Review

Bench-to-bedside review: Microvascular dysfunction in sepsis – hemodynamics, oxygen transport, and nitric oxide

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Abstract

The microcirculation is a complex and integrated system that supplies and distributes oxygen throughout the tissues. The red blood cell (RBC) facilitates convective oxygen transport via co-operative binding with hemoglobin. In the microcirculation oxygen diffuses from the RBC into neighboring tissues, where it is consumed by mitochondria. Evidence suggests that the RBC acts as deliverer of oxygen and 'sensor' of local oxygen gradients. Within vascular beds RBCs are distributed actively by arteriolar tone and passively by rheologic factors, including vessel geometry and RBC deformability. Microvascular oxygen transport is determined by microvascular geometry, hemodynamics, and RBC hemoglobin oxygen saturation. Sepsis causes abnormal microvascular oxygen transport as significant numbers of capillaries stop flowing and the microcirculation fails to compensate for decreased functional capillary density. The resulting maldistribution of RBC flow results in a mismatch of oxygen delivery with oxygen demand that affects both critical oxygen delivery and oxygen extraction ratio. Nitric oxide (NO) maintains microvascular homeostasis by regulating arteriolar tone, RBC deformability, leukocyte and platelet adhesion to endothelial cells, and blood volume. NO also regulates mitochondrial respiration. During sepsis, NO over-production mediates systemic hypotension and microvascular reactivity, and is seemingly protective of microvascular blood flow.

Keywords erythrocyte, inflammation, microcirculation, nitric oxide, oxygen

"Five to fifteen minutes after its [endotoxin] intravenous administration, there were strong waves of contraction along the small arteries, arterioles and metarterioles. These could arrest flow and last for several minutes. There would afterwards be a phase of dilatation, followed again by a strong contraction. As time went on, the phases of relaxation became more prominent until preagonally there was a general and permanent vasodilation. The circulation would slow progressively until death."

From Delauney and coworkers (1955), translated by Gilbert [1].

This early description of the microvascular response to endotoxin in guinea pig and mouse mesentery demonstrated the

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CLP = cecal ligation and perforation; D_{O_2} = oxygen delivery; d_{O_2} = microvascular oxygen demand; Hb = hemoglobin; IVVM = intravital videomicroscopy; LPS = lipopolysaccharide; MAP = mean arterial pressure; O_2ERc = capillary oxygen extraction ratio; NADH = nicotinamide adenine dinucleotide (reduced form); NO = nitric oxide; NO_2^- = nitrite; NO_3^- = nitrate; NO_x^- = nitrite/nitrate; (ec/i/n)NOS = (endothelial/inducible/neuronal) nitric oxide synthase; P_{O_2} = oxygen tension; q_{O_2} = microvascular oxygen supply; RBC = red blood cell; RSNO = nitrosothiol; SNO-Hb = nitrosated hemoglobin; S_{O_2} = oxygen saturation; SR = supply rate; V_{O_2} = oxygen consumption.

severity of the immediate arteriolar vasoconstriction response to endotoxin. Moreover, it recounted the subsequent phases of changing microvascular tone and cardiovascular collapse that occur with progression of sepsis.

With the realization that the systemic inflammatory response to bacterial infection (sepsis) or endotoxin (endotoxemia) caused plasma volume depletion came the introduction of fluid resuscitation [2]. With fluid resuscitation, both septic patients and animal models have been found, in general, to exhibit a hyperdynamic state [3–5]. This state is characterized by elevated cardiac output, increased oxygen delivery (DO_2), decreased systemic vascular resistance (with or without decreases in mean arterial blood pressure [MAP]), and increased tissue oxygen consumption (VO_2), but impaired oxygen extraction capacity [6,7] and lactic acidosis. The latter two observations have led to the concept that microvascular injury, impaired microvascular control, and maldistribution of microvascular blood flow induce a tissue oxygen debt [8], and the theory that inefficient matching of microvascular oxygen supply (qO_2) to oxygen demand (dO_2) impairs oxygen extraction [9,10]. This is manifested by a pathologic oxygen supply dependency, whereby VO_2 is dependent on DO_2, and a decreased critical oxygen extraction ratio (O_2ER) [11,12].

Global measures of hemodynamic and oxygen transport parameters, including cardiac output, arterial pressure, vascular resistance, blood gases, oxygen consumption, oxygen extraction and lactate, provide whole body information on the status of the cardiovascular system in the critically ill patient. Although serum lactate has been widely used as an indicator of tissue hypoperfusion, decreased tissue oxygenation, and anaerobic metabolism [13], recent evidence suggests that blood lactate concentration may also reflect altered pyruvate dehydrogenase [14,15] and Na^+,K^+-ATPase [16] activity, and increased glycolysis rate [17]. Global oxygen transport parameters, however, fail to measure or assess the status of the microcirculation [18], which is vital to organ function because it is the microcirculation that delivers and distributes oxygen, nutrients, and inflammatory and coagulation factors throughout the tissue and removes metabolic waste products, heat, and carbon dioxide.

To obtain information on the functional state of the microcirculation, tissue oxygenation, bioenergetic, or redox status of an organ, a variety of techniques have been used both experimentally and clinically. Laser Doppler flowmetry provides a relative signal of red blood cell (RBC) flow [19,20] from an unknown tissue volume, whereas intravital videomicroscopy (IVVM) provides real-time images of microvascular geometry and blood flow, from which microvessel diameter [21–24], functional capillary density [19,25–27], and intercapillary area [28,29] can be determined. Combined with video analysis tools and dual wavelength spectrophotometry, IVVM has allowed the quantification of capillary hemodynamics (RBC velocity, RBC lineal density, and RBC flux/supply rate)

[19,30,31] and erythrocyte hemoglobin oxygen saturation (RBC SO_2) [30]. Analysis of microvascular blood velocity in the finger nail-bed [32] and, more recently, orthogonal polarization spectral imaging of the sublingual microcirculation [33,34] have brought microvascular imaging to the bedside, but with reduced spatial resolution.

Oxygen tension (PO_2) has been assessed by oxygen microelectrodes inserted into the tissue of patients and animal models [35–37], by palladium porphyrin phosphorescence quenching, which detects the presence of intravascular or tissue PO_2 [38], and indirectly by hypoxic markers [39]. The bioenergetic status of tissue has been determined by ATP analysis [35] and inferred from nicotinamide adenine dinucleotide (reduced form; NADH) fluorescence imaging studies [38]. Although the diaphragm [40], cremaster muscle [24,41–43], heart [38,44], and now sublingual microcirculation [33,34] have been investigated, much of our current understanding of the functional state of the septic microcirculation has come from studies conducted in intestinal mucosa [10,22,23,27–29,31,38,45], liver [46–49], and skeletal muscle [19,21,25,26,30,50] because of the suitability of those tissues for *in vivo* optical examination.

This paper reviews and considers the fundamental concepts of oxygen transport and the effect of microvascular dysfunction on oxygen transport during sepsis. Aspects of nitric oxide (NO) biology relevant to microvascular function, global and local oxygen transport, and mitochondrial respiration are also discussed.

The microcirculation as a functional system for distributing blood flow

The cardiovascular system circulates blood throughout the body, but it is the microcirculation, in particular, that actively and passively regulates the distribution of RBCs and plasma throughout individual organs (Fig. 1). Blood flow into an organ is controlled upstream of the capillary networks or vascular beds by the vascular tone of the resistance vessels comprising the arteriolar network. These vessels are surrounded by smooth muscle that either constricts or relaxes in response to the balance between pressor and dilatory stimulation. Locally, vascular tone controls the diameter of the resistance vessels, the blood flow through them, and the pressure drop across the microvascular beds. Globally, vascular tone controls the systemic and pulmonary blood pressures.

Downstream of the arterioles, microvascular RBC flow is passively distributed throughout the capillary networks [51] and other vascular beds such as the liver sinusoids, according to local vessel resistance (diameter and length) and hemorheologic factors (blood viscosity and RBC deformability). RBCs are forced to deform and travel single file, often separated by plasma gaps, as they pass through vessels that are of smaller diameter then their own. This distinctive microvascular flow behavior maximizes the surface area available for gas

Figure 1
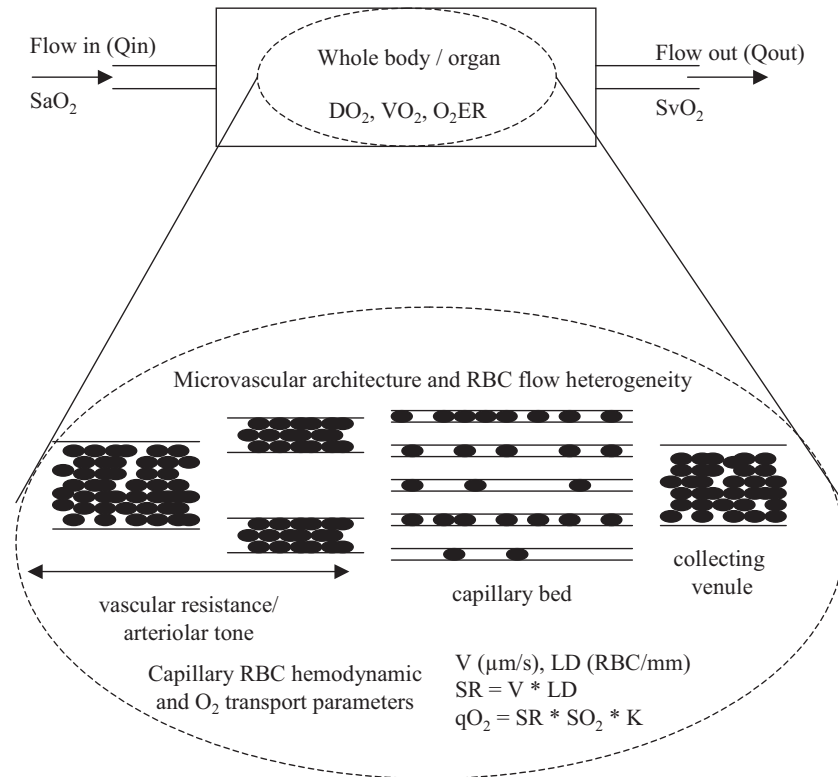
Schematic representation of global and microvascular oxygen (O_2) transport parameters. Arteriolar tone establishes the blood flow into an organ, and capillary resistance and rheology factors determine red blood cell (RBC) distribution (heterogeneity) within the capillary bed. Capillary hemodynamics are quantified as RBC velocity (V), lineal density (LD), and supply rate (SR) or flux. Capillary O_2 transport (qO_2) is determined by capillary SR, RBC hemoglobin O_2 saturation (SO_2), and the O_2 carrying capacity of the RBC (K).

