Nitric oxide-mediated vascular function in sepsis using passive leg movement as a novel assessment: a cross-sectional study

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NEW & NOTEWORTHY

This study uses a novel approach, passive leg movement (PLM), to assess NO bioavailability in patients with severe sepsis and septic shock. Our data show that both PLM and flow-mediated dilation are attenuated in septic subjects.

Sepsis is a major healthcare issue in the United States affecting ~750,000 people a year and having an estimated mortality rate of 18-25% (9, 14, 34). Sepsis results from a complex interaction between the infecting organism and the host’s immune, inflammatory, and coagulation response (9). Although there is substantial understanding of sepsis, the complete pathogenesis and pathophysiology still remain incompletely elucidated. It has, however, been recognized that vascular dysfunction plays a pivotal role in the pathophysiology of sepsis. Indeed, vascular dysfunction is largely responsible for microcirculatory impairments and ultimately organ failure, which is a hallmark of sepsis (30). Specifically, through various endotoxic and exotoxic mediators, inducible nitric oxide synthase (iNOS) is upregulated and leads to a loss of vascular tone and vasodilation. The NO-mediated vasodilation, brought on by the increased activity of iNOS, is a major mechanism responsible for hypotension and subsequent multiple organ dysfunction syndrome (MODS) in sepsis (7, 18, 21, 23, 29).

Flow-mediated dilation (FMD), stimulated by the increased shear stress following ischemic cuff occlusion, was first described in 1992 and has been interpreted as an indicator of NO bioavailability produced by endothelial NOS (eNOS) (1, 6). FMD has been used to determine endothelial function and has prognostic value, as it relates to mortality, in various diseases, such as hypertension, coronary vascular disease, and heart failure (1, 8, 13, 35). More recently, FMD, and by association NO bioavailability, has been recognized as a predictor of organ dysfunction, as well as a marker of mortality, in patients with severe sepsis and septic shock (4, 38, 39). However, it is becoming apparent that FMD does not solely reflect NO-mediated vasodilation. Indeed, it has recently been suggested that NO bioavailability may account for only ~33% of the FMD response (31, 41). The remaining vasodilation is likely due to other endothelial-dependent mechanisms, such as prostaglandins and endothelial-derived hyperpolarizing factor. Additionally, FMD can be technically challenging, as it relies on the accurate assessment of baseline brachial artery diameter and often demonstrates poor reproducibility (31, 39).

Recently, our group and others have used passive leg movement (PLM) as a reductionist model to better understand the factors controlling movement-induced hyperemia (10, 27, 37, 40). With the initial onset of passive movement there is a transient and robust increase in leg blood flow (LBF) and vascular conductance. The resulting hyperemia sets off a complex cascade of events that triggers additional peripheral hemodynamic changes including flow-mediated dilation as well as increases in heart rate and cardiac output that support the hyperemia (24, 36). By removing the increase in metabolism associated with active exercise, important findings related to the regulation of leg blood flow in both health and disease have been revealed utilizing PLM (12, 25, 40). Moreover, our group and others have found that the hyperemic response induced by PLM is highly NO dependent. Indeed, PLM has been estimated...
arginine (L-NMMA), a nonspecific NOS inhibitor (27, 37). Given the relatively simple method of assessment and analysis, as well as the largely NO-dependent mechanism, PLM has the potential to be clinically useful in patients where NO bioavailability is recognized to be of significant clinical relevance, such as in patients with sepsis and septic shock.

Consequently, this study sought to evaluate PLM as an approach to assess NO-mediated vascular function in patients with sepsis and compare this method to the more traditional FMD test. Given the dysregulation of iNOS in septic states, we hypothesized that the measurements of PLM-induced hyperemia would be attenuated in patients with sepsis compared with controls. Furthermore, we hypothesized that PLM would yield results similar to FMD, but would be less technically challenging to perform and therefore more practical clinically.

