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A M E R I C A N C O L L E G E O F  
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P H Y S I C I A N S

# Effect of L-NAME, an Inhibitor of Nitric Oxide Synthesis, on Cardiopulmonary Function in Human Septic Shock\*

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**Study objectives:** We tested the effects of continuous infusion of N<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide (NO) synthesis, on cardiovascular performance and pulmonary gas exchange in patients with hyperdynamic septic shock.

**Design:** Prospective clinical study.

**Setting:** ICU of a university hospital.

**Patients:** Eleven critically ill patients with severe refractory septic shock.

**Interventions:** Standard hemodynamic measurements were made and blood samples taken before, during, and after 12 h of continuous infusion of 1 mg/kg/h of L-NAME.

**Measurements and results:** Continuous infusion of L-NAME increased mean arterial pressure (MAP) from 65±3 (SEM) to 93±4 mm Hg and systemic vascular resistance (SVR) from 962±121 to 1,563±173 dyne · s · cm<sup>-5</sup>/m<sup>2</sup>. Parallel to this, cardiac index (CI) decreased from 4.8±0.4 to 3.9±0.4 L/min/m<sup>2</sup> and myocardial stroke volume (SV) was reduced from 43±3 to 34±3 mL/m<sup>2</sup>. Left ventricular stroke work was increased in the first hour of L-NAME infusion from 31±3 to 43±4 g · m/m<sup>2</sup> (all p<0.01 compared with baseline). Heart rate, cardiac filling pressures, and right ventricular stroke work did not change significantly (p>0.05). L-NAME increased the ratio of arterial PO<sub>2</sub> to the fraction of inspired O<sub>2</sub> from 167±23 to 212±27 mm Hg (p<0.05). Venous admixture (Q<sub>VA</sub>/Q<sub>T</sub>) was reduced from 19.4±2.6% to 14.2±2.1% (p<0.05) and oxygen extraction ratio increased from 21.1±2.4% to 25.3±2.7% (p<0.05). Oxygen delivery (DO<sub>2</sub>) was reduced following L-NAME, whereas oxygen uptake and arterial lactate and pH were unchanged.

**Conclusions:** Prolonged inhibition of NO synthesis with L-NAME can restore MAP and SVR in patients with severe septic shock. Myocardial SV and CI decrease, probably as a result of increased afterload, since heart rate and stroke work were not reduced. L-NAME can improve pulmonary gas exchange with a concomitant reduction in Q<sub>VA</sub>/Q<sub>T</sub>. L-NAME did not promote anaerobe metabolism despite a reduction in DO<sub>2</sub>. (CHEST 1998; 113:1640-46)

**Key words:** analogues of L-arginine; cardiac performance; inhibitors of nitric oxide synthesis; N<sup>G</sup>-nitro-L-arginine methyl ester; nitric oxide; pulmonary gas exchange; sepsis

**Abbreviations:** CI=cardiac index; DO<sub>2</sub>=oxygen delivery; FIO<sub>2</sub>=fraction of inspired oxygen; L-NAME=N<sup>G</sup>-nitro-L-arginine methyl ester; L-NNA=N<sup>G</sup>-nitro-L-arginine; LVSW=left ventricular stroke work; MAP=mean systemic arterial pressure; NO=nitric oxide; P(A-a)O<sub>2</sub>=alveolar-arterial oxygen pressure difference; PAP=pulmonary artery pressure; PVR=pulmonary vascular resistance; Q<sub>VA</sub>/Q<sub>T</sub>=venous admixture; RVSW=right ventricular stroke work; SVR=systemic vascular resistance; SV=stroke volume;  $\dot{V}$ O<sub>2</sub>=oxygen uptake

Sepsis and septic shock are characterized by massive systemic vasodilatation with low vascular resistance, increased cardiac output despite myocardial depression, decreased sensitivity to catecholamines, and high mortality rate.<sup>1,2</sup> Recent evi-

dence suggests that pathologic overproduction of the nitric oxide (NO) radical, a potent vasodilator formerly known as endothelium derived relaxing factor, is at least in part responsible for the cardiovascular dysfunction seen in sepsis and endotoxemia.<sup>3,4</sup>

Under normal conditions, small amounts of NO are formed from L-arginine by the constitutive NO synthase present in the vascular endothelium. This results in a constant vasodilatory tone maintaining adequate tissue perfusion. Upon stimulation by endotoxins (lipopolysaccharides) and cytokines such as tumor necrosis

