Impairments in microvascular reactivity are related to organ failure in human sepsis

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Doerschug KC, Delsing AS, Schmidt GA, Haynes WG. Impairments in microvascular reactivity are related to organ failure in human sepsis. Am J Physiol Heart Circ Physiol 293: H1065–H1071, 2007. First published May 4, 2007; doi:10.1152/ajpheart.01237.2006.—Severe sepsis is a systemic inflammatory response to infection resulting in acute organ dysfunction. Vascular perfusion abnormalities are implicated in the pathology of organ failure, but studies of microvascular function in human sepsis are limited. We hypothesized that impaired microvascular responses to reactive hyperemia lead to impaired oxygen delivery relative to the needs of tissue and that these impairments would be associated with organ failure in sepsis. We studied 24 severe sepsis subjects 24 h after recognition of organ dysfunction; 15 healthy subjects served as controls. Near-infrared spectroscopy (NIRS) was used to measure tissue 1) microvascular hemoglobin signal strength and 2) oxygen saturation of microvascular hemoglobin (StO2). Both values were measured in thenar skeletal muscle before and after 5 min of forearm stagnant ischemia. At baseline, skeletal muscle microvascular hemoglobin was lower in septic than control subjects. Microvascular hemoglobin increased during reactive hyperemia in both groups, but less so in sepsis. StO2 at baseline and throughout ischemia was similar between the two groups; however, the rate of tissue oxygen consumption was significantly slower in septic subjects than in controls. The rate of increase in StO2 during reactive hyperemia was significantly slower in septic subjects than in controls; this impairment was accentuated in those with more organ failure. We conclude that organ dysfunction in severe sepsis is associated with dysregulation of microvascular oxygen balance. NIRS measurements of skeletal muscle microvascular perfusion and reactivity may provide important information about sepsis and serve as endpoints in future therapeutic interventions aimed at improving the microcirculation.

SEVERE SEPSIS IS AN INFLAMMATORY response to infection that results in acute organ dysfunction (5). Despite hyperdynamic systemic blood flow and elevated mixed venous oxygen saturations, elevated blood lactate concentrations are common in sepsis and suggest that a maldistribution of oxygen delivery within the tissues may contribute to organ failure and death. Impaired microvascular perfusion was first identified in animal models (28), and direct visualization of vessels recently demonstrated heterogeneous flow in the sublingual microcirculation of patients with severe sepsis (11, 38), providing human data to substantiate the models. Normal capillary beds maintain homogenous flow (i.e., matching supply to demand) through signaling from endothelium to precapillary arterioles. Sepsis disrupts this signaling (40) and also mechanically disrupts flow by activating endo-

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oxy- and deoxymyoglobin in the muscle, although the latter chromophore has little influence on the total signal (6, 31). Nonetheless, nomenclature has evolved such that some literature and device manufacturers refer to tissue measurements, and we will use this nomenclature for the purpose of consistency.

A commercially available, clinical spectrometer (InSpectra Tissue Spectrometer model 325; Hutchinson Technology) was applied to the thenar eminence throughout the study. This monitor uses 15-mm spacing between emission and detection points and provides tissue attenuation measurements at four discreet wavelengths (680, 720, 760, and 800 nm). Details of this wide-gap second derivative spectroscopic method have been described previously (34). The monitor provides the total hemoglobin signal strength (TH) in the volume of tissue sensed by the probe, as well as fractions of oxy- and deoxymyoglobin through a tissue depth approximating the probe length (34). Values were recorded directly to computer every 3.5 s. The positioning of the probe on the thenar eminence was chosen because of relatively low adiposity, consistency with other studies, and because of its amenability to forearm manipulation.

A vascular cuff (Hokanson) was inflated to 250 mmHg on the forearm to achieve stagnant ischemia for 5 min and then rapidly deflated. For each subject, we assessed the following: 1) total hemoglobin signal, an indicator of blood volume in the region of microvasculature sensed by the probe (referred to as TH by the manufacturer) expressed in arbitrary units (AU). TH was measured at baseline and recorded as the average of five values immediately before ischemia; 2) microvascular hemoglobin during reactive hyperemia (TH-RH), defined as the average of five TH values obtained during the interval 18–32 s following the release of occlusion. This prospectively defined interval depicts a plateau that follows a rapid increase during the initial 14 s of flow and precedes a decline toward baseline values; 3) change in TH (ΔTH), defined as the difference of TH-RH – TH, and percent change in TH (%ΔTH), defined as ΔTH × 100/TH; 4) oxygen saturation of hemoglobin in the microvasculature, referred to as tissue oxygen saturation (StO2) by the device manufacturer, expressed as percent; 5) oxygen consumption of skeletal muscle tissue through a tissue depth approximating the probe length (34). Values were recorded directly to computer every 3.5 s. The positioning of the probe on the thenar eminence was chosen because of relatively low adiposity, consistency with other studies, and because of its amenability to forearm manipulation.