exchange between the RBC and the local environment. Surprisingly, passive rheologic mechanisms appear to play a greater role than arteriolar heterogeneity in determining capillary heterogeneity and functional capillary density, especially at low flow states [51]. Passive rheologic mechanisms are also responsible for the Fahreaus effect (i.e. the drop in vessel hematocrit along the arteriolar tree to the capillary bed). In the skeletal muscle of septic rats, we have observed that stopped-flow capillaries have lower hematocrit, or lineal density (RBC/mm), than do neighboring flowing capillaries. Neither the implications nor the cause and effect relationship of this phenomenon is clearly understood.
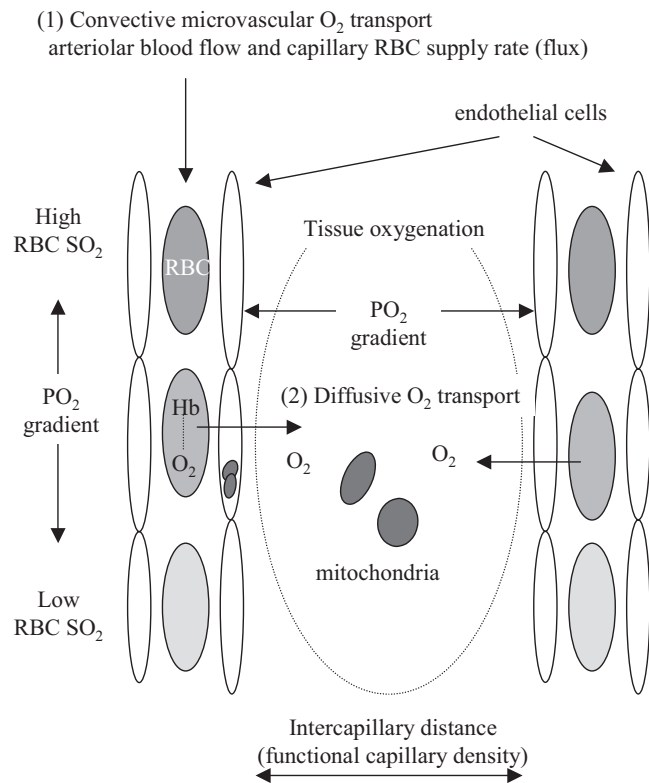
In hyperdynamic sepsis progressive arteriolar constriction in the small intestine occurs at all levels of the microvascular arteriolar network, causing a decrease in blood flow to this organ [23] and thus contributing to impaired villus microcirculation [29,45]. In cremaster muscle [24,41] and the diaphragm [40], however, there is a differential arteriolar response in which arterioles with larger diameter vasoconstrict and those with smaller diameter vasodilate. Similarly, bacteremia causes a differential response in liver microvessels in which two-thirds of the inlet periportal sinusoids and

portal venules and outlet distal centrilobular sinusoids and central venules dilate, whereas the remaining microvessels constrict [46]. In addition, Zhao and coworkers [22] reported that sepsis induces a differential sensitivity to acetylcholine in the arteriolar networks of the splanchnic circulation.

The endothelial cell is a critical component of the highly integrated microvascular system and plays an obligatory role as a signal transducer of shear stress [52,53] and vasoactive substances [54], including acetylcholine, catecholamines, prostaglandins, endothelin, bradykinin, thromboxane, adenosine, nitrosothiols, and ATP. Moreover, endothelial cells conduct and integrate local stimulatory signals throughout the microcirculation [55] via cell–cell communication. During endotoxemia endothelial dysfunction disrupts the microvascular communication system [56] and seemingly contributes to abnormal tissue perfusion [57].

The microcirculation is an integrated system designed to ensure that oxygen delivery meets or exceeds cellular oxygen demand throughout the tissue. Within the context of the cardiovascular system, oxygen transport can be considered to be a flow of oxygen from the lungs (high 'PO_2') to the tissues

Figure 2

(1) Convective microvascular O$_2$ transport
arteriolar blood flow and capillary RBC supply rate (flux)

endothelial cells

High RBC SO$_2$

Tissue oxygenation

RBC

PO$_2$ gradient

PO$_2$ gradient

Hb

(2) Diffusive O$_2$ transport

O$_2$

O$_2$

O$_2$

mitochondria

Low RBC SO$_2$

Intercapillary distance
(functional capillary density)

Schematic representation of convective and diffusive oxygen (O$_2$) transport in the microcirculation. O$_2$ is carried by the red blood cell (RBC; convective transport) from the lung microcirculation to the tissue microcirculation. As the RBC traverses the vascular bed it 'offloads' O$_2$ to the neighboring tissue; O$_2$ then diffuses from the capillary to the tissue mitochondria, where it is consumed. Local oxygen tension (PO$_2$) gradients are established along the capillary vessel, as the RBC hemoglobin (Hb) O$_2$ saturation (SO$_2$) decreases, and into the tissue with the latter acting as the driving force of O$_2$ diffusion.

(low 'PO$_2$'), involving both convective and diffusive transport mechanisms (Fig. 2).

Diffusive oxygen transport

Oxygen diffuses over relatively short distances down its partial pressure (PO$_2$) gradient. PO$_2$ gradients exist along the blood vessels and into the tissue (Fig. 2). In 1919 the physiologist August Krogh and mathematician Karl Erlang developed a mathematical model of oxygen transport based on simple cylindrical geometry and the assumption that each capillary supplied a unique tissue volume [58]. Today, we know that oxygen diffuses from arterioles [59] and capillaries in any direction based solely on the local PO$_2$ gradient; however, the Krogh model is still instructive, especially under conditions of diffusion limitation. Oxygen diffusion is limited by oxygen solubility (k), oxygen diffusivity (D), and the PO$_2$ gradient (dPO$_2$/dr). The critical oxygen diffusion distance, which is the maximum distance that mitochondria can be away from an

oxygen source without impaired function, is determined by these oxygen diffusion parameters and by capillary PO$_2$ and tissue oxygen consumption. Typical diffusion distances may range from 10 to hundreds of microns. Whether tissue is adequately oxygenated is then ultimately determined by local microvascular oxygen delivery, critical oxygen diffusion distance, and intercapillary distance.

The PO$_2$ gradient drives the net movement of oxygen from a region of high PO$_2$ to a region of low PO$_2$; as such, as the PO$_2$ gradient increases so to will the flux of oxygen, or the amount of O$_2$ that diffuses out of the vessel per unit surface area per unit time. Oxygen flux is described mathematically by Fick's first law of diffusion:

$$\text{Oxygen flux} = -kD \times dP_{O_2}/dr \qquad (1)$$

The negative sign in the expression converts the negative slope of the gradient to a positive value. It is diffusion that facilitates the movement of oxygen in the lung alveolus to RBCs in capillaries surrounding the alveolar wall and from the RBCs in the microvasculature into the tissue.

Convective oxygen transport and the erythrocyte

Because oxygen has a low solubility in plasma, it is RBC flow specifically, and not 'blood' (plasma and RBCs) in general, that determines oxygen delivery; accordingly, the oxygen carrying capacity of the erythrocyte, facilitated by hemoglobin (Hb), is essential to the convective, or bulk, transport of oxygen over large distances by the blood. Within the RBC, as it circulates between the lungs and the tissue, oxygen binds co-operatively with Hb in a manner that alters its tetrameric conformation, switching it from a relaxed, high oxygen affinity structure to a tense, low oxygen affinity structure as Hb alternately acquires oxygen and releases it to the local environment. The physiologic significance of the Hb–oxygen interaction is reflected in the sigmoidal nature of the oxygen dissociation curve. The affinity of Hb for oxygen can be affected by temperature, pH, the Bohr effect, and NO [60,61], in which *S*-nitrosohemoglobin increases the affinity of Hb for oxygen [62].

In vitro, *in vivo*, and theoretical evidence [63–66] suggests that the RBC releases vasoactive ATP and nitrosothiols in response to increased PO$_2$ gradients and mechanical deformation [67]. In theory, the erythrocyte 'senses' the local PO$_2$ gradient through a conformational change in the Hb molecule and signals the microvasculature to vasodilate. Thus, the RBC is integrated into the microvascular system as both a deliverer and sensor of oxygen (Fig. 3).

During sepsis the mechanical properties of the RBC, including membrane deformability and shape recovery, are progressively altered such that the RBC becomes less deformable [26,68,69]. Condon and coworkers [70] reported that

Figure 3
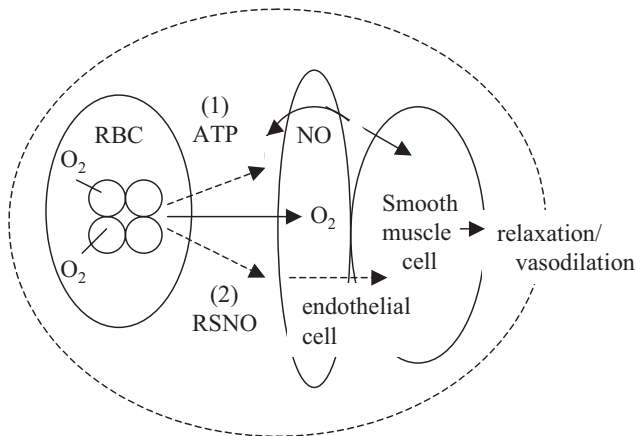
The red blood cell (RBC) as a deliverer and 'sensor' of oxygen (O_2). By altering its conformation, hemoglobin facilitates the release of nitrosothiol (RSNO; nitric oxide [NO] derivative) or ATP in response to increased O_2 tension (P_{O_2}) gradients or mechanical deformation. Both RSNO and ATP evoke vasodilation, with the latter being NO mediated.

'elderly' RBCs, comprising 20% of the circulating erythrocytes, were most susceptible to decreased deformability and that Hb content decreased in a large fraction of the RBCs during sepsis. The accumulation of rigid erythrocytes suggested to the authors that the erythrophagocytic capacity of mononuclear phagocytes had been overwhelmed during sepsis [70]. Although a cause and effect relationship has not been demonstrated, a change in blood rheology does appear to be a factor in the loss of functional capillary density [26] and peripheral shunting [68] during sepsis. What effect sepsis has on RBC oxygen sensing and signaling mechanisms is unknown.

In larger blood vessels, convective oxygen transport is calculated as the product of blood flow (Q [ml/s]), Hb concentration (g/dl), Hb S_{O_2} (%), and the oxygen-binding capacity of Hb (C [oxygen/Hb]).

$$\text{Convective oxygen transport} = Q \times Hb \times S_{O_2} \times C \qquad (2)$$

In the microcirculation, capillary hemodynamics can be quantified as either RBC flux [31] or as an RBC supply rate (SR) [30]. RBC flux or SR (RBC/s) account for both RBC velocity (V [μm/s]) and capillary hematocrit or RBC lineal density (LD [RBC/mm]). Since RBC flux implies movement of RBC per unit area, we prefer the term RBC SR.

$$SR = V \times LD \qquad (3)$$

Similar to convective oxygen transport in larger vessels, oxygen flow in capillaries (q_{O_2}) can be calculated from the RBC SR, the RBC S_{O_2}, and the oxygen carrying capacity of a single RBC (K = 0.0362 ml oxygen/RBC at 100% S_{O_2}) [30].

$$q_{O_2} = SR \times S_{O_2} \times K \qquad (4)$$

Capillary oxygen transport parameters, capillary O_2ER (O_2ERc), and capillary oxygen flux ($O_2 \text{ flux}_c$) can be calculated from capillary oxygen flow rates at the capillary entrance (en) and exit (ex) and the capillary surface area, as determined by local capillary dimensions length (L) and diameter (d), respectively.