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factor and interleukin-1, an inducible calcium independent isoform of NO synthase is formed in various cell types. This inducible enzyme differs from the constitutive isoform in that it releases massive amounts of NO over long periods of time, resulting in profound vasodilatation, hypotension, and resistance to catecholamines.<sup>3</sup> Furthermore, the early hypotension and hyporeactivity to constrictors seen after exposure to endotoxins may result from increased activity of the constitutive enzyme.<sup>5</sup> High levels of nitrite and nitrate, the stable end product of NO metabolism, are found in patients with severe sepsis, and these levels may correlate with vasodilation.<sup>6</sup> Recently, NO has been incriminated in cascade leading to the myocardial depression during sepsis, as suggested by *in vitro* studies.<sup>7,8</sup>

Both the constitutive and inducible isoform of NO synthase are competitively inhibited by N<sup>G</sup>-substituted analogues of L-arginine, such as N<sup>G</sup>-monomethyl-arginine (L-NMMA), N<sup>G</sup>-nitro-L-arginine (L-NNA), and N<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME).<sup>9,10</sup> Infusion of these analogues of L-arginine can reverse endotoxin- or cytokine-induced hypotension and can restore reactivity to catecholamines in animals.<sup>11-13</sup> In patients with septic shock, short-term administration of these inhibitors of NO synthesis has been shown to increase BP and systemic vascular resistance (SVR).<sup>14-16</sup> Furthermore, methylene blue, an inhibitor of soluble guanylate cyclase that is the effector enzyme of NO, has been shown to temporarily raise BP in patients with sepsis.<sup>17</sup> Therefore, inhibitors of NO synthesis have been suggested to be of value in the treatment of hypotension during human septic shock. However, little data are present about the effects of continued inhibition of NO synthesis on cardiovascular performance in human sepsis, and the effect on pulmonary function remains to be determined.<sup>18</sup> Since inhalation of NO can improve oxygenation in patients with ARDS, one could hypothesize that systemic inhibition of NO synthesis could compromise pulmonary gas exchange through pulmonary vasoconstriction.<sup>19</sup>

The present study was designed to assess the effects of prolonged inhibition of NO synthesis on cardiovascular performance and pulmonary gas exchange during continuous infusion of L-NAME for 12 h in patients with severe septic shock. To study the effect of L-NAME on NO production, measurements were made of serum nitrite and nitrate levels.

## MATERIALS AND METHODS

### Subjects

The hospital's Medical and Ethics Committee approved the study. First-degree relatives were informed of the nature of the study and gave informed consent. Eleven adult critically ill patients of the ICU of our hospital were included in the study.

All patients met the criteria of sepsis as described by Bone et al.<sup>20</sup> These criteria include evidence of infection, tachycardia (>90 beats/min in the absence of  $\beta$ -adrenergic receptor blockade), tachypnea (respiratory rate >20 breaths/min or the requirement of mechanical ventilation), fever or hypothermia (temperature >38.3°C or <35.6°C), plus at least one of the following signs of inadequate organ perfusion: PaO<sub>2</sub>/fraction of inspired oxygen (FIO<sub>2</sub>) <280 (without other pulmonary or cardiovascular disease as the cause), increased blood lactate levels (>2 mmol/L), and oliguria (<0.5 mL/kg/h) for at least 1 h. All patients were in shock (systolic BP <90 mm Hg or a decrease >40 mm Hg from baseline unresponsive to fluid challenge) at the time of ICU admission requiring therapy with pressor agents. At the time of the study, all patients were receiving dopamine >15  $\mu$ g/kg/min and/or norepinephrine (Noradrenaline) >0.1  $\mu$ g/kg/min. All patients had respiratory failure and required mechanical ventilation. Patients received antibiotic therapy based on culture results. Four patients required continuous hemodialysis because of renal failure. Only patients with cardiac index (CI) >3.0 L/min/m<sup>2</sup> were included since earlier reports have shown reductions of cardiac output during inhibition of NO synthesis.<sup>14-16</sup> We hypothesized that a further reduction in cardiac output would be undesirable and even dangerous in a state where cardiac output is reduced. Exclusion criteria for the study were severe coronary artery stenosis (angina pectoris grade III according to New York Heart Association classification), pregnancy, and CI <3.0 L/min/m<sup>2</sup>.