METHODS

Subjects. Seventeen patients meeting the criteria for severe sepsis or septic shock were recruited from the medical intensive care units (ICUs) at the University of Utah Hospital and the George E. Weather Department of Veterans Affairs Medical Center. All patients >18 yr of age admitted to the medical ICU were screened within 24 h by the attending physician for severe sepsis or septic shock. Once referred to the study, sepsis inclusion criteria were verified by chart review. Criteria for severe sepsis and septic shock adhered to the 2012 Surviving Sepsis Campaign International Guidelines (9). Specifically, patients had to meet at least two of four systemic inflammatory response syndrome (SIRS) criteria (body temperature >38.3°C or <36°C, respiratory rate >20 breaths/min, heart rate >90 beats/min, or white blood cell count >12 × 10^3/μl or <4 × 10^3/μl), plus evidence of a suspected or confirmed infection. Severe sepsis was defined as meeting the SIRS criteria in addition to dysfunction of at least one organ due to hypoperfusion. Septic shock was defined as persistent sepsis-induced hypotension (systolic blood pressure <90 mmHg or mean arterial pressure <70 mmHg) despite adequate fluid resuscitation (at least 30 ml/kg of intravenous fluid administration) (9). All septic subjects received an acute physiology and chronic health evaluation (APACHE) II score, which is a well-validated composite score assessing the severity of illness in ICU patients (16). Exclusion criteria included refusal or inability to consent, >48 h since admission, preexisting severe liver disease (Child-Pugh grade C), chronic end-stage renal disease requiring hemodialysis, known cancer, organ transplantation, non-English speaker, pregnancy, active hemorrhage, cardiomyopathy with a left ventricular ejection fraction of <45%, and neuromuscular disease. Measurements were made on day 1 and, when possible, day 3 of hospitalization. Sixteen age and sex-matched volunteers were recruited from the community as controls. The controls were normally active without signs or symptoms of infection. Comorbid conditions, such as hypertension, hyperlipidemia, and coronary artery disease, were not exclusion criteria to better match the controls to the sepsis cohort. The study protocol was approved by the institutional review board (IRB) committees of both the University of Utah Hospital and the George E. Weather VA Medical Center, and prior to participation, written informed consent was obtained from the subject or the legally authorized surrogate in compliance with IRB requirements.

Brachial artery flow-mediated dilation. Following published guidelines for the performance of brachial artery FMD, subjects were positioned supine and a pneumatic cuff was placed on the upper arm near the elbow, distal to the site of the ultrasound Doppler probe (11). After a 10-min rest period, baseline measurements were made, and the arm cuff was then inflated to supersystolic pressure (250 mmHg) for 5 min. Full occlusion of the artery was verified by continuous ultrasound Doppler scanning during occlusion. The cuff was then deflated, and brachial artery diameter and blood velocity measurements were continuously recorded for 2 min after cuff release. Brachial artery diameter was measured off-line using automated edge detection software (Brachial Analyzer Medical Imaging Applications, Coralville, IA) (28). Relative and absolute FMD were calculated as the percent and the absolute change, respectively, from resting artery diameter to the largest diameter achieved during the 120 s of postinflation imaging. All ultrasound vessel lumen diameter measurements were evaluated during end diastole, which was verified by the R wave from the electrocardiogram signal.

Shear stress is considered to be the mechanism that stimulates the vascular endothelium and results in subsequent vasodilation (6). Since blood viscosity was not measured, shear rate, an adequate surrogate measure (5, 32), was calculated using the following equation: Shear rate (in s⁻¹) = 8Vmean (in cm/s)/vessel diameter (in cm). Cumulative reactive hyperemia (RH) area under the curve (AUC) from cuff release to peak brachial artery diameter was integrated using the trapezoidal rule and calculated as follows: \(2\frac{1}{2}(y_1 - y_0)/x_1 - x_0\). To normalize the vasodilatation for shear rate, FMD was divided by the cumulative shear rate (%/diameter s⁻¹) (33).