$$O_2ERc = \{q_{O_2}(en) - q_{O_2}(ex)\}/q_{O_2}(en) \qquad (5)$$

$$O_2 \text{ flux}_c = \{q_{O_2}(en) - q_{O_2}(ex)\}/\pi dL \qquad (6)$$

Local differences in capillary hemodynamics give rise to microvascular flow heterogeneity and subsequently oxygen flow heterogeneity within the organ (Fig. 1). The relationships between microvascular geometry, capillary hemodynamics, functional capillary density, and oxygen transport are of particular importance in the pathophysiology of sepsis because the systemic inflammatory response induces remote microvascular derangements and dysfunction that may contribute to tissue injury and ultimately organ failure in septic patients.
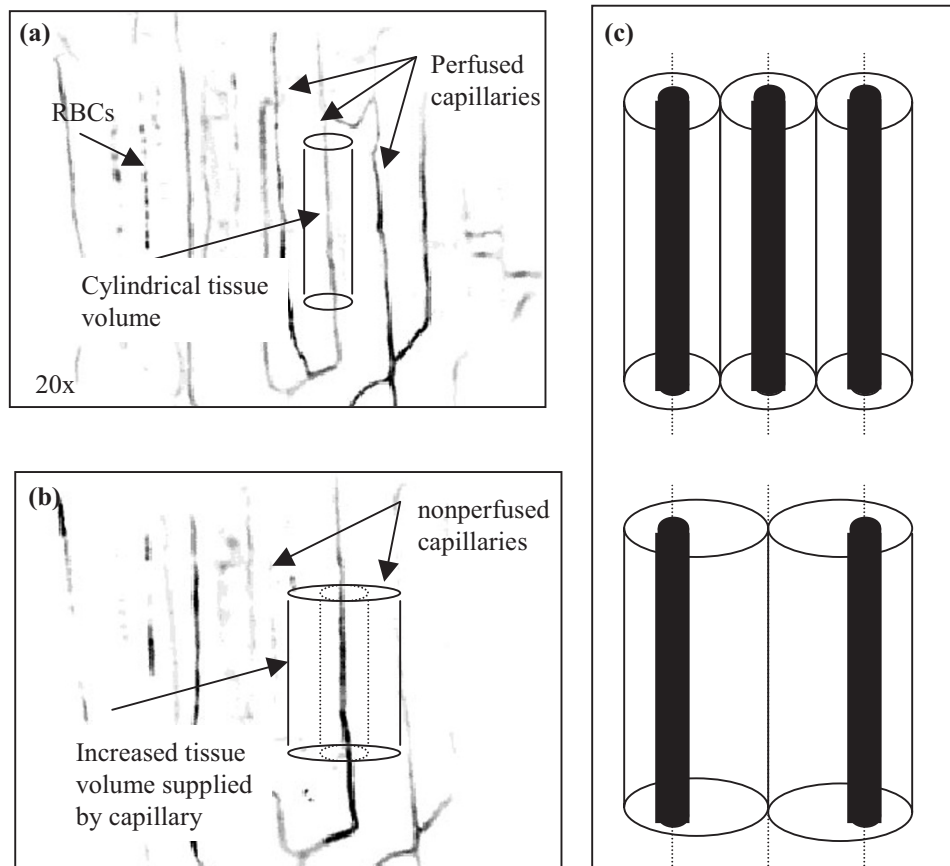
Microvascular stopped-flow in sepsis

One of the most striking manifestations of increased microvascular heterogeneity during sepsis is an increase in microvascular stopped-flow (Fig. 4), specifically capillary or sinusoidal ischemia. This is evident in skeletal muscle [19,25,26,30], intestinal villi [27–29,31,45], diaphram [40], sublingual [33,34], and liver [46,47] microcirculation. IVVM studies have found that capillary stopped-flow results in a loss of functional capillary density and an increase in intercapillary distance in skeletal muscle [19,26], increased intercapillary area in the intestinal villi [28,29], and decreased numbers of perfused liver sinusoids [46]. In septic patients it is unknown whether decreased sublingual microvascular flow index [33] or vessel density [34] correlates with putative losses of microvascular or capillary density in other organs.

The loss of functional capillary density in skeletal muscle occurs early during the progression of experimental sepsis and is associated with both a loss of RBC deformability and an over-production of NO [26]. Although Laser Doppler flowmetry has detected the presence of dysfunctional microcirculation on the basis of attenuated reactive hyperemia in skeletal muscle [19,20], it is unable to discriminate fundamental capillary stopped-flow or flow heterogeneity induced by sepsis. While the exact mechanism of microvascular stasis is still to be determined, it is clear that sepsis causes local regions of ischemia in the tissue by virtue of capillary stopped-flow.

A combination of inflammatory and coagulation mediated factors [71] may contribute to microvascular stasis, including fibrin deposition, altered RBC deformability [26], aggregation [72] or adhesion [73] properties, increased leukocyte adhesion and reduced leukocyte deformability [44], endothelial

Figure 4

Sepsis induced capillary stopped-flow and increased effective tissue volume. **(a, b)** Consecutive microvascular variance images of the same rat skeletal muscle capillary bed at different times during the progression of sepsis. (Variance images depict the change in light intensity at each pixel in the field of view, from a 30 s video sequence. Perfused capillaries appear as dark lines, and the tissue background is white.) Sepsis increased capillary stopped-flow, reduced functional capillary density, and increased the effective tissue volume supplied by the remaining vessels. The latter is depicted in **(c)**. RBC, red blood cell.

swelling, reduced or altered driving pressures in the microcirculation, or the development of putative microthrombi [74]. Neither tissue edema nor leukocyte adhesion in postcapillary venules is the apparent cause, at least in skeletal muscle [25,50]. In liver, increased leukocyte rolling and adhesion is associated with decreased sinusoidal flow velocity [75], raising the possibility that leukocytes are affecting microvascular blood flow in this organ, although the precise relationship between adhesion and flow is unknown. Although it has been proposed that platelet–fibrin clots occlude microvessels, the role that platelets play in capillary stopped-flow is also unknown. Capillary stopped-flow appears to be independent of arterial pressure because both diaphragmatic capillaries and mucosal capillaries were more likely to shut down in septic relative to control animals with a similar degree of hypotension [31,40]. In our 6-hour acute and 24-hour chronic sepsis models [26,30] we also observed reversal of capillary flow in skeletal muscle, indicating that significant fluctuations in pressure gradients have occurred across the vascular bed.

Fluid resuscitation, tissue oxygen tension, and microvascular derangements

Sepsis induces decreases in liver, gut, and skeletal muscle tissue P_{O_2} [35,36,76,77]. Fluid resuscitation has been demonstrated to apparently rescue skeletal muscle microcirculation in terms of oxygen transport by improving P_{O_2} [35,36]. However, despite this apparent improvement in microvascular function tissue ATP remained depressed and lactate remained elevated [35] (Fig. 5), suggesting that tissue P_{O_2} was not a reliable indicator of bioenergetic status during sepsis, or conceivably of anaerobic metabolism. It has been argued that increased tissue P_{O_2} during sepsis indicates a decrease in the ability of tissue to consume oxygen [35,78] and that this may be due to mitochondrial failure or cytopathic hypoxia [79].

Subsequently, a more detailed evaluation of skeletal muscle oxygen transport at the individual capillary level in a fluid resuscitated, 24-hour normotensive rat model of sepsis

Figure 5
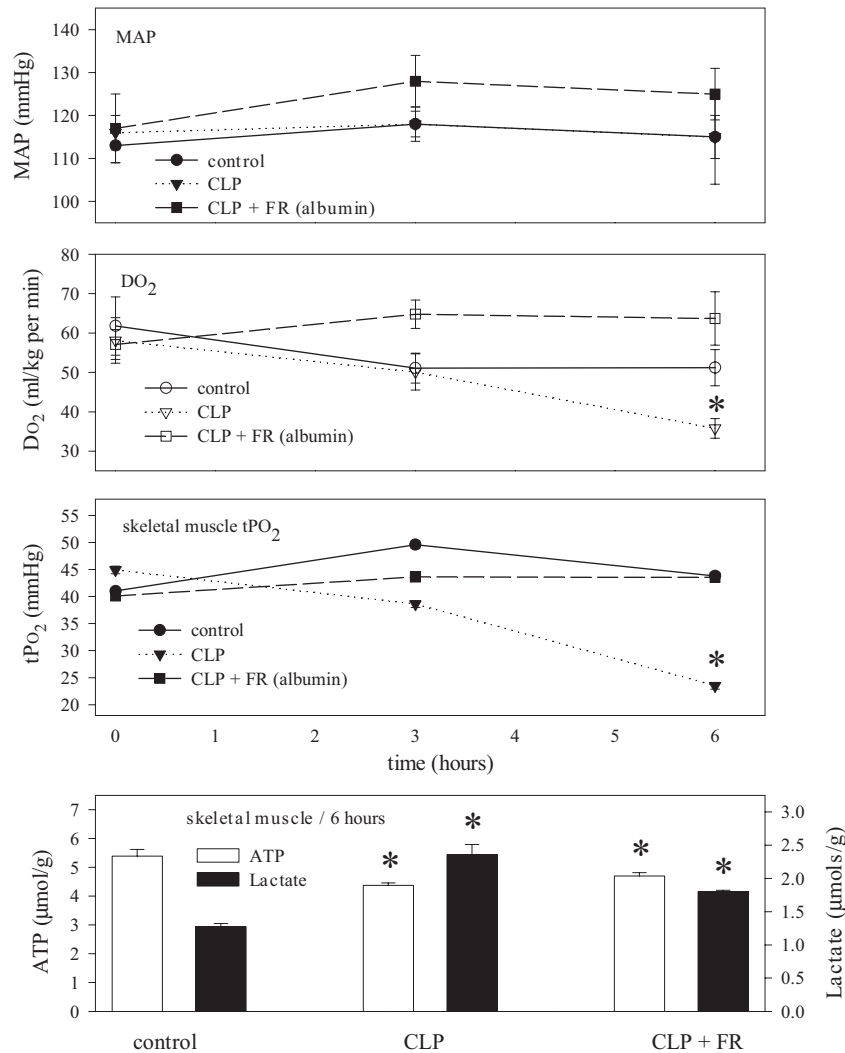
Relationships between global hemodynamics, tissue oxygen tension (P_{O_2}), and skeletal muscle lactate and ATP. In a 6-hour rat model of sepsis (cecal ligation and perforation [CLP]), fluid resuscitation was found to prevent decreases in systemic oxygen delivery (D_{O_2}) and tissue P_{O_2} in skeletal muscle (tP_{O_2}). This apparent rescue of the muscle microcirculation was belied by elevated lactate and reduced ATP. *$P < 0.05$, versus control. FR, albumin fluid resuscitation; MAP, mean arterial pressure. By permission from *Circ Shock* 1988, **26**:311-20 [35].

demonstrated that the microcirculation was not rescued by fluid resuscitation [30]. Using *in vivo* spectrophotometric imaging that simultaneously determined skeletal muscle microvascular geometry, capillary hemodynamics, and erythrocyte Hb S_{O_2} in normally perfused capillaries (i.e. capillaries with RBC velocity 20–325 µm/s), we found that O_2ERc actually increased by a factor of three in septic rats, indicating an increase in oxygen flux out of the capillary into the surrounding tissue.