### Study Protocol

All patients underwent continuous ECG monitoring and had indwelling radial artery and pulmonary artery catheters (Criti-cath; Ohmeda; Singapore). Mean systemic arterial BP (MAP), central venous pressure, and mean pulmonary artery pressure (PAP) were measured continuously. Triplicate measurements of cardiac output were made according to the thermodilution method, and the mean value was reported. Data were recorded on a computerized data system (Mennen Medical Systems; Clarence, NY). Cardiac output and pulmonary artery occlusion pressure were measured 3 and 6 h before L-NAME infusion (T=-6 and -3 h), at baseline (T=0), during L-NAME infusion (T=0.5, 1, 3, 6, and 12 h), and 3 and 6 h following L-NAME infusion (T=15 and 18 h). L-NAME was obtained from the manufacturer (Sigma Chemical; St. Louis). The hospital pharmacy prepared a sterile and pyrogen-free solution of L-NAME, 10 mg/mL, ready for infusion. Within 6 h after inclusion, baseline measurements were made and L-NAME, 1 mg/kg/h, infusion was started and continued for 12 h. If mean arterial BP increased >100 mm Hg, the administration of vasopressors was gradually reduced and the dosage noted. Concomitant therapy was at the discretion of the clinician treating the patient. Continuous infusion of L-NAME was chosen since the effect of a bolus injection of 0.15 mg/kg L-NAME lasted only 5 to 10 min (unpublished observations).

In a prior dose-finding experiment, L-NAME was given as a continuous infusion at a rate of 1.5 mg/kg/h in two patients with severe sepsis. This resulted in unacceptable high PAP in both patients for which L-NAME infusion had to be stopped (unpublished observations). Therefore, in the present study, a lower dose of L-NAME, 1 mg/kg/h, was chosen that did not result in pulmonary hypertension. Before and after administration of L-NAME, blood was withdrawn for routine measurement of serum electrolytes, creatinine, liver function tests, lactate, hemoglobin, platelets, and leukocytes. Arterial and central venous blood was withdrawn at T=0, 1, 3, 6, 12, and 15 h and blood gases, pH, and hemoglobin content were analyzed (ABL505; Radiometer; Copenhagen) for determination of pulmonary gas

exchange parameters, oxygen delivery ( $\text{DO}_2$ ), and oxygen consumption ( $\dot{V}\text{O}_2$ ). Since central venous blood gas measurements were incomplete in three patients, mean values of gas exchange parameters and related calculations are reported for only eight patients. In all patients, blood was withdrawn at T=0, 1, 3, 6, 12, 15, 18, and 24 h for determination of nitrite and nitrate levels, the stable end products of NO metabolism, and analyzed using an automated procedure based on the Griess reaction.<sup>21</sup> Serum from eight postoperative ICU patients without sepsis or shock served as control for nitrite and nitrate levels.

#### Calculations and Statistical Analysis

Calculations were performed according to standard formulas. Recorded variables were compared with baseline values using Student's paired *t* test with Bonferroni correction. Nitrite/nitrate levels were compared with control using Student's unpaired *t* test. A *p* value <0.05 was considered statistically significant. Data are expressed as mean  $\pm$  SEM.

## RESULTS

Patient characteristics are shown in Table 1. Most patients had an intra-abdominal infection as the cause for sepsis. Cultures of the infective focus revealed both Gram-negative and Gram-positive

**Table 1—Patient Characteristics\***

Characteristics	No.
Age, yr	51.7 $\pm$ 3.9
Sex, M/F	10/1
APACHE II score <sup>†</sup>	20.7 $\pm$ 1.0
Sepsis Severity Score <sup>‡</sup>	20.8 $\pm$ 0.9
Source of sepsis	
Peritonitis	5
Pneumonia	2
Pancreatitis	2
Mediastinitis	1
Peritonitis+pneumonia	1
Bacteria	
Local culture	
<i>Enterococcus faecalis</i>	6
<i>Staphylococcus epidermidis</i>	3
<i>Klebsiella pneumoniae</i>	1
<i>Streptococcus pneumoniae</i>	1
<i>Escherichia coli</i>	1
<i>Clostridium difficile</i>	1
Bacteroides species	1
Blood culture	
<i>E faecalis</i>	1
<i>Pseudomonas aeruginosa</i>	1
<i>Staphylococcus aureus</i>	1
<i>Proteus vulgaris</i>	1
<i>K pneumoniae</i>	1
Mechanical ventilation	11
PEEP, <sup>§</sup> (cm H <sub>2</sub> O)	5.7 $\pm$ 2.0
Mortality (at day 28)	6/11

\*Values are displayed as mean  $\pm$  SEM of 11 patients.