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The timing and duration of intervals were defined prospectively based on previous data. Pulse rates and blood pressures were specifically recorded during testing to exclude the possibility that systemic hemodynamic changes were responsible for local perfusion changes.

Data were analyzed with GraphPad Prism software version 4.0 (San Diego, CA). Three groups were compared using one-way ANOVA. Significant differences (α <0.05) were evaluated with Tukey’s multiple-comparison test. Two sepsis subjects had ischemia for <5 min (one mechanical malfunction, one human error); for these subjects, we analyzed preischemia data but excluded postischemia data.

RESULTS

Clinical data. Twenty four subjects with severe sepsis were enrolled during the study period. Demographic and clinical data are listed in Table 1. The mortality of sepsis subjects was 33%, consistent with a group at high risk of death. Taken as a whole, these data describe a heterogeneous, critically ill population typical of severe sepsis.

TH. TH was significantly reduced in severe sepsis subjects at baseline (see Fig. 1). There was no correlation between TH and blood hemoglobin concentration. Preischemic TH did not differ between septic subjects with severe organ failure and those with only modest dysfunction; however, it was lower in those subjects receiving vasoconstrictor infusions than in those who did not receive these medications [13.4 AU(SD3.5) vs. 18.4 AU(SD5.7), P = 0.01]. Among subjects with severe sepsis, there was no relationship between TH and age (linear regression r² = 0.005, P = 0.74).

TH-RH. During and after the period of stagnant ischemia, there were no observed changes in systemic pulse rate or blood pressure. Following the release of the forearm vascular cuff and the subsequent return of blood flow, TH increased rapidly (within 30 s) to baseline levels or higher and remained elevated for ~2 min in both severe sepsis and control subjects. TH-RH was significantly lower in septic subjects with both extensive and modest organ dysfunction compared with control subjects (P = 0.0002, see Table 2), but there was no significant difference between the two septic subgroups. When we examined the incremental change in TH from baseline to reactive hyperemia (ΔTH), subjects with severe sepsis culminating in extensive organ dysfunction tended to have less increase in TH compared with either those with modest organ failure or control subjects. Because TH was lower in severe sepsis subjects, however, when ΔTH was expressed as a percent of baseline (%ΔTH), the values were similar in all three groups. We examined the effect of vasoconstrictor infusions on TH-RH and found this measure to be somewhat lower in subjects receiving these medications [16.8 AU(SD6.0)] compared with those who did not receive these medications [22.3 AU(SD6.5); Student’s t-test, P = 0.05]. However, we found virtually no effect of vasoconstrictor use on ΔTH (P = 0.7) and %ΔTH (P = 0.91).

Table 1. Clinical data of severe sepsis subjects

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Median</th>
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<tbody>
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<td>Age, yr</td>
<td>40</td>
<td>85</td>
<td>56</td>
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<td>Mean arterial pressure, mmHg</td>
<td>55</td>
<td>90</td>
<td>70</td>
<td>69</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>72</td>
<td>121</td>
<td>93</td>
<td>92</td>
</tr>
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<td>SpO2, %</td>
<td>94</td>
<td>100</td>
<td>98</td>
<td>98</td>
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<tr>
<td>Hemoglobin, g/dl</td>
<td>7.9</td>
<td>14.7</td>
<td>10.7</td>
<td>10</td>
</tr>
<tr>
<td>Blood lactate*</td>
<td>1.1</td>
<td>10.3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>SOFA score</td>
<td>2</td>
<td>18</td>
<td>9.6</td>
<td>9</td>
</tr>
</tbody>
</table>

SpO2, oxygen saturation of microvascular hemoglobin; SOFA, sequential organ failure assessment; n, no. of subjects. *n = 11 subjects. †Severe organ failure defined as SOFA ≥10. ‡Vasoconstrictor use defined as SOFA cardiovascular component ≥3.

Tissue oxygenation before, during, and after stagnant ischemia. Baseline, or resting, StO2 in control subjects was 84% (SD 10), consistent with a vascular bed that includes pre- and postcapillary vessels, and with previous reports (34). Resting StO2 was similar in severe sepsis subjects [82% (SD13), see Fig. 2]. In parallel, resting StO2 measured 24 h after the onset of organ dysfunction was not associated with organ failure or survival at 7, 14, or 30 days.