In the same muscle capillary beds we observed a threefold increase in the ratio of fast flow (i.e. RBC velocity >325 µm/s) to normal flow capillaries, and a fourfold increase in capillary density of stopped-flow. Technical limitations pre-

vented the determination of hemodynamic parameters in the fast flow vessels, but their appearance indicated that some local regions of tissue were clearly over-supplied with oxygen whereas other areas – those supplied by capillaries exhibiting increased O_2ERc – were clearly under-supplied with oxygen. This was evidence of a maldistribution of RBC flow at the capillary level and a mismatching of local oxygen supply with local oxygen demand. These findings also indicated that the septic microcirculation had lost its ability to regulate capillary blood flow because it was unable to redistribute RBCs to regions of low P_{O_2} and increased oxygen demand. The presence of an increased proportion of fast flow vessels, adjacent to stopped-flow capillaries, suggested that oxygen might have been shunted through the capillary beds via these

vessels. Mathematical modeling of these vessels may provide insight into their oxygen transport properties.

This putative shunting mechanism in skeletal muscle is suspected to be quite different from that proposed to exist in either the gut or the heart. In these organs it has been proposed that fundamental 'microvascular weak units' [38] exist that are bypassed during sepsis or low flow conditions. Although the exact microvascular geometry of such microvascular weak units has not been demonstrated, entire microvascular beds, rather than single capillaries, may be compromised. In the liver, Unger and coworkers [46] reported an intrahepatic redistribution of blood flow in which blood was channeled away from constricted vessels to vasodilated vessels. Taken together, these data demonstrate that sepsis creates microvascular heterogeneities or inefficiencies in the matching of local oxygen delivery to local oxygen demand in these organs, which may contribute to a local tissue oxygen debt and organ dysfunction.

Capillary stopped-flow and increased capillary oxygen extraction

Although increased local oxygen consumption [6,7] could account for increased capillary oxygen extraction in skeletal muscle in 24-hour septic rats [30], a positive correlation between capillary oxygen extraction and the degree of regional capillary stopped-flow (Fig. 6) indicates that, as stopped-flow increases, the remaining functionally normal capillaries offload greater amounts of oxygen to the surrounding tissue. There is no evidence of capillary recruitment. That vessels with high flow were unable to compensate for the loss of perfused capillaries is supported by the correlation between increased oxygen extraction and stopped-flow. The dysfunctional aspect of oxygen transport in the septic microcirculation is realized with the Krogh cylinder model (Fig. 4c). The effective tissue volume supplied by a capillary actually increases as the degree of capillary stopped-flow increases; hence, local capillary oxygen extraction necessarily increases to meet the increased oxygen demand imposed on it by the increased tissue volume surrounding it.

A loss of functional capillary density has been found to increase intercapillary distances in skeletal muscle and intestinal villi [19,31], but whether convective oxygen flow was sufficient and oxygen diffused over these increased distances is unknown. In the ileum of septic rats, oxygen transport is further compromised by increased flow motion with longer periods of capillary stopped-flow [29]. Although Hotchkiss and coworkers [39] did not detect cellular hypoxia using [18F]fluoromisonidazole in either the skeletal muscle, brain, liver, heart, or diaphragm of septic rats, it is possible that a mismatch in tissue qO_2/dO_2 impaired oxygen extraction in the gut and heart [9,10].

It is important to realize that fluid resuscitation in the animal model did not prevent loss of functional capillary density, or

Figure 6
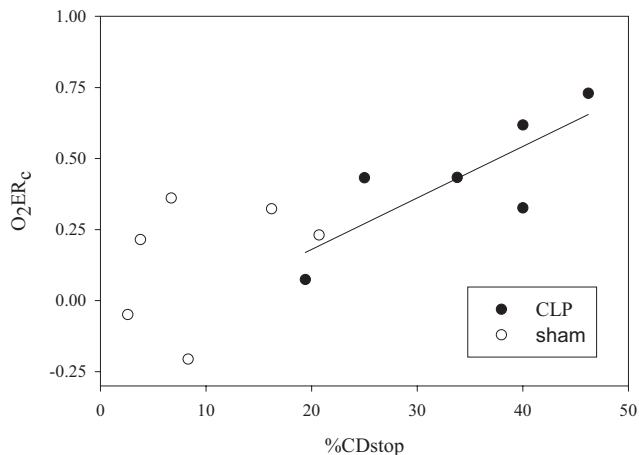
Local capillary oxygen extraction ratio (O_2ERc) versus regional capillary stopped-flow (%CDstop). In a 24-hour rat model of sepsis (cecal ligation and perforation [CLP]), capillary oxygen extraction from normally flowing capillaries (capillary velocity between 20 and 325 μm/s) was found to correlate with the degree of regional capillary stopped-flow. As capillary stopped-flow increased, the RBCs offloaded increased amounts of oxygen. (CLP group: $y = 0.018x - 0.18$; $r^2 = 0.64$; $P < 0.05$.) By permission from *Am J Physiol Heart Circ Physiol* 2002, **282**:H156-H164 [30].

restore microvascular regulation or the qO_2/dO_2 mismatch in the tissue [30]. A similar impairment in microvascular perfusion was detected in septic patients, despite restoration of intravascular volume [33]. A question that remains unanswered is at what stage of sepsis does the microcirculation actually fail to ensure adequate oxygenation. The report that early goal-directed therapy in septic patients reduces 60 day mortality, and fewer patients succumbed to cardiovascular collapse (in-hospital mortality) [80], raises the possibility that microvascular dysfunction persists throughout the entire septic process. The severity of microcirculatory dysfunction may therefore ultimately determine the severity of organ dysfunction. In light of the above observations, it is important to consider local geometry, hemodynamics, and oxygen transport as well as the average tissue PO_2 when assessing the extent of tissue oxygenation or the functional state of the microcirculation during sepsis.

Nitric oxide properties: synthesis, diffusion, and transport

The gaseous molecule NO is a potent regulator of vascular tone, a cytotoxic agent, a neurotransmitter, an antioxidant (in that it reacts with superoxide anion to form peroxynitrite), and, seemingly, a modulator of overall microvascular integrity, function, and oxygen transport. This review focuses primarily on the latter role of NO.

NO is synthesized by the L-arginine pathway in a variety of cell types including macrophages, neutrophils, platelets,

endothelial, and smooth muscle and parenchymal cells by a family of nitric oxide synthase (NOS) enzymes. They are categorized as type I (calcium dependent, inducible, neuronal, nNOS), type II (calcium independent, inducible, iNOS), or type III (calcium dependent, endothelial or constitutive, ecNOS, eNOS, or cNOS). L-arginine analogs function as nonspecific NOS inhibitors; guanidine (aminoguanidine), isothiourea (aminoethyl-isothiourea), acetamide (N-[3-(aminomethyl) benzyl]acetamide.2HCl, or 1400W), and lysine (L-N6-[1-iminoethyl]lysine, or L-NIL) derivatives have partial iNOS inhibition selectivity; and nitroinadozole has partial nNOS selectivity.

Under normal conditions, a small amount of NO is produced in endothelial cells by the constitutively expressed NOS enzyme in response to receptor mediated and shear stress vasodilatory stimuli [81,82]. As a gas, NO diffuses down its concentration gradient, from endothelial cell to smooth muscle cell, where it opposes sympathetic or chemically mediated vasoconstriction of arterioles by relaxing smooth muscle in a guanylate cyclase mediated reaction. NO also diffuses into the microvascular lumen where it regulates RBC [83] and leukocyte deformability [44], leukocyte–endothelial adhesion in mesenteric and skeletal muscle postcapillary venules [84,85], and platelet adhesion and aggregation [86,87]. NO has also been reported to both maintain and, during endotoxemia, increase vascular permeability in the intestine, heart, liver, and kidney [88,89]. Taken together, NO is an important factor in maintaining the integrity of blood flow through the microcirculation by regulating resistance vessel diameter, blood rheology, interaction between cellular blood elements and the vascular wall, and blood volume.

NO has been reported to be transported from the lungs to the tissue via S-nitrosation of the Hb β-chain cysteine 93 thiol group (SNO-Hb) [65,90] and released in the microcirculation as a low molecular weight nitrosothiol (RSNO) with vasorelaxant properties. The mechanisms of formation and release of NO from RSNO species are not completely understood [91]. Feelisch and coworkers [92] have also found considerable species variation in the amounts and ratios of RSNOs, nitrosamines, and nitrosylhemes. Although providing evidence that intravascular transfer of NO occurs between SNO-Hb and nitrosated albumin, Gladwin and coworkers [93] concluded that in human circulation these nitrosated species had little effect on regulating vascular tone.

In human NO breathing experiments, in which exogenous NO counteracted the vasopressor effect of nonspecific NOS inhibition in the forearm, Cannon and coworkers [94] reported that NO reacted predominantly with the heme moiety of Hb forming either nitrosyl(heme)hemoglobin (Hb[FeII]NO) with deoxyhemoglobin or, consistent with a NO scavenging role by the RBC [95], methemoglobin (FeIII) and nitrate (NO_3^-) with oxyhemoglobin. The authors argued that remote vasodilation was mediated either by direct NO release from Hb(FeII)NO, indirectly via a SNO-Hb intermediate, or by nitrite (NO_2^-) bioconversion to NO [94]. In similar human studies, intravenous infusion and bolus injection of NO and RSNO in the brachial artery increased both artery diameter and forearm blood flow [96,97]. Here, the authors concluded that NO was transported as both free and nitrosative, RSNO, forms.

Caveats with respect to interpreting data from functional studies investigating NOS inhibition are that NO levels in tissue or plasma are often unknown, inhibition of NO over-production is significant but incomplete, and the degree and specificity of NOS inhibition are unknown. Additionally, the reader should bear in mind that, during sepsis, NO functions in a 'sepsis milieu' of increased levels of reactive oxygen species and endogenous vasopressors, especially endothelin, which is upregulated [98] during sepsis and independent of NO inhibition [99]. Comparison of data can also be confounded by differences in animal models and the timing of the NOS inhibition (i.e. pretreatment versus delayed administration).

Nitric oxide over-production and time course during sepsis

During the progression of sepsis, proinflammatory cytokines tumor necrosis factor-α and interleukin-1, and lipopolysaccharide (LPS) stimulate the upregulation of iNOS [100] throughout the organs [101] and nNOS in brain and skeletal muscle [102]. Various LPS and cecal ligation and perforation (CLP) models of sepsis have shown rapid but transient increases in iNOS and nNOS mRNA expression followed by or commensurate with increased NOS activity and increased levels of NO oxidized metabolites, namely NO_2^- and NO_3^- (NO_x^-), in tissue and plasma.

Following LPS treatment, iNOS mRNA in rat kidney appeared by 60 min, peaked at 2–4 hours along with plasma NO_x^-, and declined by 16 hours [103]; nNOS mRNA in rat brain increased by 2 hours whereas iNOS mRNA increased by 3 hours, with both NOS isoforms returning to baseline by 12 hours [104]; and iNOS mRNA in the small intestine was detected at 1 hour, peaked at 4 hours, and was faint at 24 hours [105]. In dogs treated with LPS, iNOS activity was increased in the liver by 4 hours and in the heart by 6 hours [106]. Infusion of *Staphylococcus aureus* cell wall components caused progressive increases in iNOS activities in thoracic aorta, lung and liver, and plasma NO_x^- from 2 to 6 hours [107].

In rats subjected to CLP, Sheih and coworkers [108] found that tissue NO_x^- levels increased from 5 to 10 hours in kidney, gut, heart, liver, and lungs commensurate with increased iNOS mRNA and increased plasma NO_x^-. Also in CLP rats, thoracic aorta and lung iNOS protein and activity increased by 6 hours and remained elevated for 48 hours, whereas cNOS protein and activity progressively decreased from 6 to 48 hours. Concomitantly, plasma NO_x^- increased by 6 hours, peaked at 12 hours, and remained elevated for 48 hours [109]. The progressive loss of cNOS protein was

consistent with downregulation of ecNOS protein and mRNA in bovine coronary venular endothelial cells 8 hours after exposure to LPS [110].

