□Review Article□

Ventilatory Muscle Contractile and Vascular Dysfunctions in Different Types of Shock

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Introduction

Shock can be broadly defined as a state of reduced vascular perfusion which leads to generalized impairment of cellular function. The clinical syndrome of shock encompasses diverse pathologies such as cardiogenic, hemorrhagic, anaphylactic and septic shock. The term septic shock include several clinical conditions among which the unifying theme is the presence of bacterial infection. Unlike other forms of shock which are usually manifested as severe reduction in arterial pressure and cardiac output, the vascular manifestations of septic shock is divided into two phases, an initial hyperdynamic phase during which hypotension is usually associated with a significant rise in cardiac output and a decline in total vascular resistance. The late (hypodynamic) phase of human sepitc shock, on the other hand, is associated with severe hypotension, low cardiac output and a significant rise in peripheral vascular resistance.

Respiratory insufficiency is known to develop in various forms of shock but it is particularly serious complication in patients with septic shock. Traditionally, respiratory insufficiency is attributed to lung injury and is manifested in the early stages of septic shock as hypoxemia, elevated pulmonary arterial pressure, increased pulmonary shunting and decreased lung compliance. In addition to lung injury, there is growing evidence that shock state is associated with ventilatory pump failure. In patients with fulminating infection and sepsis, Burke *et al*[11]. described the development of hypercapneic respiratory failure in the presence of normal function and arterial PO_2. In a separate study, Pontopiddan and colleagues[46] proposed that inspiratory muscles may fail during shock because of an imbalance between energy supplies and demands. More recently, Cohen and colleagues[14] described clinical as well as electromyographic evidence of diaphragmatic contractile failure in patients with acute respiratory failure or severe sepsis who could not be weaned from mechanical ventilation[14]. The failure of ventilatory muscles in septic shock could be attributed in part to bacterial infection itself, irrespective of the hemodynamic and metabolic derangements associated with septic shock syndrome. This notion is supported by the findings that limb muscle endurance capacity and maximum force decline significantly in

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response to bacterial infections in humans[18].

In addition to these observations in humans, there is strong evidence that ventilatory muscle contractile performance is compromised in animals with cardiogenic, hemorrhagic or septic shock. For instance, Aubier and colleagues[1] were the first to report that cardiogenic shock initiated by cardiac tamponade in dogs is associated with impaired diaphragmatic contractility. Similarly, our group reported that reduction of mean arterial pressure to about 50 mmHg by inflating a balloon in the inferior vena cava of dogs was associated with significant reduction in diaphragmatic contractility measured by artificial phrenic nerve stimulation[25]. There is also ample evidence that experimental septic shock, which is usually created by infusion of live bacteria and/or bacterial endotoxin in experimental animals, is associated with depressed contractile performance of limb and ventilatory muscles. We reported for the first time that systemic injection of a bolus dose of *Escherichia coli* endotoxin in dogs elicits a progressive decline in arterial pressure and cardiac output over several hours with a decline in the ability of the ventilatory muscles to ventilate the lungs, eventually leading to hypercapneic respiratory failure[27]. In an another study, Leon and colleagues[36] addressed the acute (less than 60 minutes) effects of *E. coli* endotoxemia on ventilatory muscle contractility in mechanically ventilated rats. They reported that endotoxin infusion reduced the ability of the diaphragm to generate force in response only to high frequency ($\geq$30 Hz) stimulation, whereas *in-situ* generated diaphragmatic force in response to low frequency ($\leq$20 Hz) stimulation was not influenced by acute endotoxemia[36]. Diaphragmatic endurance, on the other hand, was attenuated by about 25% in response to acute *in-vivo* exposure to endotoxin[36] (**Figure 1**). The exact cause of the differences between the findings of Leon *et al* and those of Hussain and colleagues is not clear but could be related to methodological considerations as well as differences in the metabolic requirements of the ventilatory muscles in the two studies (mechanically ventilated vs spontaneously breathing animals).

In addition to the acute effects of endotoxin infusion, several investigators assessed the influence of prolonged (over 3 days) endotoxemia on ventilatory muscle performance. The degree of contractile failure in response to prolonged systemic exposure to low concentrations of endotoxin, however, appears to vary, depending on many factors such as endotoxin concentration, methodological considerations and animal species. For instance, while Boczkowski *et al*[7]. reported a decline in diaphragmatic force in response to high frequency stimulation after 3 days of endotoxemia in rats, Shindoh and colleagues[48] studied diaphragmatic contractility and fatigue resistance after 3 days of endotoxin injection in hamsters. These authors found that *in-vitro* isolated diaphragmatic strips obtained from endotoxemic hamsters generated significantly lower tension in response to a wide range of stimulation frequencies when compared with strips obtained from normal hamsters. Both groups of investigators, however, agree that diaphragmatic endurance in response to low frequency stimulation is significantly attenuated after prolonged endotoxemia in hamsters and rats[7][48][52].

