Impaired mitochondrial function induced by serum from septic shock patients is attenuated by inhibition of nitric oxide synthase and poly(ADP-ribose) synthase*

Michael Boulos, MD; Mark E. Astiz, MD, FCCM; Rajat S. Barua, MD; Mohammed Osman, MD

Objective: **The purpose of this study was to determine the role of nitric oxide and poly(ADP-ribose) synthase on impaired mitochondrial function in septic shock.**

Design: **Human umbilical vein endothelial cells were incubated with serum from ten healthy controls, 20 patients with septic shock, and seven critically ill patients who were not septic. The experiment was repeated after pretreatment with 3-aminobenzamide, a poly(ADP-ribose) synthase inhibitor, or N^G-methyl-L-arginine, a nonspecific nitric oxide synthase inhibitor.**

Measurements: **Mitochondrial respiration was measured using a modified MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay.**

Setting: **Research laboratory.**

Main Result: **Endothelial cell mitochondrial respiration was significantly depressed by septic serum and averaged 61% ± 6% of control values ($p < .05$). Incubation with septic serum as compared with control serum also significantly decreased cellular adenosine triphosphate levels (6.7 ± 1.2 nM vs. 13.5 ± 1.9 nM, $p < .01$). The level of mitochondrial respiration in endothelial cells exposed to septic serum did not correlate with arterial lactate concentration but was correlated with both cardiac output ($r_s = .52$, $p < .05$) and mixed venous oxygen saturation ($r_s = .61$, $p < .05$). Pretreatment with N^G-methyl-L-arginine significantly increased mitochondrial respiration in endothelial cells treated with septic serum from 63% ± 6% of normal to 88% ± 6% ($p < .05$) of normal values. Similarly, pretreatment with 3-aminobenzamide increased mitochondrial respiration in endothelial cells treated with septic serum from 64% ± 6% to 100% ± 4% ($p < .01$) of normal values. Endothelial cells incubated with serum from nonseptic critically ill patients did not demonstrate a significant decrease in mitochondrial respiration.**

Conclusion: In vitro **mitochondrial respiration was significantly depressed by septic serum. The addition of N^G-methyl-L-arginine, a nitric oxide synthase inhibitor, and 3-aminobenzamide, a blocker of the poly(ADP-ribose) synthase pathway, significantly attenuated this suppression. These data suggest that nitric oxide and poly(ADP-ribose) synthase activation may play an important role in the inhibition of mitochondrial respiration in septic shock. (Crit Care Med 2003; 31:353–358)**

KEY WORDS: septic shock; nitric oxide; peroxynitrite; mitochondria; poly(ADP-ribose) synthase; endothelial cells

Septic shock is characterized by impaired oxygen extraction, despite evidence of apparent tissue hypoperfusion. Two mechanisms have been postulated to be responsible for the inability to maximally extract oxygen. The first mechanism is maldistribution of blood flow at either a regional or a microvascular level with resulting tissue hypoperfusion (1, 2). Clinical evidence of splanchnic hypoperfusion and of impaired microvascular blood flow in skeletal muscle support the importance of this mechanism in patients with septic shock (3–6).

The second, and more controversial, mechanism involves direct impairment of mitochondrial function by the mediator substances released in septic shock (1, 7–9). Several observations suggest that this mechanism may also contribute to impaired oxidative metabolism in septic shock. First, increasing oxygen delivery in patients with septic shock does not consistently increase oxygen consumption and decrease the level of anaerobic metabolism as measured by arterial lactate concentration (10). Second, normal levels of tissue oxygen tension have been reported in patients with septic shock and elevated arterial lactate concentrations (11). Third, a number of the mediators implicated in septic shock have been demonstrated to directly impair mitochondrial function. Early studies examined the cytotoxic effects of endotoxin, and more recent research has focused on cytopathic pathways related to nitric oxide (12–16). Both nitric oxide and its derivative, peroxynitrite (which is formed from the interaction between nitric oxide and superoxide), directly impair the function of mitochondrial electron transport complexes (17, 18). In addition to inhibiting mitochondrial function, peroxynitrite causes single-strand DNA breakage, thereby activating the nuclear enzyme poly(ADP-ribose) synthase (PARS) (19). The subsequent ADP-ribosylation of nuclear proteins depletes nicotine adenine dinucleotide and high-energy phosphates with resulting cellular injury (14, 20). Studies in experimental models of endotoxemia suggest that these pathways may play an important role in interfering with mitochondrial oxidative metabolism in septic shock (14–16, 20). The purposes of this study were to extend these prior observations by examining whether serum from patients in septic shock impaired endothelial cell mitochondrial activity and to examine the possible role of nitric oxide and PARS activation on this impairment.