<sup>†</sup>APACHE II=Acute Physiology and Chronic Health Evaluation, Knaus et al.<sup>22</sup>

<sup>‡</sup>Sepsis Severity Score as described by Elebute and Stoner.<sup>23</sup>

<sup>§</sup>PEEP=positive end-expiratory pressure.

bacteria with positive blood cultures in 45% of patients. Mortality at 28 days was 55% and one patient died at day 34 when treatment was discontinued because of inoperable surgical problems. No obvious link was noted between deaths and infusion of L-NAME.

Continuous infusion of L-NAME resulted in an increase in BP (MAP), SVR, PAP, and pulmonary vascular resistance (PVR) that was direct in onset (Table 2; *p*<0.01). Parallel to these changes, CI and myocardial stroke volume (SV) were reduced (Table 2 and Fig 1; *p*<0.01). Left ventricular stroke work (LVSW) was increased only in the first hour of L-NAME infusion whereas right ventricular stroke work (RVSW) was not significantly changed (Fig 1). Heart rate and cardiac filling pressures did not change significantly (Table 2). L-NAME infusion resulted in improved arterial oxygenation (Fig 2 and Table 3) with a maximum increase in the ratio of PaO<sub>2</sub>/FIO<sub>2</sub> from 167 $\pm$ 23 to 212 $\pm$ 27 mm Hg after 3 h of L-NAME infusion (*p*<0.05). Venous admixture (QVA/Q<sub>T</sub>) was reduced from 19.4 $\pm$ 2.6% to a minimum of 14.2 $\pm$ 2.1% (*p*<0.05) after 6 h of L-NAME infusion (Fig 2) and oxygen extraction ratio increased from 21.1 $\pm$ 2.4% to 25.3 $\pm$ 2.7% (*p*<0.05). No significant changes were seen in partial PaCO<sub>2</sub> and alveolar-arterial oxygen pressure difference (Table 3). The positive end-expiratory pressure level was not significantly changed during L-NAME infusion (5.7 $\pm$ 2.0 vs 4.7 $\pm$ 2.0 cm H<sub>2</sub>O at the end of L-NAME infusion, *p*>0.05). Other ventilatory parameters such as mean airway pressure, peak inspiratory pressure, and minute volume were unchanged (not shown).  $\dot{V}\text{O}_2$  was unchanged despite a reduction in ( $\text{DO}_2$ ) (Table 3). Vasopressor support was significantly reduced during L-NAME administration (dopamine from 1,920 $\pm$ 170 to 1,630 $\pm$ 200  $\mu\text{g}/\text{min}$  and norepinephrine [Noradrenaline] from 8.3 $\pm$ 2.1 to 7.5 $\pm$ 2.3  $\mu\text{g}/\text{min}$  at the end of L-NAME infusion; both *p*<0.05). During continuous infusion of L-NAME, the hemodynamic effects persisted, although the effects on MAP were most pronounced in the early stages of L-NAME administration. CI and SV remained depressed throughout the whole 12-h infusion period. At baseline, nitrite/nitrate levels ranged from 13 to 86 (median, 37)  $\mu\text{mol}/\text{L}$  in the study group, which was increased compared with control (range, 5 to 17 [median, 11]  $\mu\text{mol}/\text{L}$ ; *p*<0.05). Serum nitrate and nitrite showed no significant changes during L-NAME infusion (Fig 3). Urine output increased from 92 $\pm$ 27 mL/h to a maximum of 157 $\pm$ 59 mL/h during L-NAME infusion, which was not statistically significant (*n*=7; *p*=0.09). Arterial lactate (from 3.7 $\pm$ 1.2 to 3.0 $\pm$ 0.7 mmol/L) and arterial pH (from 7.36 $\pm$ 0.02 to 7.36 $\pm$ 0.02) were not significantly changed after the 12 h of L-NAME