In addition to bacterial endotoxin, systemic bacteremia induced by infusion of live bacteria is also associated with significant impairment of ventilatory muscle contractile performance. Boczkowski *et al*[6]. studied *in-situ* generated transiaphragmatic pressure in response to artificial phrenic nerve stimulation after 3 days of

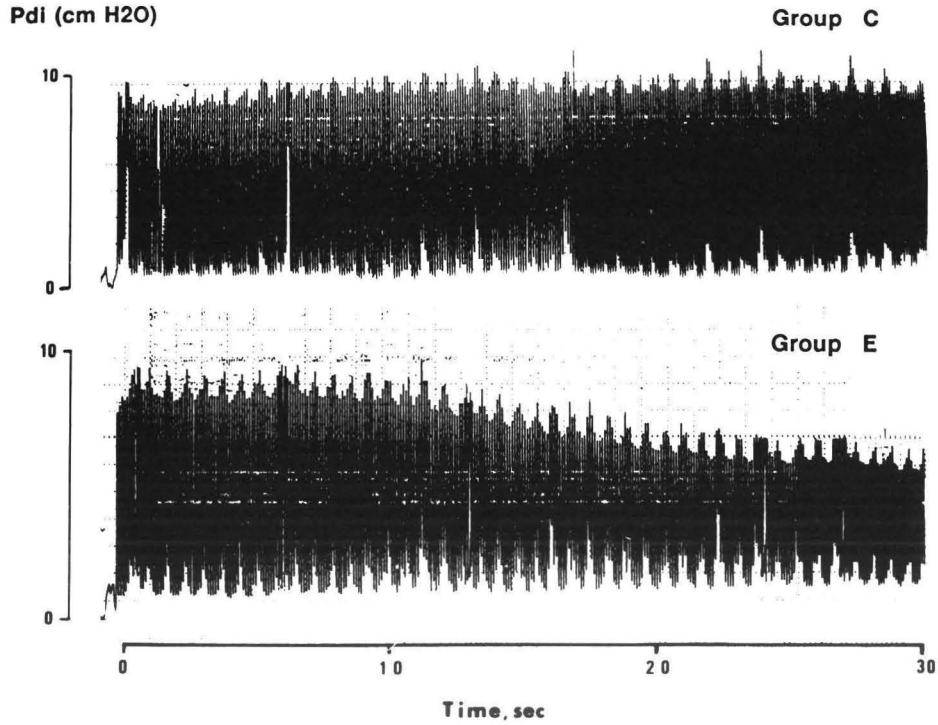

Figure 1：Influence of sepsis on diaphragmatic endurance in mechanically ventilated rats. Typical tracing showing changes in Pdi (transdiaphragmetic pressure) during a contious 30-s stimulation of the phrenic nerves at 10 Hz in control animals (group C) and an endotoxic animal (group E) at T 75. Baseline variations are artifacts due to mechanical ventilation. (Leon and colleagues, J Appl Physiol 72(4)：1466-1472, 1992)

subcutaneous inoculation with *Streptococcus pneumoniae* in rats. They found that, although muscle weight was not influenced by sepsis, the diaphragmatic force-frequency relationship shifted significantly to the right when compared to control animals (**Figure 2**). In addition, diaphragmatic endurance capacity measured during 30-seconds stimulation at 10 Hz was attenuated by more than 25% compared with control animals. The influence of bacterial infusion on ventilatory muscle contractility has also been confirmed by Murphy *et al*[41]. who infused group B *streptococcus* bacteria in piglets and reported a significant decline in diaphragmatic contractility after hour of bacterial infusion. This decline in force persisted throughout the 4- hours observation period.

Site of Muscle Failure

The exact cellular site of ventilatory muscle mechanical failure in shock state appears to vary between different studied depending on the methodology, animal species and experimental conditions. For instance, our group[27] measured both phrenic neural activity and electromyographic activity of the diaphragm and concluded that the site of ventilatory muscle mechanical failure during endotoxemic shock is beyond the neuromuscular junction because the ratio of phrenic neural activity and diaphragmatic electromyographic activity remained unchanged. A similar conclusion