***See also p. 632.**

From Saint Vincent's Catholic Medical Centers of New York, New York Medical College, Valhalla, NY.

DOI: 10.1097/01.CCM.0000050074.82486.B2

METHODS

This study was approved by the Institutional Research Board of Saint Vincent's Hospital and Medical Center, New York, NY. The study population consisted of three groups. The first group included 20 patients meeting entrance criteria for septic shock (SS), the second group consisted of seven patients meeting the entrance criteria for critically ill nonseptic patients, and the third group consisted of ten normal control subjects. Sepsis was defined by an identifiable site of infection and evidence of a systemic inflammatory response as manifested by three of the following variables: 1) temperature of ≥38°C or ≤30°C, 2) heart rate of >90 beats/min, 3) respiratory rate of >20 breaths/min, and 4) white cell count of >12,000 mL/mm or <4,000 mL/mm. Septic shock was defined as sepsis-induced systolic blood pressure of <90 mm Hg persisting, despite fluid resuscitation, and requiring vasopressor therapy.

Blood was collected in vacutainer tubes and centrifuged at 3000 rpm for 15 mins at 4°C. The serum was collected and stored at −70°C until used. Primary cultures of human umbilical vein endothelial cells and culture medium were obtained from Biowhittaker (Walkersville, MD). The cells were initially cultured in tissue culture flasks and later subcultured after a single passage in 96-well tissue culture plates (200 μL of media added per well) until they reached 90% confluence. Mitochondrial respiration was assessed using a commercially available assay kit (Chemicon, Temecula, CA). This test is based on the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay and involves cleavage of the tetrazolium salt WST-1 to formazan by mitochondrial dehydrogenases. The formazan dye produced by viable cells can be quantified in a multiwell spectrophotometer (microplate reader) by measuring the absorbance of the dye solution at 450 nm with a reference wavelength at 630 nm.

Adenosine triphosphate (ATP) levels were measured in the cultured endothelial cells by chemiluminescence using a commercially available assay kit (ATP Bioluminescence Assay Kit HS II, Roche Diagnostics, Indianapolis, IN). Endothelial cells, 5000 cells per well, were subcultured in 96-well culture plates for 24 hrs. After incubation, the serum was discarded and 35 μL of media and 35 μL of lysis reagent were added to each well. After 10 mins, 50 μL of the contents of each well was transferred to a white microliter plate (Thermo Lab Systems, Helsinki, Finland). Fifty microliters of luciferase was added to each well by automated injection, and the ATP content was measured using a microplate photometer (Anthos Lucy 1, Anthos Labtec Instruments, Salzburg, Austria).

Cardiac output was determined by thermodilution. Mixed venous oxygen saturation was determined by co-oximetry. Nitric oxide levels were determined in the serum by the chemiluminescence method using an nitric oxide analyzer (Sievers, Model 2280) (21). All samples were done in duplicate, and the nitric oxide concentration was expressed in micromoles per liter.

In preliminary experiments, a 4-hr incubation period was found to be associated with equivalent endothelial cell viability in cells exposed to serum from normal controls and cells exposed to serum from patients with septic shock. Cell viability was independently assessed using a neutral red–based assay kit (Basic Red 5, Sigma, St. Louis, MO) (22). Viable cells take up the neutral red by active transport and incorporate the dye into lysosomes, whereas nonviable cells do not incorporate the dye. An increase or decrease in the number of cells or their physiologic state results in a concomitant change in the amount of dye incorporated by the cells. Sodium dodecyl sulfate (Sigma, St Louis, MO) in a 1% solution was used as a cellular toxin and a positive control.

In two separate experiments, endothelial cells were pretreated with 3-aminobenzamide (3-AB), a PARS inhibitor, and N^G-methyl-L-arginine (L-NMMA) (Sigma, St. Louis, MO), a nonspecific nitric oxide synthase inhibitor. Different concentrations (0, 0.1 mM, 1.0mM) were added to the cells for a 30-min period followed by a 4-hr incubation time with the serum and inhibitors. After the 4-hr incubation period, the plate contents were emptied and a solution of 200 μL of media and 20 μL of WST-1 was added to each well, and the absorbance was measured as above. Readings were obtained at baseline, 1, and 2 hrs. All measurements were done in duplicate. The best response with both L-NMMA and 3-AB was observed at the 0.1 mM concentration. Accordingly, for the purpose of this study, the 2-hr points and 0.1-mM data are used for analysis.