**Table 2—Effect of L-NAME Infusion on Hemodynamic Variables in Patients With Severe Sepsis\***

	Continuous L-NAME Infusion, 1 mg/kg/h									
	Baseline	0.5 h	1 h	3 h	6 h	12 h	15 h	18 h	24 h	
CI, L/min/m <sup>2</sup>	4.8±0.4	4.4±0.4 <sup>†</sup>	4.3±0.4 <sup>†</sup>	3.9±0.4 <sup>†</sup>	4.0±0.4 <sup>†</sup>	4.1±0.4 <sup>†</sup>	4.2±0.4 <sup>†</sup>	4.2±0.3 <sup>†</sup>	4.4±0.4	
MAP, mm Hg	65±3	93±4 <sup>†</sup>	87±4 <sup>†</sup>	80±4 <sup>†</sup>	75±3 <sup>†</sup>	71±3 <sup>†</sup>	66±3	66±3	68±3	
SVR, dyne·s·cm <sup>-5</sup> /m <sup>2</sup>	962±121	1,563±173 <sup>†</sup>	1,531±183 <sup>†</sup>	1,483±172 <sup>†</sup>	1,410±173 <sup>†</sup>	1,206±137 <sup>†</sup>	1,088±127	1,092±124	1,096±131	
PAP, mm Hg	31±2	35±2 <sup>†</sup>	36±2 <sup>†</sup>	36±2 <sup>†</sup>	33±2	31±2	30±2	30±2	30±2	
PVR, dyne·s·cm <sup>-5</sup> /m <sup>2</sup>	329±31	432±51 <sup>†</sup>	463±51 <sup>†</sup>	475±53 <sup>†</sup>	469±57 <sup>†</sup>	418±49	380±38	376±43	374±41	
HR, beats/min	112±4	110±4	108±4	112±4	110±4	110±4	107±5	108±5	108±5	
CVD, mm Hg	12.9±1.0	14.1±1.1	13.5±1.2	14.1±1.3	11.8±1.0	11.7±1.3	11.7±0.9	11.9±0.7	11.6±1.3	
PAOP, mm Hg	12.7±0.9	14.1±0.9	13.9±0.9	14.4±1.2	12.7±0.9	12.3±1.1	12.2±0.8	12.6±0.8	12.9±1.0	

\*HR=heart rate, CVD=central venous pressure; PAOP=pulmonary artery occlusion pressure. Values are displayed as mean±SEM of 11 patients.

<sup>†</sup>p<0.05 for comparison to the baseline value.

infusion. Also, serum electrolytes, urea, creatinine, liver function test results, platelets, and leukocytes were not significantly changed at the end of L-NAME infusion (results not shown). During administration of L-NAME, no changes were seen in the ECG. No side effects were seen during administration of L-NAME.

## DISCUSSION

The mortality rate from septic shock remains high despite progress in antibiotic and vasopressor therapy, and therefore new treatment modalities are warranted.<sup>1</sup> Increased production of NO by the

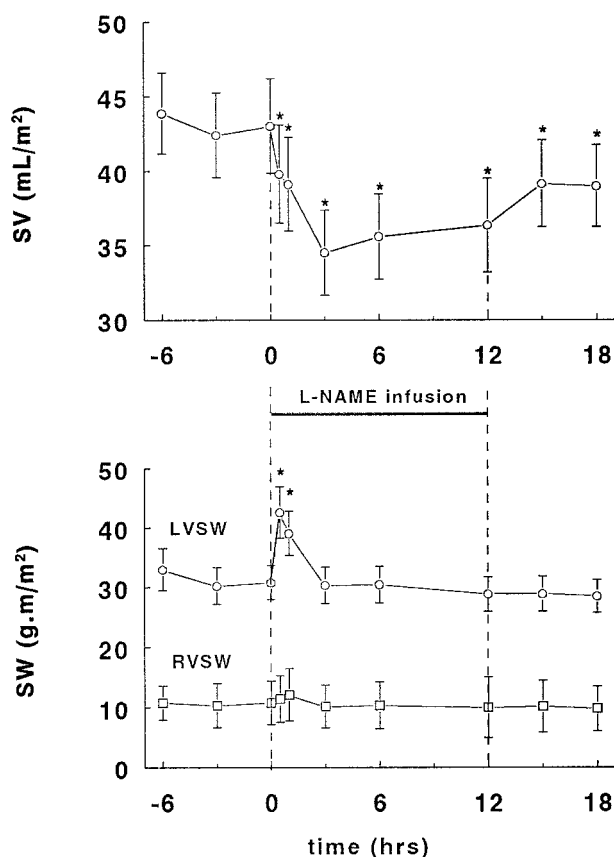


FIGURE 1. Effects of continuous infusion of 1 mg/kg/h of L-NAME on cardiac performance in 11 patients with severe septic shock. SW=stroke work. Time of L-NAME infusion is from t=0 to t=12 h. Values are displayed as mean±SEM. Asterisk: p<0.05 for comparison to the baseline value.