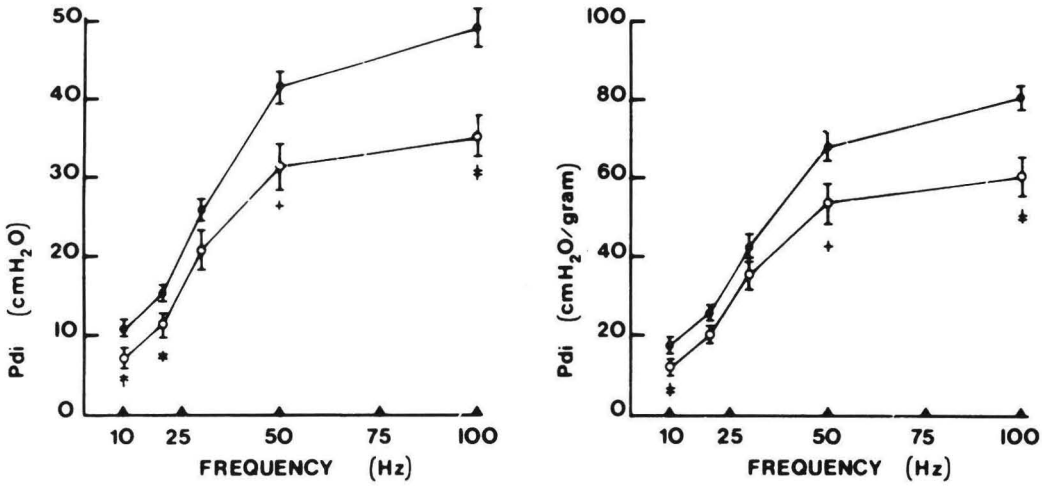

Figure 2：Influence of 3-day pneumococcal infection on diaphragmatic strength. Relationship between frequency of stimulation and transdiaphragmatic pressure (Pdi) expressed in absolute values (cmH_2O) and normalized (cmH_2O)/g) in control (closed circles) and septic (open circles) animals. Each point represents mean±SEM. Asterisk indicates significantly differenet from control, $P<0.05$. Cross indicates significantly different from control, $P<0.01$. Double cross indicates significantly different from control, $P<0.001$. (Boczkowski et al. Am Rev Resp Dis 138：260-265, 1988)

was drawn regarding the site of the ventilatory muscle failure in animals with cardiogenic shock[1]. In comparison, Leon and colleagues[36] concluded that neuromuscular transmission failure is the main site of decreased diaphragmatic strength and endurance in endotoxemic mechanically ventilated rats. These authors attributed endotoxemia-induced transmission failure to a significant elevation of muscle membrane potential. Whether neuromuscular transmission failure and/or failure of excitation-contraction coupling are involved in depressed ventilatory muscle contractile failure in human septic shock remains to be evaluated.

Mediators of Muscle Failure

The precise factor(s) causing depression of ventilatory muscle contractile performance in shock state have yet to be elucidated. One of the early candidates has been bacterial endotoxin (lipopolysaccharide) which is known to be an important mediator of hemodynamic changes in septic shock. Although endotoxin was initially proposed to depress muscle contractility by initiating metabolic and hemodynamic alterations inside muscle fibers leading eventually to muscle failure, there is no clear evidence implicating their direct impact on muscle function. This is confirmed by Diaz and colleagues[16] who reported that *E. coli* endotoxin had no direct effects on the *in-vivo* contractility of isolated rat diaphragm. These authors, however, examined the direct action of endotoxin after only a short period of exposure (60 minutes). Cytokines such as tumor necrosis factor (TNF-α) and interlukins are another group of factors which has been proposed to mediate, directly or indirectly, sepsis-induced muscle contractile failure. TNF-α is released from immune cells such as macrophages, monocytes and mast cells in response to endotoxin infusion and is consid-