The data between groups was compared using Kruskal Wallis and Mann-Whitney *U* tests as appropriate. Relationships between variables were examined using the Spearman's rank correlation coefficient (r_s). Data are presented as mean ± SE.

RESULTS

A total of 20 patients with septic shock and ten normal volunteers were initially studied. All of the patients with septic shock required vasopressor therapy and mechanical ventilation. The age of the patients was 66 ± 2 yrs. Their Acute Physiology and Chronic Health Evaluation (APACHE) II score was 34 ± 1, their arterial lactate concentration was 6 ± 0.9 mEq/L, and they had a mortality rate of 65%. The source of the infections was intraabdominal in eight patients, pulmonary in nine patients, skin and soft tissue in two patients, and urinary tract in one patient.

Studies done with neutral red indicated that viability was similar in endothelial cells treated with serum from normal controls, 0.67 ± 0.07 absorbance (abs), cells treated with serum from septic patients, 0.63 ± 0.05 abs, and cells treated with media, 0.63 ± 0.07 abs. Endothelial cells treated with sodium dodecyl sulfate demonstrated a significant decrease in neutral red uptake, 0.34 ± 0.03 abs ($p < .05$), as compared with all three groups.

Septic serum significantly decreased endothelial cell mitochondrial respiration as compared with serum from normal controls (Figs. 1 and 2). Levels of mitochondrial respiration did not correlate with arterial lactate concentration or APACHE II score. In patients with arterial lactate concentrations of >3 mEq/L, endothelial cell mitochondrial respiration was 0.651 ± 0.085 abs, and in patients with arterial lactate concentrations of <3 mEq/L, mitochondrial respiration was 0.604 ± 0.087 abs (not significant). Mitochondrial respiration was significantly correlated with both the mixed venous oxygen saturation ($r_s = .606$, $p = .019$) (Fig. 3) and cardiac index ($r_s = .517$, $p = .039$).

Serum nitric oxide levels were significantly increased in septic shock patients, 38 ± 8 μmol/L, as compared with controls, 15 ± 1.3 μmol/L ($p < .05$). However, these values were not correlated with *in vitro* mitochondrial respiration. High background levels from the septic

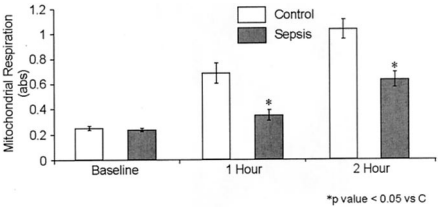

Figure 1. Mitochondrial respiration measured by mitochondrial-dependent reduction of WST-1 in endothelial cells incubated with serum from ten normal controls (*C*) and 20 patients with septic shock for 4 hrs followed by 2 hrs with WST-1 and media. Measurements were taken at baseline, 1 hr, and 2 hrs after adding the WST-1. Data presented in mean ± SEM. *abs*, absorbance.

serum precluded measuring nitric oxide levels in the cell culture supernatants.

The effect of L-NMMA on septic serum-induced mitochondrial depression was examined. Mitochondrial respiration, 100% ± 5% of baseline values, did not change significantly from baseline in endothelial cells exposed to normal serum and L-NMMA. In contrast, at a concentration of 0.1 mM, L-NMMA significantly increased mitochondrial respiration in cells exposed to septic serum from baseline levels of 74% ± 5% to 98% ± 5% of control values ($p < .05$). To more accurately assess the effect of L-NMMA, serum from septic patients was excluded that did not inhibit mitochondrial respiration. In this secondary analysis of serum from septic patients who decreased mitochondrial respiration by at least 15% from control values, similar levels of improvement were demonstrated (Fig. 4).

In a separate set of experiments, endothelial cells were incubated with septic serum and 3-AB, a PARS inhibitor. Incubation with 3-AB did not significantly alter mitochondrial respiration, 108% ± 7% of baseline values, in endothelial cells exposed to normal serum. In contrast, a 0.1-mM concentration of 3-AB significantly increased mitochondrial respiration in cells incubated with septic serum from baseline levels of 70% ± 3% of control values to 106% ± 6% of control values ($p < .05$). Analysis limited to serum from septic shock patients, which decreased mitochondrial respiration by at least 15% from control values, demonstrated similar levels of improvement (Fig. 5).