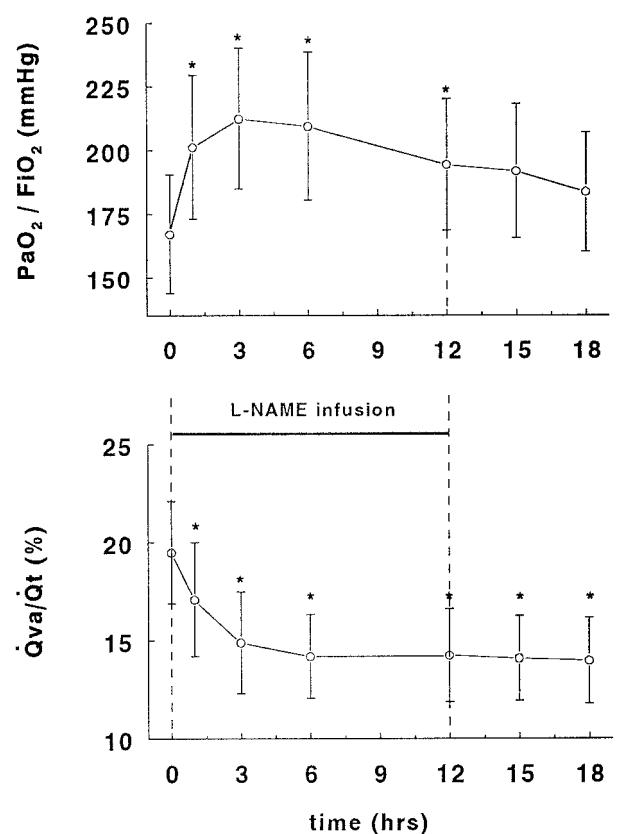


FIGURE 2. Effect of continuous infusion of 1 mg/kg/h of L-NAME on pulmonary gas exchange in patients with severe septic shock. PaO<sub>2</sub>/FiO<sub>2</sub>, n=11; Q<sub>va</sub>/Q<sub>t</sub>, n=8. Time of L-NAME infusion is from t=0 to t=12 h. Values are displayed as mean±SEM. Asterisk: p<0.05 for comparison to the baseline value.

**Table 3—Effect of L-NAME Infusion on Pulmonary and Metabolic Variables in Patients With Severe Sepsis\***

	Continuous L-NAME Infusion						
	Baseline	1 h	3 h	6 h	12 h	15 h	18 h
FI <sub>O</sub> <sub>2</sub> , %	57±6	57±6	56±6	51±5	50±5	51±5	50±5
PaO <sub>2</sub> , mm Hg	81.0±5.4	99.4±10.6 <sup>†</sup>	107.6±13.5 <sup>†</sup>	93.1±7.9 <sup>†</sup>	84.8±7.5	81.5±4.6	77.6±4.8
PaCO <sub>2</sub> , mm Hg	34.9±1.3	35.5±1.3	36.2±1.4	35.5±1.1	35.4±1.4	34.7±1.5	36.1±1.5
P(A-a)O <sub>2</sub> , mm Hg	292±48	273±48	258±42 <sup>†</sup>	234±40 <sup>†</sup>	236±39 <sup>†</sup>	246±46	242±46
PvO <sub>2</sub> , mm Hg <sup>†</sup>	41.3±1.8	41.8±1.7	40.7±2.0	39.3±1.9	40.3±1.6	40.0±1.4	39.6±1.6
QVA/QT, % <sup>†</sup>	19.4±2.6	17.1±2.9 <sup>†</sup>	14.9±2.6 <sup>†</sup>	14.2±2.1 <sup>†</sup>	14.3±2.3 <sup>†</sup>	14.1±2.2 <sup>†</sup>	13.9±2.2 <sup>†</sup>
O <sub>2</sub> ER, % <sup>†</sup>	21.1±2.4	23.3±2.7	24.7±2.6 <sup>†</sup>	25.3±2.7 <sup>†</sup>	24.1±2.1	24.4±2.5	23.5±2.1
DO <sub>2</sub> , mL/min/m <sup>2†</sup>	713±68	658±64 <sup>†</sup>	600±51 <sup>†</sup>	621±55 <sup>†</sup>	644±60	673±57	653±49
VO <sub>2</sub> , mL/min/m <sup>2†</sup>	158±12	153±13	151±10	162±12	156±16	167±19	159±19

\*P(A-a)O<sub>2</sub>=alveolar arterial oxygen difference; PvO<sub>2</sub>=partial pressure of venous oxygen; O<sub>2</sub>ER=oxyg extraction ratio. Values are displayed as mean±SEM of 11 († or eight) patients.