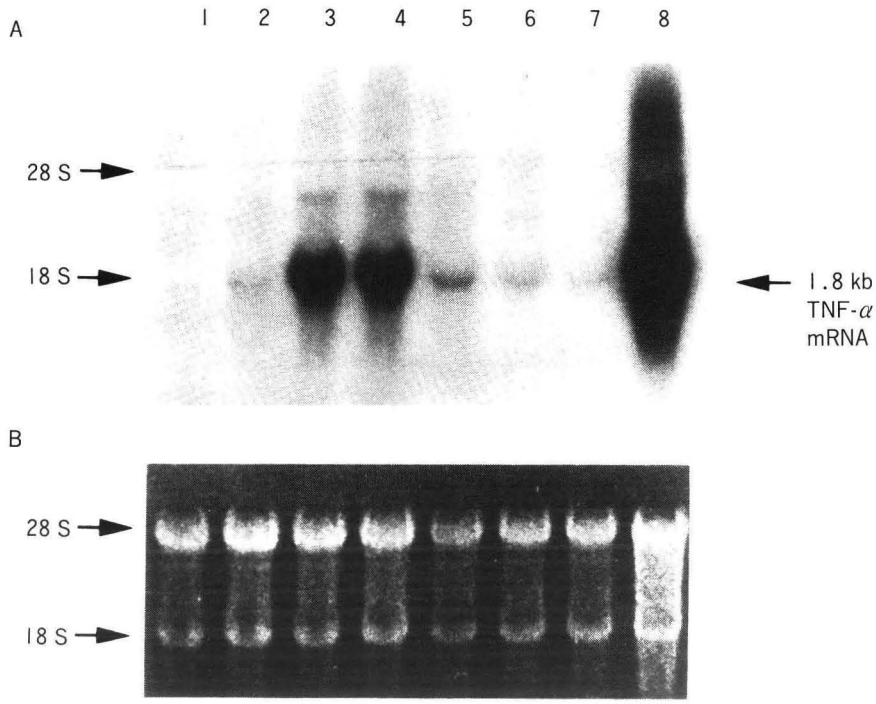

Figure 3：Tumor Necrosis Factor (TNF)-α mRNA gene expresson in diaphragmatic tissue after endotoxin (20 mg/kg) injection. 20 μg of total RNA was loaded on each lane and electrophoresed in 1% agarose gel under denaturing conditions. A：The TNF-α mRNA was evaluated by Northern blot analysis with ^{32}P-labeled mTNF-α cDNA probe. Lane 1：control；lane 2, 0.5 h；lane 3, 1 h；lane 4, 1.5 h；lane 5, 2 h；lane 6, 4 h；lane 7, 6 h；lane 8 positive control, in which TNF-α mRNA expression in rat lung stimulated with LPS was used. B：acridin orange staining indicated almost equl loading amount (20 μg) of RNA was electrophoresd in the gel. 28 S and 18 S indictate 28 S ribosome and 18 S ribosome, respectively. (Shindoh et al. Am J Crit Care Resp Med 152：1690-1694, 1995)

ered to be a central mediator of immune and inflammatory responses. The involvement of TNF-αin diaphragmatic mechanical failure has been examined by Wilcox *et al*[59]. who reported that 3 hours after systemic TNF-α infusion, diaphragmatic pressure and muscle shortening were substantially depressed when compared to control animals. This decline in contractility appears to be the result of neuromuscular transmission failure since peak to peak amplitude of muscle action potential declined significantly after several hours of TNF-α infusion. The involvement of TNF in muscle dysfunction during septic shock was confirmed by Shindoh *et al*[49]. who described a significant elevation of TNF-α messenger RNA in the diaphragm after 3 hours of endotoxin infusion (**Figure 3**). In addition, these authors found that preinjection of anti-TNF-α antibodies partially reversed endotoxin-induced diaphragmatic hypocontractility. Their results imply an important role for TNF-α in depressed muscle

contractile performance in sepitc shock or endotoxemia. It is not clear yet, however, whether TNF-α acts directly or indirectly on muscle contractile machinery. Diaz and colleagues[16] excluded a direct influence of TNF-α on muscle contractility by showing no change in the force-frequency relationship of the *in-vitro* isolated rat diaphragm after 60 minutes of incubation with TNF-α. On the other hand, it is possible that TNF-α may act by inducing secondary messenger molecules such as superoxide, hydrogen peroxide and hydroxyl radicals. These radicals are known to be synthesized in the mitochondria of skeletal muscle fibers and could also be released by activated neutrophils.

There is increasing evidence that reactive oxygen species play a significant role in contractile dysfunction associated with septic shock. The direct influence of these species on muscle contractility was studied by Supinski *et al.* and Shindoh and colleagues[52)48)] who reported that infusion of anti-oxidant enzymes and scavengers such as superoxide dismutase, catalase and dimethylsulfoxied attenuate the decline in diaphragmatic force and endurance in response to endotoxin infusion. It should be emphasized that these results imply a role for oxygen radicals, however, these radicals may be acting in concert with other mediators such as nitric oxide (NO), resulting in the formation of peroxynitrite which is known to inhibit mitochondria respiration.

Bacterial endotoxin could alter arachidonic acid metabolism, stimulating the release of prostaglandins such as prostacyclin and thromboxane A_2. The importance of arachidonic acid metabolites in mediating diaphragmatic dysfunction in animals with septic shock was confirmed by Boczkowski *et al*[7]. who reported that pretreatment with indomethacin, an inhibitor of cyclooxygenase, completely reversed the decline in diaphragmatic force generating capacity and prolongation of twitch relaxation time observed after 3 days of sublethal endotoxemia. Similar findings with indomethacin pretreatment were described during acute bacteremia in piglets[41]. Murphy *et al*[41]. however, attributed the effects of indomethacin to the prevention of thromboxane A_2 release since infusion of thromboxane A_2 analogue elicited similar changes in diaphragmatic force generation similar to those observed after acute bacteremia.

Finally, there is evidence that shock state in general and septic shock in particular is associated with enhanced nitric oxide (NO) synthesis inside skeletal muscle fibers which in turn may lead to impaired muscle contractility. NO is a secondary messenger molecule which is synthesized by a group of flavoproteins known as nitric oxide synthase (NOS). The role of NO in hemodynamic, metabolic and contractile derangements of septic shock is discussed below.