Another set of experiments was done to determine whether serum from other critically ill nonseptic patients depressed mitochondrial respiration. Serum from eight normal volunteers was compared with serum from seven nonseptic patients and serum from fifteen patients with septic shock, including seven samples remaining from the original study population. The underlying diagnoses of these nonseptic patients included gastrointestinal bleed, acute stroke, hypoxic brain injury, and chronic obstructive lung disease requiring mechanical ventilation but without underlying pneumonia. At 2 hrs, mitochondrial respiration in cells exposed to control serum was 0.819 ± 0.0580 abs, as compared with 0.645 ± 0.105 abs (not significant) in cells exposed to serum from critically ill nonseptic patients. However, cells exposed to serum from septic patients demonstrated lower levels of mitochondrial activity, 0.462 ± 0.056 abs ($p < .05$) than controls ($p < .01$) and nonseptic patients ($p = .06$).

A final set of experiments was done to examine the relationship of decreases in mitochondrial respiratory activity as measured by conversion of WST-1 to formazan to alterations in high-energy phosphate levels. Serum remaining from 12 patients in septic shock and seven normal controls taken from the previous group was studied. ATP levels, 6.7 ± 1.2 nM, in endothelial cells exposed to septic cells were significantly lower than those exposed to serum from normal controls, 13.5 ± 1.9 nM (Fig. 6). A significant correlation between ATP levels and mitochondrial activity measured by WST-1 conversion was also observed ($r_s = .637$, $p = .007$).

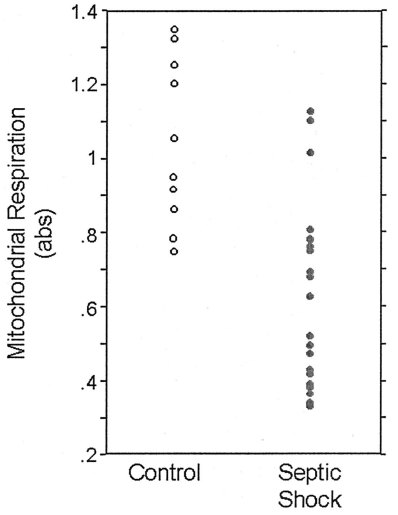

Figure 2. Individual values for endothelial cell mitochondrial respiration in cells incubated with serum from ten normal controls and serum from 20 patients with septic shock. Measured as mitochondrial-dependent reduction of WST-1 (*abs*, absorbance) at 2 hrs.

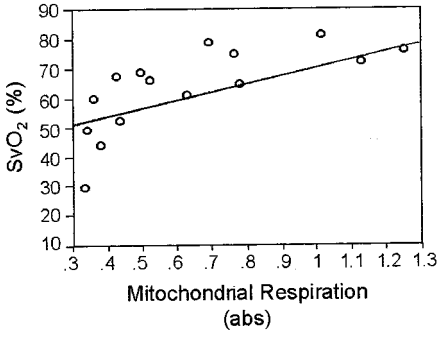

Figure 3. Mixed venous oxygen saturation (Svo_2) and mitochondrial respiration measured as mitochondrial-dependent reduction of WST-1 (*abs*, absorbance) for 15 patients with septic shock. Spearman's rank correlation coefficient, $r_s = .61$, $p < .05$.

DISCUSSION

The mechanisms of the underlying defects in oxygen extraction in septic shock have been an area of controversy and active interest since the initial clinical descriptions of this syndrome (1, 8, 23–25). Studies done on isolated mitochondria initially suggested that endotoxin depressed levels of mitochondrial respiration and produced uncoupling of mitochondrial oxidative phosphorylation (12, 13). Subsequent experiments confirmed the presence impaired mitochondrial function in endotox-

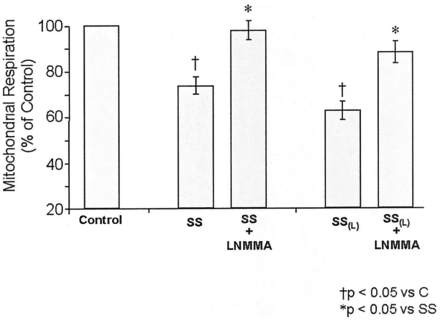

Figure 4. Effect of N^G-methyl-L-arginine (*LNMMA*; 0.1 mM) on the decrease in mitochondrial respiration in response to serum from 20 patients with septic shock (*SS*) expressed as percentage of respiration in endothelial cells exposed to serum from normal controls (*C*). Experiments involved 15 patients with serum producing a 15% decrease in mitochondrial respiration (SS_L). Data presented as mean ± SEM.