In septic patients no such NOS/NO_x^- time course profiles have been determined; however, increased plasma NO_x^- [111] and increased skeletal muscle tissue NO_x^- [78] have been reported. In a longitudinal patient study, fluctuating plasma NO_x^- levels were associated with recurring bacterial infection over a 28-day period [112]. Plasma NO_x^- was also reported to be inversely related to systemic vascular resistance [113] and positively related to cardiac output [114] in septic patients.

Effect of nitric oxide over-production on microvascular reactivity

Under septic conditions iNOS and nNOS are upregulated in endothelial and muscle cells, respectively [102,115], leading to over-production of NO in the microvasculature and arteriolar dysfunction. In a rat CLP model of sepsis, Gocan and coworkers [102] reported that microvascular reactivity to acetylcholine, quantified as changes in arteriolar diameter and downstream capillary RBC velocity, was impaired in skeletal muscle by NO and restored by nNOS inhibition. Interestingly, acetylcholine increased the proportion of perfused microvessels in the sublingual microcirculation of septic patients [34]. Hollenberg and coworkers [42,43] found that NOS inhibition in CLP rats reversed arteriolar hyporesponsiveness to catecholamines and endothelin in cremaster muscle, as measured by changes in arteriolar contraction. In another study, iNOS deficient mice were resistant to vascular hypocontractility [116]. All of these findings suggest that NO over-production was a factor in refractory hypotension in sepsis.

The effect of nitric oxide synthase inhibition on hemodynamics and oxygen transport in sepsis

In 1990, Kilbourn and coworkers [117] reported that tumor necrosis factor induced hypotension in dogs could be reversed by nonspecific NOS inhibition. Subsequently, numerous clinical [118–122] and animal studies of sepsis [4,5,123] demonstrated that NOS inhibition prevented or reversed hypotension, even in septic patients unresponsive to conventional vasoconstrictor therapy [118,122]. In 48-hour sheep models of sepsis, both nonspecific and iNOS specific NOS inhibition reversed hyperdynamic sepsis and normalized cardiac indices, systemic vascular resistance, and oxygen extraction [124–126] by peripheral vasoconstriction. In a clinical study of septic shock patients, Broccard and coworkers [118] reported that nonspecific NOS inhibition increased MAP and decreased cardiac output, allowed gradual withdrawl of α-adrenergic support, decreased DO_2 and increased O_2ER, but had no effect on VO_2, lactate, or splanchnic oxygenation. Despite a favorable outcome on MAP in septic shock patients, a nonspecific NOS inhibition phase III clinical trial was terminated because of increased mortality arising from increased cardiovascular failure [127]. Interestingly, activated protein C, which is currently the only treatment to reduce patient mortality in severe sepsis [128], has been found to reduce increased lung iNOS mRNA and activity and prevent hypotension in a 3-hour LPS rat model of sepsis [129].

The effect of inhibiting NO over-production on microvascular geometry, hemodynamics, and oxygen transport is less well characterized. Nonspecific NOS inhibition normalized cardiac output but exacerbated vasoconstriction in the small intestine of rats infused with *Escherichia coli* [130]. In dogs treated with LPS for 3 hours, Walker and coworkers [131] reported that nonspecific NOS inhibition normalized gut and hind limb vascular resistance with negligible effect on oxygen extraction or oxygen uptake. In *ex vivo* rat endotoxemic hearts, nonspecific NOS inhibition resulted in decreased coronary blood flow and myocardial ischemia as measured by increased NADH fluorescence [132]. The same reduction in coronary blood flow in normal hearts had no effect, suggesting that hyperdynamic sepsis masked an underlying myocardial microvascular dysfunction. In liver, nonspecific NOS inhibition in 2-, 3-, and 8-hour models of sepsis decreased sinusoidal blood flow and increased both the number of nonperfused sinusoids and leukocyte adhesion in sinusoids and postsinusoidal venules [48,49,75]. Huang and coworkers [133] also reported that both nonspecific and partially specific NOS inhibition with aminoguanidine exacerbated 7 hour LPS induced decreases in blood velocity and hemoglobin oxygenation in liver sinusoids. Liver hypersensitivity to the pressor effect of endothelin may account, in part, for NOS inhibition exacerbating decreased liver hemodynamics during sepsis [98]. Although fluid resuscitation restored skeletal muscle tissue PO_2 in a 3.5-hour rat LPS model of sepsis, concurrent nonspecific NOS inhibition reduced tissue PO_2 [36].

Maintaining plasma NO_x^- at baseline in a 6-hour rat CLP model of sepsis using the iNOS inhibitor aminoguanidine attenuated the loss of functional capillary density in skeletal muscle [26]. In the ileum, aminoguanidine was found to attenuate LPS induced decreased oxygen consumption [134]. However, the specific iNOS inhibitor 1400W had no effect on decreased intestinal perfused villi or oxygen extraction in a 24-hour pig LPS model of sepsis [135]. The effectiveness of NOS inhibition in this study, however, is uncertain because portal venous NO_x^- levels remained elevated. In 6-hour endotoxemic pigs, the more selective iNOS inhibitor aminoethyl-isothiourea normalized hepatic artery blood flow and partially restored portal venous flow [136]. Although liver oxygen extraction remained above baseline, interestingly, liver oxygen consumption increased. In an 8-hour rat ischemia/reperfusion LPS model in which nonspecific and partially specific iNOS inhibition by aminoethyl-isothiourea reduced plasma NO_x^- by 33% and 44%, respectively, liver microvascular blood flow,

as assessed by Laser Doppler flowmetry, decreased dramatically in both cases [137].

Alternatively, evidence from NO donor studies suggest that increased NO production may preserve or protect microvascular blood flow. Sodium nitroprusside attenuated the loss of perfused liver sinusoids in a rat LPS model of sepsis [47], administration of nitroglycerin to fluid resuscitated septic patients increased sublingual microvascular flow index [33], and the NO donor 3-morpholinosydnonimine decreased critical oxygen delivery and increased critical oxygen extraction ratio in dogs treated with LPS [4], indicating that increased NO improved the matching of microvascular oxygen delivery with oxygen demand. NO over-production may also have caused an apparent rebound in liver perfusion at a later stage of sepsis [138]. Taken together with the deleterious effects of NOS inhibition, these results suggest that NO over-production may have a protective effect on microvascular blood flow by counteracting increased endogenous vasopressor activity during sepsis; clearly, however, more microcirulatory research needs to be conducted to assess the role of endogenous NO during sepsis.

Relationship between nitric oxide, mitochondrial dysfunction, and oxygen supply

Oxygen is primarily consumed at the inner membrane of the mitochondrion by the redox reaction, which simultaneously oxidizes cytochrome c and reduces oxygen to water. The reaction is catalyzed by the terminal enzyme of the electron transport chain, namely cytochrome c oxidase (or cytochrome a,a_3). Several *in vitro* studies have reported that NO inhibits cytochrome c oxidase [139–142]. Moreover, Torres and coworkers [140] found that the degree of inhibition is determined by the oxygen concentration (Fig. 7). Their data also indicate that inhibition is reversible and suggest that NO and oxygen compete for the same binding site on cytochrome c oxidase, although the precise nature of the inhibition is unknown. *In vitro* studies of rat aorta endothelial cells have shown that NO inhibits mitochondrial respiration in an oxygen dependant manner [143]. From the standpoint of the septic microcirculation, these findings raise the intriguing possibility that sepsis induced microvascular oxygen transport dysfunction and NO over-production contribute to both tissue hypoxia and mitochondrial inhibition.

Near infrared spectroscopy of baboon forearm muscle in an *E. coli* infusion and fluid resuscitated model of sepsis found progressive changes in the redox state of cytochrome a,a_3 that correlated with ultrastructural changes in the mitochondria despite minimal changes in global Do_2, Vo_2, and oxygen extraction [144]. The authors concluded that abnormalities in muscle oxygen metabolism and mitochondrial function were the result of an early defect in oxygen supply followed by a progressive loss of cytochrome a,a_3 function. In human septic patients it was recently reported that increased NO levels in muscle (measured as NO_x^-) are associated with mitochondr-

Figure 7
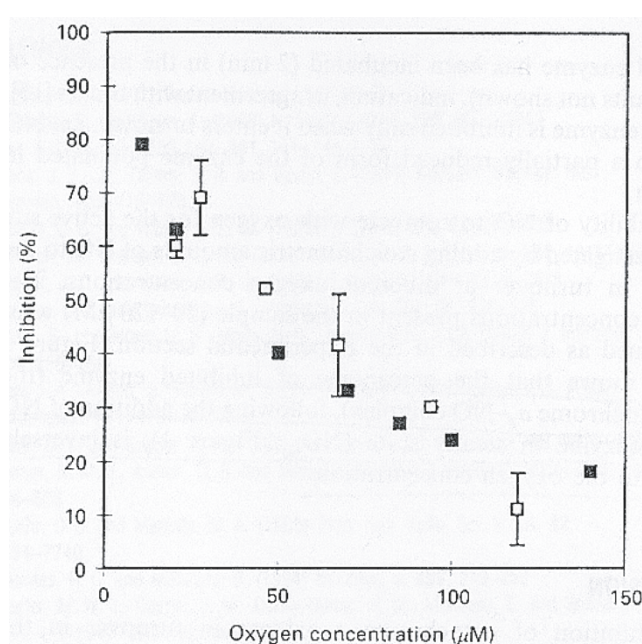
Dependence on oxygen concentration of the percentage of inhibition of cytochrome c oxidase by nitric oxide (NO). For equal concentrations of cytochrome c oxidase and NO (1.2–1.8 μm) oxygen was found to compete with NO when the enzyme enters turnover. Each data point represents a different experimental setup. The results suggest that, under physiologic conditions, in which oxygen concentration is low, nanomolar concentrations of NO can regulate mitochondrial respiration. By permission from *Biochem J* 1995, **312**:169-173 [140]. © The Biochemical Society.

ial dysfunction, decreased ATP concentration, organ failure, and eventual outcome [78].

Conclusion

Since the mid-1950s it has been known that sepsis induces profound derangements in cardiovascular function. More recently, information acquired on the functional state of the microcirculation in intestine, liver, and skeletal muscle has shown that sepsis induces profound changes in microvascular geometry, hemodynamics, and oxygen transport. Increased microvascular stopped-flow results in a maldistribution of RBC flow within the microcirculation and a mismatching of local oxygen delivery with oxygen demand. Remaining functional capillaries compensate for decreased functional capillary density by offloading more oxygen to the surrounding tissue; nevertheless, increased oxygen flow heterogeneity seemingly impairs oxygen extraction by increasing critical oxygen delivery and decreasing the critical oxygen extraction ratio. Abnormal microvascular oxygen transport also indicates that regulatory mechanisms have become dysfunctional and suggests that local cellular environments, as such, have been dramatically altered. The loss of capillary blood flow may

potentiate the effects of proinflammatory mediators by increasing their residence time in the microcirculation and tissue.