Ventilatory, Metabolic and Hemodynamic Factors

Contractile dysfunction of the ventilatory muscles in shock state is likely to be due to several factors working in concert to increase muscle metabolic demands, interfere with muscle perfusion and inhibit several metabolic processes which are essential for generation of muscle force.

It is well known that ventilatory demands increase substantially during the course of hypotension as a result of a combination of increased minute ventilation and alterations in pulmonary and airway mechanics. These changes lead to increased requirements for blood flow and O_2 delivery to the ventilatory muscles.

Another important factor which may be

involved in causing depressed muscle contractile failure is alterations in muscle blood flow. Maintaining normal delivery of O_2 and other metabolites is critically dependent on normal muscle blood flow which under normal conditions is regulated through changes in perfusion pressure and muscle vascular resistance. Under normal ranges of arterial pressure, ventilatory muscle perfusion is autoregulated independently of changes in perfusion pressure[26]. At low blood pressure values, muscle blood flow becomes a function of arterial pressure despite the significant decline in muscle vascular resistance[26]. It is, therefore, predictable that as arterial pressure declines during the course of shock, ventilatory muscle blood flow may be inadequate to meet muscle metabolic demands, leading to limitation of muscle aerobic capacity and greater reliance on anaerobic metabolism. While reduction in muscle ventilatory muscle blood flow is likely to be an important factor in determining muscle contractility in hemorrhagic shock, our results indicate that ventilatory muscle blood flow in septic animals is elevated out of proportion to the increase in metabolic demands even though arterial pressure declines substantially[24]. This condition of substantial vasodilation has also been observed in other skeletal muscles during the course of endotoxemia and has been termed "vascular decompensation"[10]. Several mechanisms are known to be involved in casuing vascular decompensation in septic shock. These include direct inhibition of the sympathetic system by circulating bacterial endotoxin[3], direct suppression of vascular smooth muscle metabolism[50], increased metabolic demands with release of local vasodilator substances[10], and augmented NO release resulting from activation of the inducible or constitutive NOS[4)31)29].

The exact contribution of vascular decompensation to depressed muscle contractile performance in septic shock remains under investigation. Vascular dilation can lead to heterogenous intramuscular blood flow distribution which, in turn, divert nutritive flow away from active muscle fibers depriving these fibers of necessary energy sources. Increased blood flow heterogeneity may also lead to enhanced microembolization of capillaries by platelets and white cell aggregates which, in turn, leads to reduced capillary density and poor O_2 extraction. Both, reduced capillary recruitment and depressed O_2 extraction have been documented in the diaphragm of endotoxemic rats and dogs[9)32]. Impaired O_2 extraction is also likely to increase the dependence of ventilatory muscle O_2 consumption on local O_2 delivery. Under normal conditions, local O_2 consumption is independent of changes in O_2 delivery because of compensatory changes in O_2 extraction. At a certain critical O_2 delivery, the increase in O_2 extraction can no longer maintain O_2 consumption constant, hence, further reduction of O_2 delivery below this critical value will result in limitation of local O_2 consumption. We reported recently[32], that critical diaphragmatic O_2 delivery in normal dogs averaged 9 ml/kg/min. In septic shock, this value increases to an average of 14.8 ml/kg/min. We also found that this pathological dependence on O_2 delivery is not limited to the diaphragm only since a similar elevation of critical systemic O_2 delivery has been observed in endotoxemia[32].

Impaired blood flow distribution during shock state also leads to the dependence of muscle metabolims on anaerobic energy sources which in turn cause a rise in lactic acid production. Our group and that of Aubier et al[2)24]. reported that ventilatory muscle lactate concentration increases significantly after several hours of cardiogenic or septic shock. This

rise in lactate concentration was associated with depletion of ventilatory muscle glycogen stores[2)24)]. These results suggest that these muscles rely heavily on intramuscular energy stores for their metabolic needs in the state of shock. Furthermore, septic shock is associated with other metabolic derangements such as impaired carbohydrates utilization. Indeed, a significant elevation of blood glucose and a degree of glucose intolerance occur during the early phases of septic shock and are believed to be due to the depressed ability of skeletal muscles to utilize glucose. In addition to abnormalities in glucose metabolism, the late stages of septic shock are usually associated with impaired free fatty acid utilization by skeletal muscles. As a result of these aberrations in carbohydrate and lipid metabolism, skeletal muscles become more dependent on proteins as energy sources, leading to increased muscle wasting and elevated blood levels of non-branched amino acids, especially during the late stages of septic shock. other metabolic defects associated with septic shock include depression of pyruvate dehydrogenase activity, a defect which is likely to cause increased intramuscular lactate concentration. In addition, significant alterations in mitochondrial morphology and functions have been observed in septic shock, including depression of cytochrome oxidase activity, reduced Ca^{++} trans port, poor coupling of respiration and phosphorylation as well as marked distortion of inner and outer mitochondrial membranes[38)]. Besides mitochondrial defects, various processes necessary for normal functioning of the muscle contractile machinery may be directly inhibited during shock. These include suppression of myofibrillar ATPase[44)] and sarcoplasmic reticulum Ca^{++} uptake[21)] which eventually leads to interference with normal excitation-contraction coupling.