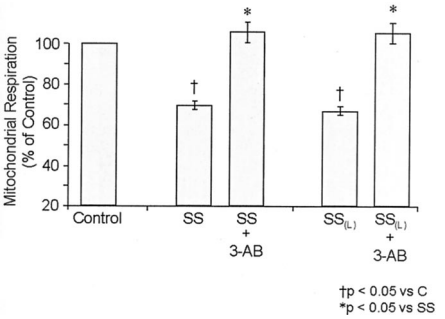

Figure 5. Effect of 3-aminobenzamide (*3-AB*) (0.1 mM) on the decrease in mitochondrial respiration in response to serum from 20 patients with septic shock (*SS*) expressed as percentage of respiration in endothelial cells exposed to serum from normal controls (*C*). Experiments involved 17 patients with serum producing a 15% decrease in mitochondrial respiration (SS_L). Data presented as mean ± SEM.

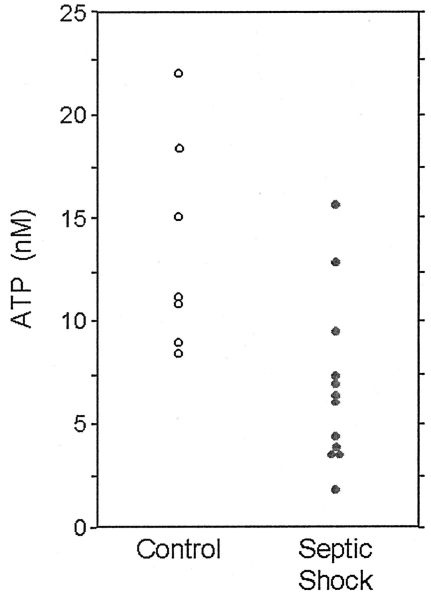

Figure 6. Individual values for endothelial cell adenosine triphosphate (*ATP*) levels (nM) in cells incubated with serum from seven normal controls and serum from 12 patients with septic shock.

emia and extended these observations to models of peritonitis and bacteremia (26–29). Reports of normal levels of tissue oxygenation in patients with septic shock and lactic acidosis and of the inability to increase oxygen consumption in the face of increased oxygen delivery support the presence of impaired oxygen utilization clinically (10, 11).

Our study provides further support of the concept that sepsis may cause direct impairment of mitochondrial respiration and extends these observations to patients with septic shock. Exposure to serum from patients with septic shock significantly impaired endothelial cell mitochondrial respiratory activity and resulted in decreases in cellular ATP levels. Similar decrements were not observed when endothelial cells were incubated with serum from a group of critically ill but nonseptic patients. Our observations are based on a modification of the MTT assay. Although the MTT assay is not a direct assay of mitochondrial respiration, it reflects the activity level of mitochondrial dehydrogenases and has been used extensively as a proxy of mitochondrial respiration in a variety of models of experimental sepsis (14, 15, 19). In endotoxin-stimulated vascular smooth muscle cells, decreases in MTT activity were associated with decreased cellular ATP and nicotine adenine dinucleotide levels (14). Similarly, decreases in mitochondrial MTT activity in intestinal epithelial cells exposed to endotoxin were associated with decreases in tissue oxygen consumption (15, 16). Our data are consistent with these previous reports and indicate that alterations in mitochondrial activity as assessed by the WST-1 assay are associated with decreases in cellular ATP levels

In our study, there was no significant correlation between arterial lactate concentration and *in vitro* measured mitochondrial respiration. Serum from patients with high (>3 mEq/L) and low arterial lactate (<3 mEq/L) concentrations resulted in similar degrees of impaired mitochondrial function. This observation probably reflects the multifactorial etiology of increased arterial lactate concentration in septic shock. Tissue hypoperfusion, decreased hepatic clearance, increased alanine flux, and impaired mitochondrial function may all contribute to lactate accumulation in septic shock (30–32). Moreover, the relative importance of these different factors may differ depending on the patient's hemodynamic status and underlying illnesses. For example, in our study, several patients were hypodynamic and had accompanying mixed venous desaturation, suggesting that in those patients, tissue hypoxia might be the major factor contributing to arterial lactate elevations. Conversely, in the more hyperdynamic patients, other factors could be more important.