During stagnant ischemia, StO2 decreased in both control and severe sepsis subjects. StO2 was similar in both groups at 30
and 60 s of ischemia, as well as at the end of ischemia (Fig. 2). Neither the absolute StO₂ nadir nor change in StO₂ during ischemia was associated with the degree of organ failure in septic subjects. However, V˙O₂tis was significantly lower in severe sepsis [233 AU(SD 156)] compared with control subjects [382 AU(SD120); P < 0.003].

With the return of blood flow, StO₂ increased rapidly and remained elevated above baseline values for ~2 min in both severe sepsis subjects and normal controls. Representative StO₂ curves of two individual subjects during RH are shown in Fig. 3. In severe sepsis subjects with only modest organ dysfunction, RR tended to be slower [3.6%/s (SD1.2)] than in normal controls [4.7%/s (SD1.1)]. Severe sepsis subjects with severe organ failure (SOFA > 10) had significant and pronounced impairments in RR [2.3%/s (SD1.5)] when compared with septic subjects with less organ dysfunction as well as normal controls (see Fig. 4). RR tended to be slower in those that did not survive hospitalization [2.5%/s (SD1.7)] than in those who survived [3.3%/s (SD1.4)], although this fell short of statistical significance (P = 0.20). RR was not associated with mean arterial pressure (r² = 0.01; P = 0.62) nor serum glucose concentration. Because exogenous norepinephrine and other vasoconstrictors may affect vasodilation and RH, we compared RR values in severe sepsis subjects receiving vasoconstrictors [2.5%/s (SD1.5)] with sepsis subjects not receiving vasoconstrictors [3.7%/s (SD1.3)] and found no significant differences (Student’s t-test, P = 0.11). There was no relation-

![Fig. 1. Tissue hemoglobin concentration is impaired in severe sepsis. The total tissue hemoglobin index (TH, arbitrary units) was measured in thenar muscle capillaries of severe sepsis and control subjects. A: TH was impaired in both septic subjects with modest organ dysfunction (SOFA < 10, light gray bar) and those with severe organ failure (SOFA ≥ 10, dark gray bar) compared with control subjects (white bar). **P < 0.001 vs. each septic subgroup. B: TH had no relationship with blood hemoglobin concentration in severe sepsis subjects. The horizontal line depicts the linear regression line r² = 0, with 99% confidence interval (broken lines).](http://ajpheart.physiology.org/)

![Fig. 2. Tissue oxygenation before and during ischemia. The oxygen saturation of tissue hemoglobin (StO₂) was measured in thenar skeletal muscles during stagnant ischemia. A: StO₂ decreased in both severe sepsis (gray bars) and control (white bars) subjects. There were no identifiable differences between the two groups at baseline, at 30 or 60 s of ischemia, nor at the end of 5 min of ischemia. The data shown depict the mean and SD at each time point. B: tissue oxygen consumption (V˙O₂tis) was calculated as the difference between tissue oxygen contents (StO₂ × tissue hemoglobin) at beginning and end of ischemia. *V˙O₂tis was reduced significantly in severe sepsis subjects (P = 0.003).](http://ajpheart.physiology.org/)

### Table 2. Total tissue hemoglobin index in reactive hyperemia

<table>
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<tr>
<th>Subject group</th>
<th>TH</th>
<th>TH-RH</th>
<th>ΔTH</th>
<th>%ΔTH</th>
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<tbody>
<tr>
<td>Control</td>
<td>22.7±2.4</td>
<td>27.7±5.1</td>
<td>5±4.2</td>
<td>21.7±17.6</td>
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<tr>
<td>Sepsis</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Modest organ failure</td>
<td>15.8±5.5*</td>
<td>19.7±6.5†</td>
<td>4.5±3.8</td>
<td>30.2±28.4</td>
</tr>
<tr>
<td>Severe organ failure</td>
<td>15.2±4.9*</td>
<td>16.9±6.9*</td>
<td>2.4±3.6</td>
<td>16.7±23.9</td>
</tr>
</tbody>
</table>

All values are means ± standard deviation. Definition of abbreviations: TH, total tissue hemoglobin index, at baseline, expressed in arbitrary units; TH-RH, TH during reactive hyperemia; ΔTH, change in TH from baseline to reactive hyperemia; %ΔTH, the ratio ΔTH × 100/TH. *P < 0.001 vs. control. †P < 0.01 vs. control.
ship between RR and age in septic subjects as investigated by linear regression ($r^2 = 0.47$, $P = 0.0006$; see Fig. 5) such that those with pronounced impairments in RR also had less total microvascular hemoglobin during RH. Interestingly, we also found a linear relationship between $V_{\text{O}_2\text{tis}}$ and RR in severe sepsis subjects ($r^2 = 0.38$, $P = 0.002$; see Fig. 6).