The involvement of nitric oxide pathway

NO is synthesized by nitric oxide synthases (NOSs), a group of hemoproteins which catalyse L-arginine conversion to NO and L-citrulline. Three main NOS isoforms have been identified so far ; 2 of them are constitutively expressed and were first described in the endothelial cells (the endothelial isoform, ecNOS) and brain cells (the neuronal isoform, nNOS). The third is an inducible (iNOS) isoform which is expressed in a variety of cells in response to bacterial endotoxin and pro-inflammatory cytokines.

In the vascular system, NO synthesis is known to regulate peripheral vascular tone by acting on smooth muscle guanylate cyclase leading to increased cGMP concentration in these muscles. In normal skeletal muscles, it has been well established that NO synthesis by the ecNOS isoform localized in the endothelial cells plays an important role in the regulation of skeletal muscle blood flow. Indeed, infusion of NOS inhibitors in the vasculature of various skeletal muscles leads to a significant rise in vascular resistance, suggesting continuous NO release from endothelial cells regulating the tone of resistance vessels[45)55)57)]. Direct measurement of NO release by several techniques has confirmed basal NO release in several vascular beds[30)]. It has also been established by various groups that NO not only regulate basal vascular resistance but also mediates augmented muscle blood flow during increased metabolic demands (active hyperemia) and reactive hyperemic responses observed following brief arterial occlusions[28)56)58)]. Finally, basal NO synthesis in normal skeletal muscle fibers also plays a role in the regulation of muscle O_2 consumption through its modulation of blood flow and mitochondrial respiration (see below)[12)].

Until 1993, the only source of NO release in skeletal muscles was assumed to be the endothelium. More recent studies indicate, however, that the source of NO in skeletal muscles may not be restricted to the endothelium. Nakane *et al*[42]. reproted that mRNA of the bNOS isoform is expressed in human skeletal muscle fibers. Western blotting analysis and measurements of NOS activity confirmed the presence of bNOS in normal muscle fibers. In a subsequent study, Kobzik *et al*[33]. confirmed that bNOS also exists in rat muscle fibers and is localized in close proximity to the sarcolemma. In addition, these authors found that bNOS expression was higher in muscles rich with type II fibers compared with muscle rich in highly oxidative (type I) fibers. One of the most important findings of Kobzik and colleagues[33] is that inhibition of endogenous NOS activity in skeletal muscles results in a significant rise in submaximal force generated during direct muscle stimulation. This observation suggests that muscle contractility is regulated in part by endogenous NO synthesis. The mechanisms through which NO modulate muscle force are still being investigated but there is strong evidence that a rise in cGMP levels is an important pathway through which NO may influence excitation-contraction coupling. In addition to the neuronal isoform, NO is synthesized inside skeletal muscle fibers by the endothelial isoform which is localized at the mitochondria[34]. The exact functional significance of muscle fiber ecNOS expression remains to be elucidated but Kobzik and colleagues[34] proposed that ecNOS regulates mitochondrial respiration by inhibiting the activity of cytochrome oxidase. This notion is supported by the observation that addition of NOS substrate, L-arginine, to isolated skeletal muscle mitochondrial preparation leads to inhibition of respiration. More recent studies using electron microscopy and immunostaining confirmed that ecNOS exists not only in skeletal muscle mitochondria but in the mitochondria of various cells such as cardiac myocytes, brain cells and hepatocytes. Despite these recent advances in the identification of various NOS isoforms in normal mus cles, the exact physiological roles of sarcolemmal bNOS expression and mitochondrial ecNOS expression in the regulation of normal muscle function remain to be thoroughly evaluated.

Role of NO in Septic Shock

Numerous investigators confirmed in the past few years that NO plays an important role in the pathogenesis of shock state particularly in septic shock. The three NOS isoforms appear to be participate in enhanced NO release during sepitc shock. The inducible isoform (iNOS) has been implicated in mediating numerous immune, hemodynamic and metabolic aberrations observed in septic shock, such as skeletal muscle vascular decompensation, depressed vascular reactivity and hypotension. These changes have been attributed to iNOS induction in smooth muscle cells, which, in turn, results in increased cyclic GMP concentration leading to muscle relaxation. The expression of iNOS has been documented in blood vessels of septic animals[29)37)]. The role of iNOS in these hemodynamic alterations has also been supported by observations that inhibitors of iNOS or guanylate cyclase in endotoxemic or septic animals restore arterial pressure and improve the depressed contractility of large arteries exposed to endotoxin[5]. iNOS expression has also been detected in various organs such as the lung, liver and spleen of septic animals[37]. The involvement of NO release in human septic shock has been recently assessed by correlating plasma nitrite and nitrate with the changes in cardiac output. In addition,

recent clinical trials on septic patients indicate that infusion of methylene blue, an inhibitor of guanylate cyclase, reversed the decline in arterial pressure and systemic vascular resistance and reduced blood lactate levels[47]. Others have, however, questioned the role of iNOS in septic shock because : a) vascular reactivity is not completely restored even when iNOS is totally inhibited ; b) iNOS expression is not always associated with depressed vascular reactivity ; and c) reduced vascular reactivity in septic animals sometimes precedes iNOS induction.