In contrast to arterial lactate concentration, a significant correlation between *in vitro* mitochondrial activity and mixed venous oxygen saturation and cardiac index was observed. This relationship may be related to the effect of tissue hypoperfusion and tissue hypoxia in augmenting the release of inflammatory mediators (33, 34). Indeed, hypoxia has been demonstrated to increase the release of interleukin-6 and reactive oxygen species in isolated endothelial cells (34). The correlation we observed may also be related to the fact that the decreases in mixed venous oxygen saturation were observed primarily in the patients with myocardial depression and low cardiac indices. Nitric oxide–mediated pathways have been implicated as an important mechanism contributing myocardial depression during sepsis and, as our data suggest, may also play a role in impaired mitochondrial function in septic shock (35, 36).

Several of the proinflammatory mediators have been implicated in mitochondrial dysfunction during sepsis, including endotoxin, tumor necrosis factor, and most recently, nitric oxide. The effects of nitric oxide may be related to its direct inhibitory effects on the cytochrome a, a_3 complex (17, 18). Nitric oxide also interacts with superoxide to form peroxynitrite, which may impair mitochondrial respiration by irreversibly inhibiting iron-containing enzymes in the mitochondrial respiratory chain (17, 18). Peroxynitrite may also cause DNA strand breakage, thereby activating PARS with resulting depletion of nicotine adenine dinucleotide$^+$ and ATP (19). Previous studies indicate that these pathways contribute to mitochondrial dysfunction in septic shock. In vascular smooth muscle cells exposed to endotoxin, incubation with L-NMMA, an inhibitor of inducible nitric oxide synthase, and 3-AB, a PARS inhibitor, reversed endotoxin-induced decreases in mitochondrial respiration and ATP concentration (14). Similarly, pretreatment of endotoxemic rats with aminoguanidine, an inhibitor of inducible nitric oxide synthase, preserved gut mucosal oxygen consumption and preserved mitochondrial MTT reduction in endotoxin-exposed intestinal epithelial cells (15, 16).

Our observations are consistent with these data. The source of the nitric oxide in our cells is not clear. Although constitutive nitric oxide synthase is considered the major source of nitric oxide in resting endothelial cells, inflammatory mediators have been demonstrated to upregulate the expression of inducible nitric oxide synthase in endothelial cells from a variety of vascular beds (37–39). The increase in nitric oxide levels in the septic shock patients reflects the increased activity of inflammatory mediators in these patients. Unfortunately, these high background levels precluded an accurate measurement of the nitric oxide generated in the cell culture supernatants of the endothelial cells incubated with septic serum.

Data support the hypothesis that septic shock can result in direct inhibition of endothelial cell mitochondrial function.

Nonetheless, the improvement in mitochondrial respiration we observed in the presence of L-NMMA and 3-AB indicate that nitric oxide and peroxynitrite play a role in the inhibition of mitochondrial respiration induced by the serum of our patients with septic shock. These observations also suggest an important role for the PARS-mediated energy depletion in affecting mitochondrial respiration in patients with septic shock.

We did not observe a significant decrease in mitochondrial respiration when endothelial cells were incubated with serum from nonseptic critically ill patients, however, this does not exclude a possible role for nitric oxide–mediated mitochondrial dysfunction in other groups of critically ill patients. Cellular injury due to PARS activation has been implicated in organ dysfunction in experimental hemorrhagic shock and reperfusion injury (40). Indeed, abnormalities of mitochondrial activity have been reported in skeletal muscle biopsies taken from patients with cardiogenic shock (41).

Although the clinical significance of the impaired mitochondrial activity is unclear, there are data to indicate that these alterations may contribute to organ dysfunction in patients with septic shock. In a recent study, alterations in monocyte mitochondrial membrane potential were linked to the development of monocyte apoptosis (42). Similarly, experimental studies have demonstrated coupling of mitochondrial dysfunction with impaired vascular contractility and increased intestinal epithelial cell permeability during endotoxemia (15, 20).