The potent vasodilator NO plays an important, yet complex, role in microvascular homeostasis. During sepsis, over-production of NO has been associated with decreases in blood pressure, impaired microvascular reactivity, abnormal RBC deformability, decreased functional capillary density, and reduced oxygen consumption. Although inhibiting NO during sepsis increases blood pressure, it also reduces microvascular blood flow and exacerbates abnormal oxygen transport. Evidence that NO donors improve microvascular hemodynamics would seem to suggest that NO over-production protects microvascular flow and oxygen transport during sepsis, but clearly more microcirculatory research must be performed to assess the role of endogenous NO during sepsis.

Competing interests

None declared.

Acknowledgements

References

1. Gilbert RP: **Mechanisms of the hemodynamic effects of endotoxin.** *Physiol Rev* 1960, **40:**245-278.
2. Carroll GC, Snyder JV: **Hyperdynamic severe intravascular sepsis depends on fluid administration in cynomolgus monkey.** *Am J Physiol* 1982, **243:**R131-R141.
3. Krishnagopalan S, Kumar A, Parrillo JE, Kumar A: **Myocardial dysfunction in the patient with sepsis.** *Curr Opin Crit Care* 2002, **8:**376-388.
4. Zhang H, Rogiers P, Smail N, Cabral A, Preiser JC, Peny MO, Vincent JL: **Effects of nitric oxide on blood flow distribution and O_2 extraction capabilities during endotoxic shock.** *J Appl Physiol* 1997, **83:**1164-1173.
5. Yang S, Cioffi WG, Bland KI, Chaudry IH, Wang P: **Differential alterations in systemic and regional oxygen delivery and consumption during the early and late stages of sepsis.** *J Trauma* 1999, **47:**706-712.
6. Samsel RW, Nelson DP, Sanders WM, Wood LD, Schumacker PT: **Effect of endotoxin on systemic and skeletal muscle O_2 extraction.** *J Appl Physiol* 1988, **65:**1377-1382.
7. Nelson DP, Samsel RW, Wood LD, Schumacker PT: **Pathological supply dependence of systemic and intestinal O_2 uptake during endotoxemia.** *J Appl Physiol* 1988, **64:**2410-2419.
8. Shoemaker WC, Appel PL, Kram HB: **Tissue oxygen debt as a determinant of lethal and nonlethal postoperative organ failure.** *Crit Care Med* 1988, **16:**1117-1120.
9. Walley KR: **Heterogeneity of oxygen delivery impairs oxygen extraction by peripheral tissues: theory.** *J Appl Physiol* 1996, **81:**885-894.
10. Humer MF, Phang PT, Friesen BP, Allard MF, Goddard CM, Walley KR: **Heterogeneity of gut capillary transit times and impaired gut oxygen extraction in endotoxemic pigs.** *J Appl Physiol* 1996, **81:**895-904.
11. Bredle DL, Samsel RW, Schumacker PT, Cain SM: **Critical O_2 delivery to skeletal muscle at high and low PO_2 in endotoxemic dogs.** *J Appl Physiol* 1989, **66:**2553-2558.
12. Schumacker PT, Samsel RW: **Oxygen delivery and uptake by peripheral tissues: physiology and pathophysiology.** *Crit Care Clin* 1989, **5:**255-269.
13. Kirschenbaum LA, Astiz ME, Rackow EC: **Interpretation of blood lactate concentrations in patients with sepsis.** *Lancet* 1998, **352:**921-922.
14. Vary TC, Hazen S: **Sepsis alters pyruvate dehydrogenase kinase activity in skeletal muscle.** *Mol Cell Biochem* 1999, **198:**113-118.
15. Vary TC: **Sepsis-induced alterations in pyruvate dehydrogenase complex activity in rat skeletal muscle: effects on plasma lactate.** *Shock* 1996, **6:**89-94.
16. James JH, Luchette FA, McCarter FD, Fischer JE: **Lactate is an unreliable indicator of tissue hypoxia in injury or sepsis.** *Lancet* 1999, **354:**505-508.
17. L'Her E, Sebert P: **A global approach to energy metabolism in an experimental model of sepsis.** *Am J Respir Crit Care Med* 2001, **164:**1444-1447.
18. Cohen ES, Hollenberg SM: **Tissue oxygenation and sepsis.** *Crit Care Med* 2001, **29:**1479-1480.
19. Lam C, Tyml K, Martin C, Sibbald W: **Microvascular perfusion is impaired in a rat model of normotensive sepsis.** *J Clin Invest* 1994, **94:**2077-2083.
20. Neviere R, Mathieu D, Chagnon JL, Lebleu N, Millien JP, Wattel F: **Skeletal muscle microvascular blood flow and oxygen transport in patients with severe sepsis.** *Am J Respir Crit Care Med* 1996, **153:**191-195.
21. Tyml K, Yu J, McCormack DG: **Capillary and arteriolar responses to local vasodilators are impaired in a rat model of sepsis.** *J Appl Physiol* 1998, **84:**837-844.
22. Zhao H, Spain DA, Matheson PJ, Vaughn C, Harris PD, Garrison RN: **Sustained infection induces 2 distinct microvascular mechanisms in the splanchnic circulation.** *Surgery* 2000, **128:**513-519.
23. Whitworth PW, Cryer HM, Garrison RN, Baumgarten TE, Harris PD: **Hypoperfusion of the intestinal microcirculation without decreased cardiac output during live *Escherichia coli* sepsis in rats.** *Circ Shock* 1989, **27:**111-122.
24. Cryer HM, Garrison RN, Kaebnick HW, Harris PD, Flint LM: **Skeletal microcirculatory responses to hyperdynamic *Escherichia coli* sepsis in unanesthetized rats.** *Arch Surg* 1987, **122:**86-92.
25. Piper RD, Pitt-Hyde M, Li F, Sibbald WJ, Potter RF: **Microcirculatory changes in rat skeletal muscle in sepsis.** *Am J Respir Crit Care Med* 1996, **154:**931-937.
26. Bateman RM, Jagger JE, Sharpe MD, Ellsworth ML, Mehta S, Ellis CG: **Erythrocyte deformability is a nitric oxide-mediated factor in decreased capillary density during sepsis.** *Am J Physiol Heart Circ Physiol* 2001, **280:**H2848-H2856.
27. Drazenovic R, Samsel RW, Wylam ME, Doerschuk CM, Schumacker PT: **Regulation of perfused capillary density in canine intestinal mucosa during endotoxemia.** *J Appl Physiol* 1992, **72:**259-265.
28. Farquhar I, Martin CM, Lam C, Potter R, Ellis CG, Sibbald WJ: **Decreased capillary density in vivo in bowel mucosa of rats with normotensive sepsis.** *J Surg Res* 1996, **61:**190-196.
29. Madorin WS, Martin CM, Sibbald WJ: **Dopexamine attenuates flow motion in ileal mucosal arterioles in normotensive sepsis.** *Crit Care Med* 1999, **27:**394-400.
30. Ellis CG, Bateman RM, Sharpe MD, Sibbald WJ, Gill R: **Effect of a maldistribution of microvascular blood flow on capillary O_2 extraction in sepsis.** *Am J Physiol Heart Circ Physiol* 2002, **282:**H156-H164.
31. Nakajima Y, Baudry N, Duranteau J, Vicaut E: **Microcirculation in intestinal villi: a comparison between hemorrhagic and endotoxin shock.** *Am J Respir Crit Care Med* 2001, **164:**1526-1530.
32. Weinberg JR, Boyle P, Thomas K, Murphy K, Tooke JE, Guz A: **Capillary blood cell velocity is reduced in fever without hypotension.** *Int J Microcirc Clin Exp* 1991, **10:**13-19.
33. Spronk PE, Ince C, Gardien MJ, Mathura KR, Oudemans-van Straaten HM, Zandstra DF: **Nitroglycerin in septic shock after intravascular volume resuscitation.** *Lancet* 2002, **360:**1395-1396.
34. De Backer D, Creteur J, Preiser JC, Dubois MJ, Vincent JL: **Microvascular blood flow is altered in patients with sepsis.** *Am J Respir Crit Care Med* 2002, **166:**98-104.
35. Astiz M, Rackow EC, Weil MH, Schumer W: **Early impairment of oxidative metabolism and energy production in severe sepsis.** *Circ Shock* 1988, **26:**311-320.
36. Anning PB, Sair M, Winlove CP, Evans TW: **Abnormal tissue oxygenation and cardiovascular changes in endotoxemia.** *Am J Respir Crit Care Med* 1999, **159:**1710-1715.

37. Boekstegers P, Weidenhofer S, Kapsner T, Werdan K: **Skeletal muscle partial pressure of oxygen in patients with sepsis.** *Crit Care Med* 1994, **22:**640-650.

38. Ince C, Sinaasappel M: **Microcirculatory oxygenation and shunting in sepsis and shock.** *Crit Care Med* 1999, **27:**1369-1377.

39. Hotchkiss RS, Rust RS, Dence CS, Wasserman TH, Song SK, Hwang DR, Karl IE, Welch MJ: **Evaluation of the role of cellular hypoxia in sepsis by the hypoxic marker [18F]fluoromisonidazole.** *Am J Physiol* 1991, **261:**R965-R972.

40. Boczkowski J, Vicaut E, Aubier M: **In vivo effects of *Escherichia coli* endotoxemia on diaphragmatic microcirculation in rats.** *J Appl Physiol* 1992, **72:**2219-2224.

41. Cryer HM, Garrison RN, Harris PD: **Role of muscle microvasculature during hyperdynamic and hypodynamic phases of endotoxin shock in decerebrate rats.** *J Trauma* 1988, **28:**312-318.

42. Hollenberg SM, Piotrowski MJ, Parrillo JE: **Nitric oxide synthase inhibition reverses arteriolar hyporesponsiveness to endothelin-1 in septic rats.** *Am J Physiol* 1997, **272:**R969-R974.

43. Hollenberg SM, Cunnion RE, Zimmerberg J: **Nitric oxide synthase inhibition reverses arteriolar hyporesponsiveness to catecholamines in septic rats.** *Am J Physiol* 1993, **264:**H660-H663.

44. Sato Y, Walley KR, Klut ME, English D, D'yachkova Y, Hogg JC, van Eeden SF: **Nitric oxide reduces the sequestration of polymorphonuclear leukocytes in lung by changing deformability and CD18 expression.** *Am J Respir Crit Care Med* 1999, **159:**1469-1476.

45. Tugtekin IF, Radermacher P, Theisen M, Matejovic M, Stehr A, Ploner F, Matura K, Ince C, Georgieff M, Trager K: **Increased ileal-mucosal-arterial PCO$_2$ gap is associated with impaired villus microcirculation in endotoxic pigs.** *Intensive Care Med* 2001, **27:**757-766.

46. Unger LS, Cryer HM, Garrison RN: **Differential response of the microvasculature in the liver during bacteremia.** *Circ Shock* 1989, **29:**335-344.

47. Gundersen Y, Corso CO, Leiderer R, Dorger M, Lilleaasen P, Aasen AO, Messmer K: **The nitric oxide donor sodium nitroprusside protects against hepatic microcirculatory dysfunction in early endotoxaemia.** *Intensive Care Med* 1998, **24:**1257-1263.

48. Corso CO, Gundersen Y, Dorger M, Lilleaasen P, Aasen AO, Messmer K: **Effects of the nitric oxide synthase inhibitors N^G-nitro-L-arginine methyl ester and aminoethyl-isothiourea on the liver microcirculation.** *J Hepatol* 1998, **28:**61-69.