<sup>†</sup>p<0.05 for comparison to the baseline value.

inducible NO synthase has been implicated as a major contributor to the cardiovascular dysfunction of endotoxin- and cytokine-induced shock in animals.<sup>11-13</sup> The present study shows that NO is responsible, at least in part, for the hemodynamic derangements in patients with severe sepsis. Prolonged inhibition of NO synthesis with L-NAME could improve cardiovascular status and pulmonary gas exchange in these severely ill patients and may provide a new treatment modality in the management of septic shock.

Although no conclusions can be made regarding mortality in this nonrandomized study with only 11 patients, the mortality rate was high (>60%), which could raise questions regarding the safety of L-NAME. However, no obvious deterioration of condition was noted during L-NAME infusion and mortality was not different from predicted death rate

as computed by the APACHE II (acute physiology and chronic health evaluation) and Sepsis Severity scores.<sup>22,23</sup> Some animal studies have shown detrimental effects and increased mortality following L-NAME and other analogues of L-arginine during septic shock.<sup>24,25</sup> The detrimental effects of these NO synthase inhibitors have contributed to their nonspecificity toward the constitutive isoform of NO synthase, and selective inhibition of the inducible enzyme has been suggested to be more effective.<sup>25,26</sup> Recent reports, however, suggest that the constitutive form of NO synthase disappears in endotoxemia and other forms of sepsis.<sup>27</sup> If this is true, then the use of a nonspecific inhibitor of NO synthase would be as effective as an inhibitor specific to the inducible isoform. In addition, the inhibition of the inducible isotype could hinder immune defense and bactericidal mechanisms that are important in sepsis.<sup>28</sup> Furthermore, the detrimental effects described in many of these studies may be explained by administration of the inhibitor in the early phase of sepsis. In this situation, the inducible NO synthase will not be fully expressed since this takes a lag-phase of several hours<sup>2</sup> and only the constitutive NO synthase will be blocked, resulting in severe and detrimental vasoconstriction. In addition, the detrimental effects are dose dependent and mostly seen in studies using extremely high doses of the NO synthase inhibitor.<sup>29</sup>

In our study, patients received L-NAME, 1 mg/kg/h, for 12 h, resulting in a total dose of 12 mg/kg of L-NAME, which is relatively low compared with the doses of NO inhibitors used in most animal studies<sup>12,13</sup> and comparable to the doses used in studies in humans.<sup>14-16,30</sup> Animal studies have shown that L-NAME is probably a prodrug that is converted to the active L-NNA, which is much more stable and has a high plasma half-life.<sup>31</sup> However, little is known about pharmacokinetics and metabolism of L-NAME in humans. To minimize the potential

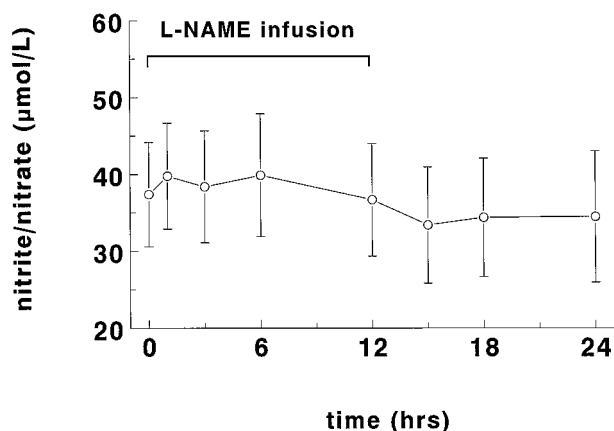


FIGURE 3. Effect of continuous infusion of 1 mg/kg/h of L-NAME on plasma levels of nitrite and nitrate, the stable end-products of NO metabolism, in patients with severe septic shock. Time of L-NAME infusion is from t=0 to t=12 h. Values are displayed as mean±SEM; for all values, p>0.05 compared with the baseline value.

chance of developing toxic reactions from high serum levels of L-NAME or L-NNA, we did not continue L-NAME infusion for a longer period than 12 h and we did not restart L-NAME therapy when patients redeveloped hypotension. At this time, we do not know whether the optimum therapeutic dose of L-NAME was used and possibly the 12-h administration period of L-NAME was too short for clinical improvement. Further pharmacodynamic studies will be necessary to find the optimum dose and schedule for L-NAME administration.

Our findings show that L-NAME can raise BP in patients with severe septic shock through preventing excess vasodilatation. Similar results have been found in previous studies using analogues of L-arginine in human sepsis,<sup>14-16</sup> although most reports describe only initial effects. The hemodynamic effects of L-NAME were direct in onset and persisted during the 12 h of infusion. However, the effect on BP was most pronounced in the first hour of infusion and tended back toward baseline after 1 h of L-NAME infusion. Since SVR remained elevated, the decline in BP was probably caused by a concomitant decrease in CI and SV, although some form of tolerance or counterregulation may have played a role.