In conclusion, our data support the hypothesis that septic shock can result in direct inhibition of endothelial cell mitochondrial function. This mitochondrial dysfunction seems to be mediated, in part, by nitric oxide and PARS activation.

REFERENCES

1. Rackow EC, Astiz M, Weil M: Cellular oxygen metabolism during sepsis and shock. *JAMA* 1988; 259:1989–1993
2. Ince C, Sinaasappel M: Microcirculatory oxygenation and shunting in sepsis and shock. *Crit Care Med* 1999; 27:1369–1377
3. Astiz M, DeGent G, Lin R, et al: Microvascular function and rheologic changes in hyperdynamic sepsis. Crit Care Med. 1995; 23: 265–271
4. Lam C, Tyml K, Martin C, et al: Microvascular perfusion is impaired in a rat model of normotensive sepsis. *J Clin Invest* 1994; 94: 2077–2083
5. DeBacker D, Creuter J, Vincent JL: Microcirculatory alterations in cardiogenic and septic shock. *Intensive Care Med* 2000; 26:S334
6. Temmesfeld-Wollbruck B, Szalay A, Mayer K, et al: Abnormalities of gastric mucosal oxygenation in septic shock: Partial responsiveness to dopexamine. Am J Respir Crit Care Med. 1998; 157(5 Pt 1):1586–1592
7. Schumer W, Erve P: Cellular metabolism in shock. *Circ Shock* 1975:109–127
8. Siegel J, Cerra F, Coleman B, et al: Physiologic and metabolic correlations in human sepsis. *Surgery* 1979; 86:163–193
9. Fink MP: Cytopathic hypoxia: Mitochondrial dysfunction as a mechanism contributing to organ dysfunction in sepsis. *Crit Care Clin* 2001; 17:219–237
10. Ronco J, Fenwick J, Wiggs B, et al: Oxygen consumption is independent of increases in oxygen delivery by dobutamine in septic patients who have normal or increased plasma lactate. *Am Rev Respir Dis* 1993; 147:25–31
11. Boekstegers P, Weidenhofer S, Kaspner T, et al: Skeletal muscle partial pressure of oxygen in patients with sepsis. *Crit Care Med* 1994; 22:640–650
12. Schumer W, Dasgupta T, Moss G, et al: Effect of endotoxemia on live mitochondria in man. *Ann Surg* 1970:875–882
13. Mela L, Bacalzao V, Miller L: Defective oxidative metabolism of rat mitochondria in hemorrhagic and endotoxic shock. *Am J Physiol* 1971; 220:517–577
14. Szabo C, Zingarelli B, Salzman A: Role of poly-ADP ribosyltransferase activation in the vascular contractile and energetic failure elicited by exogenous and endogenous nitric oxide and peroxynitrite. *Circ Res* 1996; 78: 1051–1063
15. Unno N, Wang H, Menconi M, et al: Inhibition of inducible nitric oxide synthase ameliorates lipopolysaccharide-induced gut mucosal barrier dysfunction in rats. *Gastroenterology* 1997; 113:1246–1252
16. King CJ, Tygat S, Delude R, et al: Ileal mucosal oxygen consumption is decreased in endotoxemic rats but is restored toward normal by treatment with aminoguanidine. *Crit Care Med* 1999; 27:2518–2524
17. Cassina A, Radi R: Differential inhibitory action of nitric oxide and peroxynitrite on mitochondrial electron transport. *Arch Biochem Biophys* 1996; 328:309–316
18. Brown GC: Nitric oxide and mitochondrial respiration. *Biochem Biophys Acta* 1999; 1411:351–359
19. Szabo C, Zingarelli B, O'Connor M, et al: DNA strand breakage, activation of poly-(ADP-ribose) synthetase, and cellular energy depletion are involve in the cytotoxicity of macrophages and smooth muscle cells exposed to peroxynitrite. *Proc Nat Acad Sci U S A* 1996; 93:1753–1758
20. Szabo C, Zingarelli B, Salzman A: Role of poly-ADP ribosyltransferase activation in the vascular contractile and energetic failure elicited by exogenous and endogenous nitric oxide and peroxynitrite. *Circ Res* 1996; 78: 1051–1063
21. Braman RS, Hendrix SA: Nanogram nitrite and nitrate determination in environmental and biological materials by vandium (III) reduction with chemiluminescence detection. *Anal Chem* 1989; 61:2715–2758
22. Lowik CW, Alblas MJ, van de Ruit M, et al: Quantification of adherent and nonadherent cell cultured in 96-well plates using the supravital stain neutral red. *Anal Chem* 1989; 61:2715–2758
23. Siegel J, Greenspan M, delGuercio L: Abnormal vascular tone, defective oxygen transport and myocardial failure in human septic shock. *Ann Surg* 1967; 165:504–517
24. Duff JH, Groves AC, McLean AP, et al: Defective oxygen consumption in septic shock. *Surg Gynecol Obstet* 1969; 128:1051–1060
25. Nishijima H, Weil M, Shubin H, et al: Hemodynamic and metabolic studies on shock associated with Gram negative bacteremia. *Medicine* 1973; 52:287–293
26. Astiz M, Rackow EC, Weil MH, et al: Early impairment of oxidative metabolism and energy production in severe sepsis. *Circ Shock* 1988; 26:311–316
27. Simonson S, Welty-Wolf K, Huang Y, et al: Altered mitochondrial redox response in gram negative septic shock in primates. *Circ Shock* 1994; 43:34–43
28. Kantrow S, Taylor D, Carraway M, et al: Oxidative metabolism in rat hepatocytes and mitochondria during sepsis. *Arch Biochem Biophys* 1997; 345:278–288
29. Schaefer C, Biber B, Lerne M, et al: Rapid reduction of intestinal cytochrome a, a_3 during lethal endotoxemia. *J Surg Res* 1991; 51:382–391
30. Kirshchenbaum LA, Astiz ME, Rackow EC: Interpretation of blood lactate concentration in patients with sepsis. *Lancet* 1998; 352: 921–922
31. Gutierrez G, Wulf M: Lactic acidosis in sepsis: A commentary. *Intensive Care Med* 1666; 22:6–16
32. Levraut J, Ciebiera JP, Chave S, et al: Mild hyperlactatemia in stable septic patients is due to impaired lactate clearance rather than overproduction. *Am J Respir Crit Care Med* 1999; 157:1021–1026
33. Ertel W, Morrison H, Ayala A, et al: Hypoxemia in the absence of blood loss or significant hypotension causes inflammatory cytokine release. *Am J Physiol* 1995; 265: R160–R166
34. Pearlstein DP, Mir H, Mungai P, et al: Role of mitochondrial oxidant injury in endothelial cells response to hypoxia. *Arterioscl Thromb Vasc Biol* 2002; 22:566–573
35. Fischer M, Oddis C, Jacobs T, et al: Negative inotropic effects of cytokines on the heart mediated by nitric oxide. *Science* 1992; 257: 387–389
36. Kumar A, Brar R, Wang P, et al: The role of nitric oxide and cyclic GMP in human septic serum-induced depression of cardiac myo-