49. Nishida J, McCuskey RS, McDonnell D, Fox ES: **Protective role of NO in hepatic microcirculatory dysfunction during endotoxemia.** *Am J Physiol* 1994, **267:**G1135-G1141.

50. Piper RD, Pitt-Hyde ML, Anderson LA, Sibbald WJ, Potter RF: **Leukocyte activation and flow behavior in rat skeletal muscle in sepsis.** *Am J Respir Crit Care Med* 1998, **157:**129-134.

51. Groom AC, Ellis CG, Wrigley SJ, Potter RF: **Capillary network morphology and capillary flow.** *Int J Microcirc Clin Exp* 1995, **15:**223-230.

52. Griffith TM: **Endothelial control of vascular tone by nitric oxide and gap junctions: a haemodynamic perspective.** *Biorheology* 2002, **39:**307-318.

53. Fisher AB, Chien S, Barakat AI, Nerem RM: **Endothelial cellular response to altered shear stress.** *Am J Physiol* 2001, **281:**L529-L533.

54. Furchgott RF, Zawadzki JV: **The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine.** *Nature* 1980, **288:**373-376.

55. Collins DM, McCullough WT, Ellsworth ML: **Conducted vascular responses: communication across the capillary bed.** *Microvasc Res* 1998, **56:**43-53.

56. Tyml K, Wang X, Lidington D, Ouellette Y: **Lipopolysaccharide reduces intercellular coupling in vitro and arteriolar conducted response in vivo.** *Am J Physiol Heart Circ Physiol* 2001, **281:**H1397-H1406.

57. Vallet B: **Endothelial cell dysfunction and abnormal tissue perfusion.** *Crit Care Med* 2002, **30:**S229-S234.

58. Krogh A: **The number and the distribution of capillaries in muscle with the calculation of the oxygen pressure necessary for supplying tissue.** *J Physiol (Lond)* 1919, **52:**409-515.

59. Ellsworth ML, Pittman RN: **Arterioles supply oxygen to capillaries by diffusion as well as by convection.** *Am J Physiol* 1990, **258:**H1240-H1243.

60. Kon K, Maeda N, Shiga T: **Effect of nitric oxide on the oxygen transport of human erythrocytes.** *J Toxicol Environ Health* 1977, **2:**1109-1113.

61. Kosaka H, Seiyama A: **Physiological role of nitric oxide as an enhancer of oxygen transfer from erythrocytes to tissues.** *Biochem Biophys Res Commun* 1996, **218:**749-752.

62. Patel RP, Hogg N, Spencer NY, Kalyanaraman B, Matalon S, Darley-Usmar VM: **Biochemical characterization of human S-nitrosohemoglobin. Effects on oxygen binding and transnitrosation.** *J Biol Chem* 1999, **274:**15487-15492.

63. Ellsworth ML, Forrester T, Ellis CG, Dietrich HH: **The erythrocyte as a regulator of vascular tone.** *Am J Physiol* 1995, **269:**H2155-H2161.

64. Jagger JE, Bateman RM, Ellsworth ML, Ellis CG: **Role of erythrocyte in regulating local O2 delivery mediated by hemoglobin oxygenation.** *Am J Physiol Heart Circ Physiol* 2001, **280:**H2833-H2839.

65. Stamler JS, Jia L, Eu JP, McMahon TJ, Demchenko IT, Bonaventura J, Gernert K, Piantadosi CA: **Blood flow regulation by S-nitrosohemoglobin in the physiological oxygen gradient.** *Science* 1997, **276:**2034-2037.

66. Ellsworth ML: **The red blood cell as an oxygen sensor: what is the evidence?** *Acta Physiol Scand* 2000, **168:**551-559.

67. Sprague RS, Ellsworth ML, Stephenson AH, Kleinhenz ME, Lonigro AJ: **Deformation-induced ATP release from red blood cells requires CFTR activity.** *Am J Physiol* 1998, **275:**H1726-H1732.

68. Powell RJ, Machiedo GW, Rush BF Jr, Dikdan G: **Oxygen free radicals: effect on red cell deformability in sepsis.** *Crit Care Med* 1991, **19:**732-735.

69. Baskurt OK, Gelmont D, Meiselman HJ: **Red blood cell deformability in sepsis.** *Am J Respir Crit Care Med* 1998, **157:**421-427.

70. Condon MR, Kim JE, Deitch EA, Machiedo GW, Spolarics Z: **Appearance of an erythrocyte population with decreased deformability and hemoglobin content following sepsis.** *Am J Physiol Heart Circ Physiol* 2003, **284:**H2177-H2184.

71. Hinshaw LB: **Sepsis/septic shock: participation of the microcirculation: an abbreviated review.** *Crit Care Med* 1996, **24:**1072-1078.

72. Baskurt OK, Temiz A, Meiselman HJ: **Red blood cell aggregation in experimental sepsis.** *J Lab Clin Med* 1997, **130:**183-190.

73. Eichelbronner O, Sielenkamper A, Cepinskas G, Sibbald WJ, Chin-Yee IH: **Endotoxin promotes adhesion of human erythrocytes to human vascular endothelial cells under conditions of flow.** *Crit Care Med* 2000, **28:**1865-1870.

74. Diaz NL, Finol HJ, Torres SH, Zambrano CI, Adjounian H: **Histochemical and ultrastructural study of skeletal muscle in patients with sepsis and multiple organ failure syndrome (MOFS).** *Histol Histopathol* 1998, **13:**121-128.

75. Hwang TL, Han ML: **The changes of hepatic sinusoidal microcirculation and effects of nitric oxide synthase inhibitor during sepsis.** *Hepatogastroenterology* 2003, **50:**213-216.

76. James PE, Madhani M, Roebuck W, Jackson SK, Swartz HM: **Endotoxin-induced liver hypoxia: defective oxygen delivery versus oxygen consumption.** *Nitric Oxide* 2002, **6:**18-28.

77. Vallet B, Lund N, Curtis SE, Kelly D, Cain SM: **Gut and muscle tissue PO$_2$ in endotoxemic dogs during shock and resuscitation.** *J Appl Physiol* 1994, **76:**793-800.

78. Brealey D, Brand M, Hargreaves I, Heales S, Land J, Smolenski R, Davies NA, Cooper CE, Singer M: **Association between mitochondrial dysfunction and severity and outcome of septic shock.** *Lancet* 2002, **360:**219-223.

79. Fink MP: **Bench-to-bedside review: Cytopathic hypoxia.** *Crit Care* 2002, **6:**491-499.

80. Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B, Peterson E, Tomlanovich M; Early Goal-Directed Therapy Collaborative Group: **Early goal-directed therapy in the treatment of severe sepsis and septic shock.** *N Engl J Med* 2001, **345:**1368-1377.

81. Macedo MP, Lautt WW: **Shear-induced modulation by nitric oxide of sympathetic nerves in the superior mesenteric artery.** *Can J Physiol Pharmacol* 1996, **74:**692-700.

82. Boegehold MA: **Shear-dependent release of venular nitric oxide: effect on arteriolar tone in rat striated muscle.** *Am J Physiol* 1996, **271:**H387-H395.

83. Bor-Kucukatay M, Wenby RB, Meiselman HJ, Baskurt OK: **Effects of nitric oxide on red blood cell deformability.** *Am J Physiol Heart Circ Physiol* 2003, **284:**H1577-H1584.

84. Kubes P, Suzuki M, Granger DN: **Nitric oxide: an endogenous modulator of leukocyte adhesion.** *Proc Natl Acad Sci USA* 1991, **88:**4651-4655.

85. Mitchell DJ, Yu J, Tyml K: **Local L-NAME decreases blood flow and increases leukocyte adhesion via CD18.** *Am J Physiol* 1998, **274:**H1264-H1268.

86. Radomski MW, Vallance P, Whitley G, Foxwell N, Moncada S: **Platelet adhesion to human vascular endothelium is modulated by constitutive and cytokine induced nitric oxide.** *Cardiovasc Res* 1993, **27:**1380-1382.

87. Cerwinka WH, Cooper D, Krieglstein CF, Feelisch M, Granger DN: **Nitric oxide modulates endotoxin-induced platelet-endothelial cell adhesion in intestinal venules.** *Am J Physiol Heart Circ Physiol* 2002, **282:**H1111-H1117.

88. Laszlo F, Whittle BJ, Evans SM, Moncada S: **Association of microvascular leakage with induction of nitric oxide synthase: effects of nitric oxide synthase inhibitors in various organs.** *Eur J Pharmacol* 1995, **283:**47-53.

89. Laszlo F, Whittle BJ, Moncada S: **Time-dependent enhancement or inhibition of endotoxin-induced vascular injury in rat intestine by nitric oxide synthase inhibitors.** *Br J Pharmacol* 1994, **111:**1309-1315.

90. Jia L, Bonaventura C, Bonaventura J, Stamler JS: **S-nitroso-haemoglobin: a dynamic activity of blood involved in vascular control.** *Nature* 1996, **380:**221-226.

91. Lauer T, Kleinbongard P, Kelm M: **Indexes of NO bioavailability in human blood.** *News Physiol Sci* 2002, **17:**251-255.

92. Feelisch M, Rassaf T, Mnaimneh S, Singh N, Bryan NS, Jour-d'Heuil D, Kelm M: **Concomitant S-, N-, and heme-nitros(yl)ation in biological tissues and fluids: implications for the fate of NO in vivo.** *FASEB J* 2002, **16:**1775-1785.

93. Gladwin MT, Ognibene FP, Pannell LK, Nichols JS, Pease-Fye ME, Shelhamer JH, Schechter AN: **Relative role of heme nitrosylation and beta-cysteine 93 nitrosation in the transport and metabolism of nitric oxide by hemoglobin in the human circulation.** *Proc Natl Acad Sci USA* 2000, **97:**9943-9948.

94. Cannon RO 3rd, Schechter AN, Panza JA, Ognibene FP, Pease-Fye ME, Waclawiw MA, Shelhamer JH, Gladwin MT: **Effects of inhaled nitric oxide on regional blood flow are consistent with intravascular nitric oxide delivery.** *J Clin Invest* 2001, **108:**279-287.

95. Ignarro LJ, Fukuto JM, Griscavage JM, Rogers NE, Byrns RE: **Oxidation of nitric oxide in aqueous solution to nitrite but not nitrate: comparison with enzymatically formed nitric oxide from L-arginine.** *Proc Natl Acad Sci USA* 1993, **90:** 8103-8107.

96. Rassaf T, Kleinbongard P, Preik M, Dejam A, Gharini P, Lauer T, Erckenbrecht J, Duschin A, Schulz R, Heusch G, Feelisch M, Kelm M: **Plasma nitrosothiols contribute to the systemic vasodilator effects of intravenously applied NO: experimental and clinical study on the fate of NO in human blood.** *Circ Res* 2002, **91:**470-477.

97. Rassaf T, Preik M, Kleinbongard P, Lauer T, Heiss C, Strauer BE, Feelisch M, Kelm M: **Evidence for in vivo transport of bioactive nitric oxide in human plasma.** *J Clin Invest* 2002, **109:**1241-1248.

98. Baveja R, Kresge N, Ashburn JH, Keller S, Yokoyama Y, Sonin N, Zhang JX, Huynh T, Clemens MG: **Potentiated hepatic microcirculatory response to endothelin-1 during polymicrobial sepsis.** *Shock* 2002, **18:**415-422.