In addition to the induction of iNOS, investigators have reported that enhanced NO release during septic shock may also originate from the activity of ecNOS which causes hypotension and vascular hyporeactivity in the early stages of septic shock. This notion of ecNOS involvement is based on the observation that NOS inhibitors attenuate the initial and sustained phases of septic shock in rats[53] and that hypotension and vascular hyporeactivity during the early phase of septic shock are not prevented by pretreatment with dexamethasone, an inhibitor of iNOS induction. Finally, rapid NO release by ecNOS after endotoxin infusion has been postulated to occur in the microvasculature of the isolated rat heart.

Our group and others have recently studied the importance of enhanced NO release in the pathogenesis of vascular and contractile dysfunction of the ventilatory muscles in animals with septic shock. Using *in-situ* isolated diaphragmatic preparations in dogs, Hussain noticed that 90 minutes after endotoxin infusion, O_2 consumption of the resting diaphragm rose significantly, whereas phrenic vascular resistance declined substantially[22]. When a NOS inhibitor was infused into the diaphragm, phrenic vascular dilation was eliminated, whereas the rise in diaphragmatic O_2 consumption remained unchanged, suggesting that endotoxemia-induced vascular dysregulation is mediated by enhanced NO release but elevated O_2 consumption is mediated by other unknown mechanisms. The source of enhanced NO release in that study, however, was not identified. In more recent experiments, we investigated the influence of septic shock on NOS activity and expression of various NOS isoforms in the ventilatory and limb muscles of the rats. A significant rise in Ca^{++}/ calmodulin-dependent and Ca^{++}/ calmodulin-independent NOS activity was evident in the diaphragm, intercostal, gastrocnemius and soleus muscles after 6 hours of endotoxemia[23]. These data indicate that enhanced NO release in ventilatory and limb muscles is mediated through increased activity of iNOS and both constitutive NOS isoforms (ecNOS and bNOS). Western blotting analysis confirmed the induction of iNOS in these muscles, whereas no iNOS expression was evident in the muscles of normal rats (**Figure 4**). Polymerase chain reaction experiments and western blotting analysis also revealed that both ecNOS and bNOS expression are elevated in the diaphragm and intercostal muscles of endotoxemic rats, confirming that enhanced NO formation in the ventilatory muscles during the course of endotoxemia is due not only to iNOS induction but also to increased expression of constitutive NOS isoforms. Moreover, we found that septic shock was associated with induction of GTP cyclohydrolase I which is the rate limiting enzyme in the synthesis of tetrahydrobiopterin, an essential cofactor for NOS activity[23]. Thus, elevated NOS activity in the ventilatory muscles of septic rats is not due only to increased expression of NOS proteins but also to enhanced cofactor availability.

The physiological implications of enhanced NOS activity for ventilatory and limb muscle

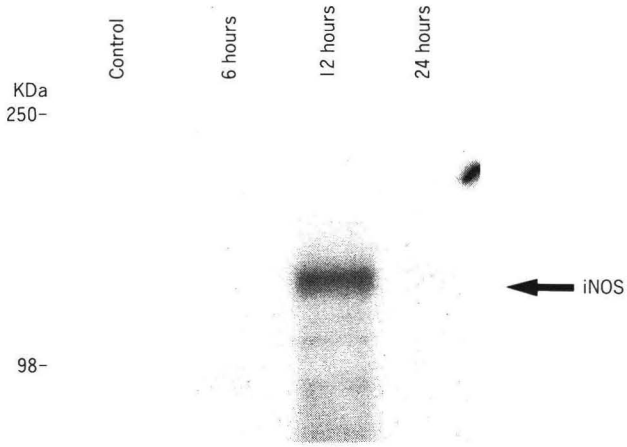

Figure 4：Expression of the inducible nitric oxide synthase (iNOS) in septic rats. Diaphragmatic samples obtained from control rats and rats sacrificed after 6, 12 and 24 of *E. coli* endotoxin injection. Diaphragmatic proteins were electophoresed under denaturing conditions and probed with anti-iNOS antibody. iNOS expression peaked 12 hrs after LPS injection. (El-Dwairi *et al.* Am J Physiol 274：C 770-C 779, 1998)