cyte contractility. *Am J Physiol* 1999; 276: R265–R276
37. Kaku Y, Nanri H, Sakimura T, et al: Differential induction of constitutive and inducible nitric oxide synthases by distinct inflammatory stimuli in bovine aortic endothelial cells. *Biochem Biophys Acta* 1997; 1356:43–52
38. Papapetropoulos A, Andreopoulos S, Go Yhoque A, et al: Regulation of the nitric oxide synthase-nitric oxide-cGMP pathway in rat mesenteric endothelial cells. *J Appl Physiol* 2001; 91:2553–2560
39. Rockey D, Chung J: Regulation of inducible nitric oxide synthase in hepatic sinusoidal endothelial cells. *Am J Physiol* 1996; 271: G260–G267
40. Szabo C: The role of peroxynitrite in the pathophysiology of shock, inflammation and ischemia- reperfusion injury. *Shock* 1996; 6:79–88
41. Corbucci G, Gasparetto A, Candiani A, et al: Shock-induced damage to mitochondrial function and some cellular antioxidant mechanism in humans. *Circ Shock* 1985; 15:15–26
42. Adrie C, Bachelt M, Vayssier-Taussat M, et al: Mitochondrial membrane potential and apoptosis peripheral blood monocytes in severe human sepsis. *Am J Respir Crit Care Med* 2001; 164:389–395

Society of Critical Care Medicine
VISION STATEMENT

SCCM envisions a health system in which
all critically ill and injured persons will obtain care
that promotes desired outcomes for individuals and society,
is consistent with emerging knowledge,
and occurs in a humane and respectful manner.

Adopted by the SCCM Council
September 28, 1997