99. Avontuur JA, Boomsma F, van den Meiracker AH, de Jong FH, Bruining HA: **Endothelin-1 and blood pressure after inhibition of nitric oxide synthesis in human septic shock.** *Circulation* 1999, **99:**271-275.

100. Beasley D, Eldridge M: **Interleukin-1 beta and tumor necrosis factor-alpha synergistically induce NO synthase in rat vascular smooth muscle cells.** *Am J Physiol* 1994, **266:**R1197-R1203.

101. Cunha FQ, Assreuy J, Moss DW, Rees D, Leal LM, Moncada S, Carrier M, O'Donnell CA, Liew FY: **Differential induction of nitric oxide synthase in various organs of the mouse during endotoxaemia: role of TNF-alpha and IL-1.** *Immunology* 1994, **81:** 211-215.

102. Gocan NC, Scott JA, Tyml K: **Nitric oxide produced via neuronal NOS may impair vasodilatation in septic rat skeletal muscle.** *Am J Physiol Heart Circ Physiol* 2000, **278:**H1480-H1489.

103. Sade K, Schwartz D, Wolman Y, Schwartz I, Chernichovski T, Blum M, Brazowski E, Keynan S, Raz I, Blantz RC, Iaina A: **Time course of lipopolysaccharide-induced nitric oxide synthase mRNA expression in rat glomeruli.** *J Lab Clin Med* 1999, **134:** 471-477.

104. Harada S, Imaki T, Chikada N, Naruse M, Demura H: **Distinct distribution and time-course changes in neuronal nitric oxide synthase and inducible NOS in the paraventricular nucleus following lipopolysaccharide injection.** *Brain Res* 1999, **821:**322-332.

105. Chen K, Inoue M, Okada A: **Expression of inducible nitric oxide synthase mRNA in rat digestive tissues after endotoxin and its role in intestinal mucosal.** *Biochem Biophys Res Commun* 1996, **224:**703-708.

106. Preiser JC, Zhang H, Vray B, Hrabak A, Vincent JL: **Time course of inducible nitric oxide synthase activity following endotoxin administration in dogs.** *Nitric Oxide* 2001, **5:**208-211.

107. Kengatharan KM, De Kimpe SJ, Thiemermann C: **Role of nitric oxide in the circulatory failure and organ injury in a rodent model of gram-positive shock.** *Br J Pharmacol* 1996, **119:** 1411-1421.

108. Shieh P, Zhou M, Ornan DA, Chaudry IH, Wang P: **Upregulation of inducible nitric oxide synthase and nitric oxide occurs later than the onset of the hyperdynamic response during sepsis.** *Shock* 2000, **13:**325-329.

109. Scott JA, Mehta S, Duggan M, Bihari A, McCormack DG: **Functional inhibition of constitutive nitric oxide synthase in a rat model of sepsis.** *Am J Respir Crit Care Med* 2002, **165:**1426-1432.

110. Lu JL, Schmiege LM 3rd, Kuo L, Liao JC: **Downregulation of endothelial constitutive nitric oxide synthase expression by lipopolysaccharide.** *Biochem Biophys Res Commun* 1996, **225:**1-5.

111. Evans T, Carpenter A, Kinderman H, Cohen J: **Evidence of increased nitric oxide production in patients with the sepsis syndrome.** *Circ Shock* 1993, **41:**77-81.

112. Strand OA, Leone A, Giercksky KE, Kirkeboen KA: **Nitric oxide indices in human septic shock.** *Crit Care Med* 2000, **28:**2779-2785.

113. Ochoa JB, Udekwu AO, Billiar TR, Curran RD, Cerra FB, Simmons RL, Peitzman AB: **Nitrogen oxide levels in patients after trauma and during sepsis.** *Ann Surg* 1991, **214:**621-626.

114. Gomez-Jimenez J, Salgado A, Mourelle M, Martin MC, Segura RM, Peracaula R, Moncada S: **L-arginine: nitric oxide pathway in endotoxemia and human septic shock.** *Crit Care Med* 1995, **23:**253-258.

115. Wu F, Cepinskas G, Wilson JX, Tyml K: **Nitric oxide attenuates but superoxode enhances iNOS expression in endotoxin- and IFN gamma-stimulated skeletal muscle endothelial cells.** *Microcirculation* 2001, **8:**415-425.

116. Gunnett CA, Chu Y, Heistad DD, Loihl A, Faraci FM: **Vascular effects of LPS in mice deficient in expression of the gene for inducible nitric oxide synthase.** *Am J Physiol* 1998, **275:**H416-H421.

117. Kilbourn RG, Gross SS, Jubran A, Adams J, Griffith OW, Levi R, Lodato RF: **NG-methyl-L-arginine inhibits tumor necrosis factor-induced hypotension: implications for the involvement of nitric oxide.** *Proc Natl Acad Sci USA* 1990, **87:**3629-3632.

118. Broccard A, Hurni JM, Eckert P, Liaudet L, Schaller MD, Lazor R, Perret C, Feihl F: **Tissue oxygenation and hemodynamic response to NO synthase inhibition in septic shock.** *Shock* 2000, **14:**35-40.

119. Lorente JA, Landin L, De Pablo R, Renes E, Liste D: **L-arginine pathway in the sepsis syndrome.** *Crit Care Med* 1993, **21:** 1287-1295.

120. Avontuur JA, Tutein Nolthenius RP, van Bodegom JW, Bruining HA: **Prolonged inhibition of nitric oxide synthesis in severe septic shock: a clinical study.** *Crit Care Med* 1998, **26:**660-667.

121. Petros A, Lamb G, Leone A, Moncada S, Bennett D, Vallance P: **Effects of a nitric oxide synthase inhibitor in humans with septic shock.** *Cardiovasc Res* 1994, **28:**34-39.

122. Petros A, Bennett D, Vallance P: **Effect of nitric oxide synthase inhibitors on hypotension in patients with septic shock.** *Lancet* 1991, **338:**1557-1558.

123. Nava E, Palmer RM, Moncada S: **Inhibition of nitric oxide synthesis in septic shock: how much is beneficial?** *Lancet* 1991, **338:**1555-1557.

124. Booke M, Hinder F, McGuire R, Traber LD, Traber DL: **Selective inhibition of inducible nitric oxide synthase: effects on hemodynamics and regional blood flow in healthy and septic sheep.** *Crit Care Med* 1999, **27**:162-167.

125. Booke M, Hinder F, McGuire R, Traber LD, Traber DL: **Nitric oxide synthase inhibition versus norepinephrine for the treatment of hyperdynamic sepsis in sheep.** *Crit Care Med* 1996, **24**:835-844.

126. Meyer J, Hinder F, Stothert J Jr, Traber LD, Herndon DN, Flynn JT, Traber DL: **Increased organ blood flow in chronic endotoxemia is reversed by nitric oxide synthase inhibition.** *J Appl Physiol* 1994, **76**:2785-2793.

127. Grover R, Lopez A, Lorente J, Steingrub J, Bakker J, Willatts S, McLuckie A, Takala J: **Multi-center, randomized, placebo-controlled, double blind study of nitric oxide synthase inhibitor 546C88: effect on survival in patients with septic shock [abstract].** *Crit Care Med* 1999, **27**:A33.

128. Bernard GR, Vincent JL, Laterre PF, LaRosa SP, Dhainaut JF, Lopez-Rodriguez A, Steingrub JS, Garber GE, Helterbrand JD: **Efficacy and safety of recombinant human activated protein C for severe sepsis.** *N Engl J Med* 2001, **344**:699-709.

129. Isobe H, Okajima K, Uchiba M, Mizutani A, Harada N, Nagasaki A, Okabe K: **Activated protein C prevents endotoxin-induced hypotension in rats by inhibiting excessive production of nitric oxide.** *Circulation* 2001, **104**:1171-1175.

130. Spain DA, Wilson MA, Bar-Natan MF, Garrison RN: **Nitric oxide synthase inhibition aggravates intestinal microvascular vasoconstriction and hypoperfusion of bacteremia.** *J Trauma* 1994, **36**:720-725.

131. Walker TA, Curtis SE, King-VanVlack CE, Chapler CK, Vallet B, Cain SM: **Effects of nitric oxide synthase inhibition on regional hemodynamics and oxygen transport in endotoxic dogs.** *Shock* 1995, **4**:415-420.

132. Avontuur JA, Bruining HA, Ince C: **Inhibition of nitric oxide synthesis causes myocardial ischemia in endotoxemic rats.** *Circ Res* 1995, **76**:418-425.

133. Huang TP, Nishida T, Kamike W, Kosaka H, Seiyama A, Morimoto Y, Tanaka S, Obunai S, Takei Y, Shiga T, Matsuda H: **Role of nitric oxide in oxygen transport in rat liver sinusoids during endotoxemia.** *Hepatology* 1997, **26**:336-342.

134. King CJ, Tytgat S, Delude RL, Fink MP: **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.

135. Pittner A, Nalos M, Asfar P, Yang Y, Ince C, Georgieff M, Bruckner UB, Radermacher P, Froba G: **Mechanisms of inducible nitric oxide synthase (iNOS) inhibition-related improvement of gut mucosal acidosis during hyperdynamic porcine endotoxemia.** *Intensive Care Med* 2003, **29**:312-316.

136. Saetre T, Gundersen Y, Thiemermann C, Lilleaasen P, Aasen AO: **Aminoethyl-isothiourea, a selective inhibitor of inducible nitric oxide synthase activity, improves liver circulation and oxygen metabolism in a porcine model of endotoxemia.** *Shock* 1998, **9**:109-115.

137. Wang Y, Lawson JA, Jaeschke H: **Differential effect of 2-aminoethyl-isothiourea, an inhibitor of the inducible nitric oxide synthase, on microvascular blood flow and organ injury in models of hepatic ischemia-reperfusion and endotoxemia.** *Shock* 1998, **10**:20-25.

138. Tadros T, Traber DL, Herndon DN: **Hepatic blood flow and oxygen consumption after burn and sepsis.** *J Trauma* 2000, **49**:101-108.

139. Lizasoain I, Moro MA, Knowles RG, Darley-Usmar V, Moncada S: **Nitric oxide and peroxynitrite exert distinct effects on mitochondrial respiration which are differentially blocked by glutathione or glucose.** *Biochem J* 1996, **314**:877-880.

140. Torres J, Darley-Usmar V, Wilson MT: **Inhibition of cytochrome c oxidase in turnover by nitric oxide: mechanism and implications for control of respiration.** *Biochem J* 1995, **312**:169-173.

141. Brown GC, Cooper CE: **Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase.** *FEBS Lett* 1994, **356**:295-298.

142. Cleeter MW, Cooper JM, Darley-Usmar VM, Moncada S, Schapira AH: **Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. Implications for neurodegenerative diseases.** *FEBS Lett* 1994, **345**:50-54.

143. Borutaite V, Matthias A, Harris H, Moncada S, Brown GC: **Reversible inhibition of cellular respiration by nitric oxide in vascular inflammation.** *Am J Physiol Heart Circ Physiol* 2001, **281**:H2256-H2260.

144. Simonson SG, Welty-Wolf K, Huang YT, Griebel JA, Caplan MS, Fracica PJ, Piantadosi CA: **Altered mitochondrial redox responses in gram negative septic shock in primates.** *Circ Shock* 1994, **43**:34-43.