.7	≤1.3
Coagulation					
Platelets ($\times 10^3/mm^3$)	>150	101–150	51–100	21–50	≤20
Liver					
Bilirubin (mg/dl)	<1.2	1.2–1.9	2.0–5.9	6.0–11.9	≥12.0
(μmol/l)	<20	20–32	33–101	102–204	≥204
Cardiovascular					
Hypotension	No hypotension	MAP <70 mmHg	Dopamine ≤5 or dobutamine (any dose)[a]	Dopamine >5	Dopamine >15
Central nervous system					
Glasgow coma score	15	13–14	10–12	6–9	< 6
Renal					
Creatinine (mg/dl)	<1.2	1.2–1.9	2.0–3.4	3.5–4.9	>5.0
(μmol/l)	<110	110–170	171–299	300–440	>440
or urine output				<500 ml/day	<200 ml/day

[a]Adrenergic agents administered for at least 1 h (doses are in μg/kg/min). MAP=mean arterial pressure.

Results

In total, 52 critically ill patients entered the study. Demographic data of the study population are given in Table 2.

Median plasma concentrations of arginine, fibrinogen, lactic acid, and PT in critically ill patients are shown in Table 3. From the patients who were in the highest quartile of arginine concentrations ($arg_{(\geq 109)}$: 109–454 μmol/l), six patients suffered from hepatic failure and four patients from renal failure.

Patients who were in the highest quartile of arginine had, as liver function variables, significantly lower fibrinogen concentrations ($arg_{(\geq 109)}$: 2.5 g/l, IQR 1.1–4.4; $arg_{(<109)}$: 4.4 g/l, IQR 3.5–5.7, $P=0.013$), higher lactic acid concentrations ($arg_{(\geq 109)}$: 3.80 mmol/l, IQR: 1.47–7.35; $arg_{(<109)}$: 1.70 mmol/l, IQR 1.20–2.20, $P=0.029$), and longer PT ($arg_{(\geq 109)}$: 22.10 s, IQR: 19.90–39.09; $arg_{(<109)}$: 18.70 s, IQR: 15.40–25.00, $P=0.03$). However, there were no significant differences for the indicators of hepatic enzyme abnormalities (AST, ALT, bilirubin, and alkaline phosphatase) and renal function (creatinine and urea).

Multiple regression analysis showed that plasma concentrations of arginine in all critically ill patients were independently associated with the presence of hepatic failure and renal failure (Table 4). Of note is the negative sign for renal failure, indicating a reduction of plasma arginine concentrations when renal failure is present.

Arginine concentration in all patients was positively correlated with lactic acid ($r=0.338$, $P=0.014$) and negatively correlated with fibrinogen ($r=-0.306$, $P=0.027$), but not to the other indicators of hepatic enzyme abnormalities or hepatic and renal function. By multiple regression analysis, lactic acid proved to be an independent determinant of plasma arginine concentration (Table 4).

Table 2 Demographic data of the patient population

Number of patients	52
Gender: male/female	34/18
Age (y): median (range)	58 (16–81)
Type of admission	
Complicated course after elective surgery	17 (32.7%)
Polytrauma	10 (19.2%)
Peritonitis, multiple perforations	10 (19.2%)
Decompensated liver cirrhosis	5 (9.6%)
Necrotizing pancreatitis	4 (7.7%)
Other	6 (11.5%)
Organ failures (SOFA score per organ sytem ≥3)	
Hepatic failure	14 (26.9%)
Renal failure	19 (36.5%)
Hepatic and renal failure	10 (19.2%)
Neurological failure	15 (28.9%)
Respiratory failure	32 (61.5%)
Coagulation failure	10 (19.2%)
Cardiovascular failure	33 (63.5%)
Organ failures (assessed by SOFA score)	
0–1	16 (30.8%)
2–3	25 (48.1%)
≥4	11 (21.2%)
ICU death	21 (40.4%)

Data are number of patients. Numbers in parentheses are percentages of total patient population unless otherwise stated. SOFA=sequential organ failure assessment; ICU=intensive care unit.

Discussion

In this study, we clearly demonstrate seriously elevated (up to 454 μmol/l) plasma concentrations of arginine in critically ill patients. Plasma arginine concentrations were independently associated with the presence of both liver and renal

Table 3 Concentrations of arginine (μmol/l), lactic acid (mmol/l), fibrinogen (g/l), and PT (s)

	Arginine	*Lactic acid*	*Fibrinogen*	*PT*
All patients (*n*=52)	68 (46–109)	1.8 (1.2–3.0)	4.1 (2.7–5.4)	19.9 (15.9–25.1)
No liver or renal failure (*n*=29)	73 (49–106)	1.6 (1.1–2.2)	4.4 (3.6–5.7)	19.2 (15.4–23.5)
Liver failure, no renal failure (*n*=4)	285 (72–319)	7.3 (3.2–7.9)	1.4 (1.1–4.0)	28.3 (20.8–38.3)
Renal failure, no liver failure (*n*=9)	54 (33–89)	1.7 (1.1–3.5)	4.6 (2.8–5.8)	18.7 (16.2–27.0)
Liver and renal failure (*n*=10)	58 (47–181)	3.2 (1.6–7.3)	3.6 (1.6–4.0)	20.0 (17.6–54.7)

Data are presented as median (IQR). PT=prothrombin time.