contractile performance during septic shock have recently been confirmed by the observations that muscle contractile dysfunction is either reversed or prevented by inhibitors of NOS isoforms[8)17)19)] (**Figure 5**). We propose the following mechanisms through which increased NO production may depress muscle contractile function in septic shock. *First,* NO attenuates mitochondrial respiration by inhibiting several enzymes such as cytochrome oxidase and aconitase[13)34)43)54)]. *Second,* NO interacts with superoxied anions leading to the formation of peroxynitrite, a potent radical species which targets various molecules such as thiols, lipids and protein containing aromatic amino acids[15)]. Several investigators have described the formation of peroxynitrite in the aorta and lungs of endotoxemic rats[15)]. It is possible that iNOS induction in the muscles of endotoxemic rats leads to the formation of peroxynitrite which, in turn, causes depressed muscle contractile function. *Third,* induction of iNOS in vascular smooth muscle cells could cause depressed vascular reactivity and poor matching of blood flow to metabolic demands in endotoxemic animals[20)]. Enhanced NO release has indeed been implicated by Hussain in endotoxin-induced diaphragmatic dysfunction[22)]. *Fourth,* it has recently been demonstrated that NO inactivates glyceraldehyde-3-phosphate dehydrogenase, a key enzyme of glucose metabolism[40)]. *Fifth,* ：NO has recently been shown to inhibit Ca^{++} release from the skeletal muscle sarcoplasmic reticulum[39)]. It is, therefore, likely that increased NO release will lead to reduction in the availability of Ca^{++} for excitation-contraction coupling and the contractile machinery and, result in reduced muscle force generation. Clearly, more detailed evaluation of the role of NO release in

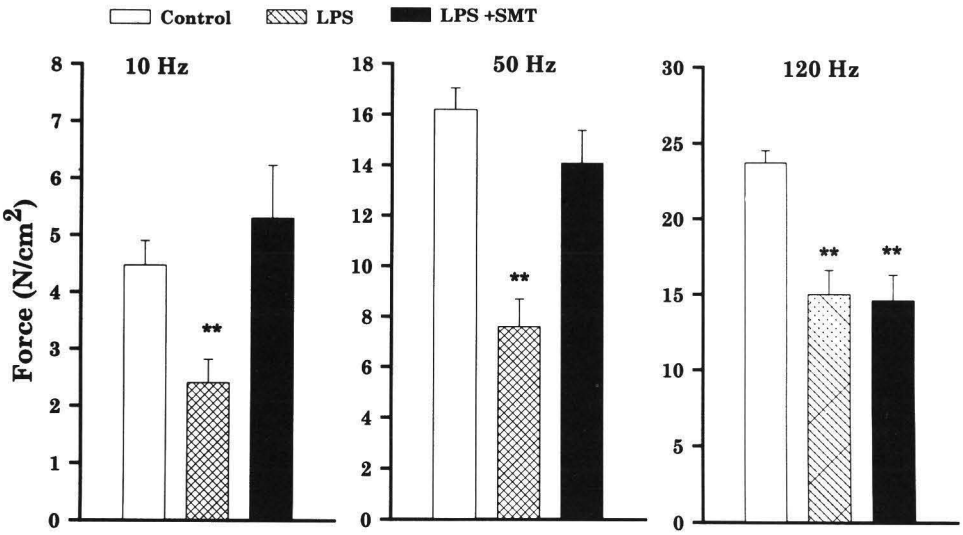

Figure 5：The involvement of NO in sepsis-induced diaphragmatic contractile dysfunction. Diaphragmatic strips were isolated from control rats and rats injected with E. coli endotoxin. Diaphragmatic contractility was assessed by supramaximal direct stimulation at 10, 50 and 120 Hz. In the endotoxemic group, muscle contractiligy was assessed before (hateched columns) and after (filled columns) exposure to 1 mM of S-methylisothioures (SMT) (nitric oxide synthase inhibitor). **P<0.01 compared with control values. Note that SMT exposure restored submaximal force with no influence on maximal diaphragmatic force generation. (El-Dwairi *et al.* Am J Physiol 274：C 770-C 779, 1998)

modulating muscle contractile performance under normal conditions and in septic shock is needed.

In summary, there is good evidence that ventilatory muscle contractile performance is depressed in septic shock and this is likely to cause hypercpaneic respiratory failure. Depression of muscle contractile performance in septic shock is the product of two main pathways：minor pathway, which is the increase in ventilatory muscle metabolic demands, and a major pathway involving numerous cellular defects, immune, metabolic and hemodynamic aberrations which interfere with several processes necessary for the generation of muscle force. Evidence from the literature indicates that these aberrations are mediated by a complex interaction between multiple molecules such as bacterial endotoxin, inflammatory cytokines, oxygen reactive species, products of arachidonic acid metabolisms and NO. The exact role of each of these molecules in the pathogenesis of depressed muscle performance remains to be determined.

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