DISCUSSION

We have shown that microvascular function is abnormal in septic subjects studied within 24 h of organ dysfunction. First we found that the skeletal muscle tissue hemoglobin signal is reduced in sepsis. Most blood volume and thus hemoglobin is likely to be contained within postcapillary venules in skeletal muscle, and therefore these data imply impaired venous vessel capacitance. However, this is in contrast to common descriptions of increased venous capacitance in the macrovasculature during septic shock (13). Catecholamine infusions decrease venous capacitance (9) and hence could explain some of the differences in tissue hemoglobin found in our septic subjects. However, septic subjects not receiving these medications also had impaired tissue hemoglobin compared with controls. Several characteristics of sepsis, such as anemia, and abnormal microvascular perfusion due to vessel constriction, decreased microvascular density, or venular leukostasis could also account for this reduced signal. Because we could show no correlation between TH and blood hemoglobin concentration, we suspect that microvascular perfusion abnormalities underlie this finding.

In addition to deficits in tissue hemoglobin during sepsis, we demonstrated abnormal tissue oxygen balance during and after stagnant ischemia. Baseline $S_O_2$ was normal in resuscitated sepsis, a finding that parallels findings of normal or high central venous oxygen saturations after resuscitation (37). Despite normal resting $S_O_2$, $V_{\text{O}_2\text{tis}}$ was significantly reduced during ischemia in septic subjects. Measurements of oxygen consumption in the absence of flow should be interpreted carefully, since flow-induced vasomotion may increase oxygen expenditures (8). However, our finding is in line with several studies showing that oxygen extraction and consumption are indeed low (24, 25), in contrast to the teaching of a decade ago.

Our most provocative finding is that the rate of tissue reoxygenation is markedly impaired in sepsis and that RR is related to the degree of organ dysfunction. Although our method does not measure blood flow, per se, an acute rise in $S_O_2$ can only reasonably be explained by an influx of oxygen-rich arterial blood. The NIRS method does not detect hemoglobin in a single vessel but rather the bulk of hemoglobin

Fig. 3. Tissue oxygen saturation during reactive hyperemia. $S_O_2$ was measured in thenar skeletal muscles following forearm stagnant ischemia. $S_O_2$ increased rapidly during the initial period of reactive hyperemia, to a level at or above baseline. Shown are representative individual $S_O_2$ curves during the initial seconds of reactive hyperemia in a septic subject with severe organ failure (black) and a control subject (gray). Reoxygenation rates (broken lines) represent the average rate of $S_O_2$ increase in the first 14 s of reactive hyperemia ($S_O_2$ at 14 s $- S_O_2$ at end ischemia, divided by 14 s). The represented subjects were chosen by the reoxygenation rate that best approached the mean for the represented group.

Fig. 4. Reoxygenation rate (RR) is related to organ injury in sepsis. The rate of increase of tissue oxygen saturation during the first 14 s of reactive hyperemia (RR) was measured in the thenar skeletal muscles of septic and control subjects. A: RR was impaired significantly in sepsis subjects with severe organ failure (SOFA $\geq 10$, dark gray bar) compared with septic subjects with modest organ dysfunction (light gray bar, *P < 0.05) and control subjects (white bar). †$P < 0.001$ (ANOVA and Tukey’s multiple-comparison test). Data shown depict mean and interquartile range for each group.
is associated with reduced influx of hemoglobin to the tissue bed.

an imbalance of O2 delivery relative to the needs of the tissue.

stands to reason that impaired RR may represent either reduced
(bulk) arterial influx to the tissue or rapid desaturation of
hemoglobin within the capillaries; both explanations describe
the contribution of exogenous catecholamines to arteriolar con-
striction may be small; and 3) additional patient factors beyond
 catecholamines likely contribute to impaired microvascular
blood flow.

Tissue oxygen transport is exceedingly complex in sepsis
since tissue perfusion (37), oxygen diffusion, and mitochond-
drial function (19) are disturbed and interdependent. Mate-
rical models from experimental sepsis predict that vessel
density, flow heterogeneity, and oxygen consumption all play
a role in tissue hypoxia in skeletal muscle (22, 23). Specifi-
cally, the regulation of capillary density in response to changes
in oxygen delivery is impaired in septic animals (15) and may
contribute to impaired oxygen extraction. Meanwhile, if oxy-
gen consumption is limited as we found in our subjects, RH
may be impaired, since flow-mediated dilation has tremendous
as a cause for impaired RR.