Table 4 Multiple regression models for both organ failure (SOFA score per organ sytem ≥ 3) and biochemical markers of hepatic and renal function as determinants of plasma arginine concentration

	b	*Standardized* b	P	r^2
Model 1: Organ failures				
Hepatic failure	0.307	0.450	0.030	0.103
Renal failure	−0.185	−0.294	0.048	0.172
Model 2: Biochemical markers				
Lactic acid	0.308	0.338	0.014	0.114

As potential explanatory variables for plasma arginine concentration, organ failures (hepatic, renal, coagulation, respiratory, cardiovascular, and neurological failure) or biochemical markers of hepatic (AST, ALT, bilirubin, alkaline phosphatase, fibrinogen, PT, and lactic acid) and renal (creatinine and urea) function were entered in the analysis.

failure. Furthermore, lactic acid, as an indicator of hepatic function, was an independent determinant of arginine concentration.

Critically ill patients may suffer from failure of the liver and kidney. These organs are important in the metabolism of arginine. The kidney synthesizes arginine from citrulline and a nitrogen donor, usually aspartic acid (Borsook & Dubnoff, 1941). In patients with chronic renal failure, it has been shown that a net citrulline uptake was balanced by an equal net arginine output, which on the overall was 40% less than in healthy humans (Tizianello *et al*, 1980). In our study, we show that plasma concentrations of arginine are independently and negatively associated with the presence of renal failure in critically ill patients. This finding is in concordance with the role of the kidney as arginine synthesizing organ as demonstrated by Prins *et al* (2002). In that study, it was demonstrated in a rat model that arginine production was impaired after ischemia–reperfusion injury of the kidney. Thus, reduced arginine levels in this patient group could be explained by a deteriorated synthesizing function of the kidney. In addition, we proved that high arginine concentrations are independently associated with the presence of hepatic failure. The liver is a crucial organ in the metabolism of arginine because it contains large amounts of the enzyme arginase, which converts arginine into ornithine and urea in the urea cycle (Jackson *et al*, 1986). In physiological conditions, large amounts of arginine are taken up from the hepatic circulation, most likely to serve as substrate for arginase. However, the effect of liver injury on plasma arginine concentrations is still not clear. Results of studies on the level of arginine concentrations after liver surgery are contradictory. Roth *et al* (1994) reported very low arginine concentrations (3.8 μmol/l) after liver transplantation due to liberation of high amounts of arginase from the implanted graft. Arginine deficiency also occurred in bile duct-ligated rats undergoing surgery (Houdijk *et al*, 1997). The decline of arginine was caused by high liver arginase activity in plasma. Administration of cholestyramine, as inhibitor of gut-derived endotoxemia, in these rats, prevented the arginine deficiency by reducing arginase activity through the inhibition of additional endotoxin-mediated hepatocellular damage after surgery. On the other hand, high concentrations of arginine have also been reported; in a rat model, Kupffer-cell depletion resulted in a higher arginase release from the remnant liver after partial hepatectomy, indicating a hepatocellular protective function of Kupffer cells. Despite this arginase release, increased arginine concentrations were present (Prins *et al*, 2000b).

In critically ill patients, we found high arginine concentrations and, therefore, dietary enrichment with arginine in the treatment of these patients needs to be questioned.

It could be argued that the need for additional arginine seems to be determined by an increased demand during stressed states. During stress, cationic amino acids as arginine are released from skeletal muscle, and are transported into the liver by cationic amino-acid transporters

(CAT) of the system y^+ (Closs *et al*, 2000). Interestingly, Hattori *et al* (1999) showed that the expression of CAT-1 and CAT-2B mRNA was significantly increased in lung, heart, and kidney by injection of lipopolysaccharide (LPS), whereas in the liver CAT-2A mRNA was abundantly expressed, independently of LPS administration. Therefore, changes in the expression of CAT mRNA may influence arginine transport, and the abundant expression of CAT-2 mRNA in the liver points to a potentially high uptake of arginine in this organ. Thus, transport mechanisms may determine intracellular concentrations, and are therefore probably important as regulators of the arginine–NO pathway. Interestingly, arginine transporters have been held responsible for the 'arginine-paradox', the observation that endothelial NO synthesis can be regulated by varying the extracellular arginine concentration, despite the fact that the reported intracellular arginine concentrations (0.1–1.0 mM) greatly exceed the K_m of endothelial nitric oxide synthase (NOS) for arginine (2.9 μM) (Wu & Morris Jr, 1998). Interstingly, Lee *et al* (2003) recently demonstrated in an *in vitro* model that arginine concentration upregulates iNOS expression via translational control of iNOS mRNA.