Passive leg movement (PLM). Subjects were moved into an upright sitting position for ~10 min before the start of the data collection and remained in this position throughout the entire protocol. The protocol consisted of 60 s of resting baseline data acquisition followed by a 2-min bout of passive leg extension. PLM was achieved by a member of the research team moving the subject’s lower leg through a range of motion, defined by 90° and 180° knee joint angles, at a rate of 1 Hz. Throughout the protocol, the nonmoving leg remained fully extended and supported. Real-time feedback to the investigator was provided by a metronome to maintain the cadence. The Doppler ultrasound was placed on the femoral artery distal to the inguinal ligament and proximal to the bifurcation of the deep and superficial femoral arteries. Before commencing, and throughout the protocol, subjects were encouraged to remain passive and resist any urge to assist with leg movement. To avoid a startle reflex and active resistance to the PLM, subjects were made aware that the assessment would start in the next minute, but to minimize the chance of an anticipatory response, they were also informed of exactly when movement would begin (37).

Measurements of arterial blood velocity and vessel diameter were performed in both the brachial artery FMD and PLM protocols with either a Logic 7 or a Logic e ultrasound system (General Electric Medical Systems, Milwaukee, WI). Both the Logic 7 and Logic e were equipped with linear array transducers operating at an imaging frequency of 9–14 MHz. Vessel diameter was determined at a perpendicular angle along the central axis of the scanned area. All blood velocity measurements were obtained with the probe appropriately positioned to maintain an insolation angle of 60° or less. The sample volume was maximized according to vessel size and was centered within the vessel on the basis of real-time ultrasound visualization. Mean velocity (Vmean) values (angle-corrected and intensity-weighted AUC) were automatically calculated using commercially available software (Logic 7 and Logic e). Using arterial diameter and Vmean, the blood flow in the brachial and femoral arteries was calculated as follows: Blood flow = Vmeanπ(vessel diameter)² × 60, where blood flow is in milliliters per minute.

Statistical analysis. Statistics were performed using commercially available software (SPSS, v. 17.0, Chicago, IL). An independent t-test (α < 0.05) was used to compare FMD between the septic and control groups. To adjust for differences in the baseline diameters between the two groups, ANCOVA was utilized as described by Atkinson et al. (3) to obtain an allometrically scaled FMD. As peripheral responses to PLM were transient, data from septic subjects and controls were only compared for the first 40 s. Before analysis all data were smoothed using a rolling 3-s average (12). Cumulative AUC was calculated as the summed second by second response during the first 40 s of passive.
Two-way repeated-measures ANOVA was used to determine significant differences between control and septic groups. When a significant main effect was observed, further Tukey post hoc analysis was performed. A Pearson’s product moment coefficient was calculated to evaluate for correlations between FMD, PLM, and APACHE II scores. All data are expressed as means ± SD except in the figures, where data are presented as means ± SE.

RESULTS

Subjects. As outlined in Fig. 1, a total of 91 patients were screened for inclusion in this study. Seventy-two patients met exclusion criteria. Written informed consent was obtained for 19 patients (from patient or authorized representative; see methods) to participate in the study, and 2 patients withdrew their consent prior to measurements taking place. Thus 17 patients underwent analysis with Doppler ultrasound. On day 1 of hospitalization, FMD testing was performed on all 17 patients while PLM was obtained for 13 patients. Two patients could not tolerate passive movement of the knee because of pain from osteoarthritis, while 1 patient had a soft tissue infection in the leg that prevented PLM measurements and 1 patient could not relax completely during passive movement. On day 3 of hospitalization, FMD and PLM were obtained for 6 and 5 patients, respectively. The mean time from admission to measurements on day 1 was 25 ± 13 h and 77 ± 15 h on day 3. Microbiology analyses were positive for 9 of the 17 subjects. Of the positive blood cultures, 5 grew gram-negative rods, 4 grew gram-positive cocci, and 1 had a positive respiratory viral panel for H1N1 influenza.