The reduction in CI has been reported with other inhibitors of NO synthesis in both animals and humans.<sup>12-16</sup> So far, the precise mechanism underlying this reduction in CO remains unclear. In our study, it could partially have resulted from a reflex change due to the increased SVR and afterload, or a reduction in vasopressor support. Since LVSW was improved during L-NAME infusion and heart rate and RVSW were not significantly changed, direct cardiac depression by L-NAME seems unlikely. Myocardial depression secondary to myocardial ischemia<sup>32</sup> could have played a role, although no changes on the ECG were observed during L-NAME infusion. The reduction in CI and concomitantly  $\text{DO}_2$  may be harmful since tissue perfusion may be reduced. Therefore, we included only patients with hyperdynamic sepsis as characterized by increased CI. The observations that  $\dot{V}\text{O}_2$  was unaffected and arterial lactate and pH were unchanged suggest that L-NAME did not promote anaerobic metabolism and that L-NAME preferentially caused vasoconstriction in metabolically inactive tissues. However, these observations include only indirect parameters that do not necessarily reflect what happens at capillary or cellular level. Thus, despite these findings, local areas of tissue underperfusion could have existed during L-NAME infusion.

NO contributes to pulmonary vascular tone and inhaled NO can be used to reduce pulmonary hypertension and improve oxygenation in patients with

ARDS.<sup>19,33</sup> That NO vasodilates the pulmonary vasculature is underscored by the finding that L-NAME increased PAP and PVR. Pulmonary vasoconstriction is an undesirable effect that may lead to pulmonary hypertension and related deleterious effects on circulation, particularly on right-sided cardiac function. Indeed, in prior pilot experiments, we found that higher doses of L-NAME resulted in severe pulmonary hypertension. The pulmonary vasoconstriction seen with L-NAME and other inhibitors of NO synthase<sup>12-15</sup> may limit their clinical application, especially in patients with high PAP. Since NO inhalation may improve oxygenation in patients with ARDS, we hypothesized that inhibition of NO synthesis might lead to problems in pulmonary gas exchange. However, we found that arterial oxygenation was improved during L-NAME infusion despite pulmonary vasoconstriction. Parallel to this, there was a reduction in QVA/QT that implicates a decrease of the pulmonary right-to-left shunt. This may suggest that L-NAME caused a redistribution of intrapulmonary blood to well-ventilated alveoli, improving ventilation-perfusion mismatch. Similar effects of L-NAME on arterial oxygenation were described by Sprague et al<sup>34</sup> using a model of unilateral alveolar hypoxia in anesthetized rabbits. Furthermore, in a study by Radermacher et al,<sup>35</sup> it was found that the increase in CO by systemic administration of nitrovasodilators worsened ventilation-perfusion mismatching and gas exchange in patients with ARDS. The combined use of NO inhalation with systemic inhibition of NO synthesis may prove to be effective therapy in the future.<sup>36</sup>

Nine of 11 patients had increased levels of nitrite and nitrate in the serum as compared with control subjects. Although this could reflect increased systemic NO production,<sup>6</sup> some reservations must be made, since NO is but one of the ways that nitrite and nitrates are formed. L-NAME infusion did not result in reduced serum levels of nitrite and nitrate, despite profound vasoconstriction. At this time, we cannot explain this finding, but it could suggest that L-NAME is not an effective inhibitor of NO synthesis and other mechanisms, independent of systemic inhibition of NO synthesis, may play a role in the increase in MAP and SVR by L-NAME.<sup>25</sup> Furthermore, serum levels of nitrite and nitrate may not directly reflect the local amount of NO released or inhibited since active excretion through kidneys and GI tract takes place.<sup>37</sup>

In conclusion, prolonged inhibition of NO synthesis with L-NAME may improve cardiovascular status with improvement of pulmonary gas exchange in patients with severe septic shock. Mortality rate was high, which could suggest only limited effects of L-NAME on outcome. Furthermore, the increase in

PAP and the reduction in CO are potentially harmful effects that may hinder the clinical utility of L-NAME and other NO synthase inhibitors. More randomized clinical studies will be required to further investigate whether inhibition of NO synthesis is beneficial in human septic shock.

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**Effect of L-NAME, an inhibitor of nitric oxide synthesis, on cardiopulmonary function in human septic shock**

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