As the liver plays a crucial role in the metabolism of arginine, hepatic failure may affect arginine concentration. To determine liver function in our study population, we measured multiple hepatic parameters. Patients who were in the highest quartile of arginine concentrations had lower fibrinogen concentrations, longer PT, and higher lactic acid concentrations, indicating impairment of hepatic function. In addition, high lactic acid concentrations were an independent determinant of arginine concentrations.

In our opinion, the crucial role of the liver in the metabolism of arginine is neglected in studies on arginine-enriched nutrition in critical illness. Moreover, hepatic failure may be a contraindication for the supplementation of arginine-enriched nutrition. Additional arginine could be potentially harmful instead of beneficial in this condition.

How can arginine possibly be harmful? Cationic amino acids such as arginine cause movement of potassium ions from (muscle) cells to the extracellular compartment. This displacement is associated with an immediate increase in potassium excretion by the kidney (Alberti *et al*, 1967). When the excretion of potassium is impaired, as in end-stage renal disease, detrimental increases in potassium concentrations have been reported (Hertz & Richardson, 1972). Moreover, hyperkalemia has also been reported in patients with severe liver failure and only moderate renal insufficiency (Bushinsky & Gennari, 1978). Other reported disturbing effects of arginine on electrolyte metabolism include hyponatremia (Alberti *et al*, 1967) and hypophosphatemia (Massara *et al*, 1980). Thrombocytopenia (Mudge, 1980) and hypotension (Nakaki *et al*, 1990) due to arginine supplementation have also been described. It has also been reported that caution should be taken in recommending arginine-enriched nutrition as a method to reverse clinical immunosuppression. Pharmacological doses of arginine have been shown to be immunosuppressive by reversible inhibition of lymphocyte proliferation *in vitro* (Wiebke *et al*, 1997). In burned rats, arginine enrichment decreases expression of inflammatory cytokines in organs (Cui *et al*, 2000). Furthermore, arginine is known to be a stimulator of growth hormone synthesis. The administration of growth hormone can attenuate the catabolic response during injury, surgery, and sepsis. Nevertheless, in critically ill patients, high doses of growth hormone resulted in adverse outcome (Takala *et al*, 1999). Another possible negative effect of arginine can be overstimulation of NO synthesis in organs that already are challenged by large amounts of NO produced by the enzyme-inducible NO synthase. NO is able to react with so-called reactive oxygen species, thereby forming peroxynitrite that may damage cell membranes, oxidize lipids, and inhibit enzyme function. Other harmful effects of overproduction of NO affect the cardiovascular system and consist of coagulation disorders and systemic vasodilation with therapeutically refractory hypotension.

Nutritional support with arginine has been shown to improve outcome in surgical patients (Heyland *et al*, 2001). However, Nelson (1998) points out that findings in patients undergoing surgery cannot be extrapolated to patients who are critically ill. This statement is supported by a recent meta-analysis of Heyland *et al* (2001), who showed that the treatment effect of immune-modulating diets containing arginine in surgical patients was significantly different from the treatment effect in critically ill patients. Moreover, the authors postulated that this arginine-enriched nutrition may be harmful. Previous studies support the finding that immunonutrition with arginine may have adverse effects. In a randomized trial, Bower *et al* (1995) compared immune-enhancing nutrition with standard enteral nutrition. Of 147 patients who received the experimental formula, 23 (15.7%) died compared to 10 of 132 (7.6%) patients in the control group. In a subgroup analysis, the mortality in critically ill patients was 11.7% in the experimental group, whereas 6.9% died in the control group. Furthermore, in a study by Dent *et al* (2003), 170 critically ill patients were randomized to receive either immunonutrition containing arginine or isonitrogenous control nutrition. In the arginine-enriched group, there were significantly more deaths than in the control group (23.0 *vs* 9.6%, $P=0.03$). However, at baseline, there were more patients with pneumonia in the experimental group in which most deaths occurred (arginine-enriched group 38.5% *vs* control group 0%). In a very recent interim analysis of a multicenter, randomized, unblinded trial on 39 patients with severe sepsis, the effect of immune-enhancing (containing arginine) enteral feed was compared with parenteral feed (Bertolini *et al*, 2003). The ICU mortality proved to be significantly higher in the enteral feeded group (44.4%) than in the parenteral feeded group (14.3%). These results show that additional arginine should not be used in septic patients (Heyland & Samis, 2003), whereas it may be beneficial in other groups of ICU patients.

Our data show that in different clinical states, arginine is abundantly available or lacking. Therefore, arginine supplementation is questionable in patients with hepatic failure, whereas it may be potentially indicated in patients suffering from renal failure.

In conclusion, we found elevated plasma concentrations of arginine in critically ill patients. High arginine concentrations proved to be associated with the presence of hepatic failure, whereas low arginine concentrations proved to be associated with the presence of renal failure. Arginine supplementation is presented to be advantageous. However, these data indicate that arginine-enriched enteral formulas may not be used as a standard nutritional regimen in each ICU patient. Possibly, the choice to treat patients with additional arginine should be tailored to the individual patient.

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