These data suggest that the thenar microvasculature is un-
able to respond appropriately to ischemia by augmenting blood
flow, especially in the sickest subjects. Microvascular pertur-
ration resulting from abnormal vasoconstriction in sepsis is evi-
dent in studies describing improved microvascular flow af-
after administration of vasodilators (10, 39). Sepsis is associ-
ated with increased nitric oxide (NO) synthesis via inducible
nitric oxide synthase in many cells and tissues, which down-
regulates endothelial NOS (17, 30), leading to impaired reac-
tivity (2).

Catecholamines will counteract NO and impair RH. It is
interesting that microvascular reoxygenation rates of subjects
receiving vasoconstrictor infusions were somewhat lower than
of those subjects not receiving these medications, but this dif-
fERENCE did not exceed that which may occur through sta-
tistical chance. Similarily, the total microvascular hemoglobin
during RH was statistically lower in subjects on vasoconstric-
tors compared with the same measure in their counterparts. Yet
neither the absolute change nor relative change in microvas-
cular hemoglobin was affected by vasoconstrictors, suggesting
this difference may reflect differences in baseline microvascular
hemoglobin (including both pre- and postcapillary vessels)
more than the hyperemic response. We suspect that the fol-
lowing three factors contribute to a lack of evidence of cate-
cholamine effects on the microvascular hyperemic response in
severe sepsis: 1) adrenergic receptors have decreased binding
affinity for norepinephrine (20) and are downregulated in
sepsis (7), thus diminishing the response to norepinephrine and
other catecholamines; 2) endogenous vasoconstrictors are
prevalent in the septic bloodstream (43), and thus the relative
contribution of exogenous catecholamines to arteriolar con-
Fig. 5. RR is related to microvascular hemoglobin during reactive hyperemia. The tissue hemoglobin index (TH-RH; arbitrary units) and the rate of increase of tissue oxygen saturation (reoxygenation rate, RR; %/s) were measured in the thenar muscles of severe sepsis subjects during reactive hyperemia. RR correlated with TH-RH ($r^2 = 0.47$, $P = 0.0006$), demonstrating that low RR is associated with reduced influx of hemoglobin to the tissue bed.

Fig. 6. RR is related to tissue oxygen consumption. $V_{O_2}$ was calculated by the rate of decrease of oxygen content during 5 min of ischemia in thenar skeletal muscles. Following ischemia, the rate of increase of tissue oxygen saturation during the first 14 s of reactive hyperemia (RR) was measured in the thenar skeletal muscles of septic subjects. RR had a significant linear relationship with $V_{O_2}$ ($r^2 = 0.38$, $P = 0.002$).
used to calculate data from the absorption data (6). Accordingly, great care should be used when comparing reports generated from different spectrometers.

NIRS does not measure individual vessels, which may limit its use in mechanistic studies of individual vessel responses during sepsis. However, blood flow is variable between tissues (32) as well as within the tissues of septic humans (4). Individual vessels therefore very likely have different impairments in reactivity as hemostatic activation (33), impaired red blood cell deformation (36), leukoadherence (26), and perturbations of endothelial vasomotor function all potentially affect microvascular perfusion and contribute to heterogeneous blood flow. The importance of this heterogeneous flow is demonstrated in studies that show that the ratio of fast-flow to stop-flow capillaries relates to tissue oxygenation in animal models of sepsis (16). To this end, NIRS may be better suited to measure bulk microvascular regulation in a heterogeneous tissue bed than instruments that look at individual or select groups of vessels.

Our study is limited by the lack of systemic, macrovascular, and hemodynamic data other than blood pressure, reflecting the global trend away from the use of invasive cardiac output monitoring (14). It is noteworthy that mean arterial pressure was not associated with RR just as mean arterial pressure >65 has not been shown to affect organ perfusion or oxygen kinetics in other studies (29). Low cardiac output does occur in severe sepsis, and this would certainly lead to decreased perfusion and RH. However, because the incidence of low-output states in resuscitated sepsis is low (13, 37), decreased systemic flow seems an unlikely explanation of our observations.

We have shown that microvascular perfusion and reactivity to ischemia are impaired in humans with severe sepsis. Importantly, impaired microvascular reactivity is related to tissue dysoxia as well as organ dysfunction. NIRS is an effective tool to study these impairments, providing important information in physiological studies, and could measure relevant end points in therapeutic interventions aimed at improving the microcirculation in severe sepsis.

GRANTS

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