Subject characteristics are provided in Table 1. The mean age was 59 ± 14 yr and 59 ± 15 yr for septic subjects and controls, respectively. Males made up 59% and 56% of the subjects in the septic and control groups, respectively. There were statistically significant differences in body mass index (BMI) (31 ± 8 compared with 25 ± 4), heart rate (110 ± 23 beats/min compared with 74 ± 8 beats/min), respiratory rate (21 ± 5 breaths/min compared with 18 ± 2 breaths/min), temperature (37.3 ± 1.1°C compared with 37 ± 0.3°C), and white blood cell count (18 ± 9 × 10³/µl compared with 5 ± 1 × 10³/µl) between the septic and control groups, respectively (P < 0.05). There was no significant difference in the hemoglobin (13 ± 2.7 g/dl compared with 14.4 ± 0.9 g/dl) or mean arterial pressure (71 ± 18 mmHg compared with 78 ± 6 mmHg) between the sepsis and control groups. In the septic cohort, 70% had a smoking history, and 35% were still smoking prior to admission to the ICU, compared with 19% of the control group who were former smokers. Comorbid conditions in the septic patients included hypertension (65%), diabetes (41%), hyperlipidemia (29%), chronic obstructive pulmonary disease (18%), and coronary artery disease (18%). Comorbid conditions in the control group included hypertension (44%), hyperlipidemia (44%), diabetes (6%), and coronary artery disease (19%). In the septic group, initial blood lactate was 2.5 ± 2.3 mmol/l, pH was 7.37 ± 0.08, glucose was 135 ± 54 mg/dl, PaO₂ was 93 ± 13 mmHg, and the A-a gradient was 210 ± 132 mmHg. Upon inclusion into the study, 14 patients met criteria for severe sepsis, and 3 met criteria for septic shock and were on vasopressor medications. Other than sinus tachycardia, there were no heart arrhythmias. The average APACHE II and sequential organ failure assessment (SOFA) scores were 17 ± 7 and 6 ± 3, respectively. The Charlson comorbidity index was 3 ± 2 in the septic group compared with 2 ± 2 in the control group (P > 0.05). All patients who participated were followed up after 3 mo, and there were no deaths.

Vascular measurements. Percent FMD was 1.1 ± 1.7% compared with 6.8 ± 1.3% (P < 0.05) in septic and control subjects, respectively (Fig. 2A). There continued to be a significant difference in FMD when normalized for shear. FMD/shear was 0.022 ± 0.18%/s⁻¹ in the sepsis group compared with 0.26 ± 0.22%/s⁻¹ (P < 0.05) in the control subjects (Fig. 2B). Baseline brachial artery diameter in the septic group was 4.8 ± .25 mm compared with 4.0 ± .3 mm in the controls (P < 0.05). Additionally, brachial artery blood flow was significantly elevated (P < 0.05) in the septic group (165 ± 22 ml/min) compared with the control group (43 ± 9 ml/min). However, despite the differences in baseline measurements, there continued to be a significant difference in the allometri-
Femoral LBF in the patients at baseline was significantly lower than controls (196 ± 33 ml/min compared with 238 ± 20 ml/min, P < 0.05). PLM-induced peak blood flow (460 ± 40 ml/min compared with 685 ± 99 ml/min, P < 0.05), change in blood flow (defined as peak LBF - baseline LBF) (133 ± 28 ml/min compared with 482 ± 86 ml/min, respectively, P < 0.01), and blood flow AUC (15.7 ± 8 ml compared with 143 ± 33 ml, P < 0.01), were significantly attenuated in the patients with sepsis compared with the controls (Fig. 3). Of note, when the analysis of PLM-induced AUC was restricted to only those subjects that had comorbid conditions (n = 7 in each group), there continued to be a significant difference between the patients with sepsis and the controls (3 ± 7 ml compared with 146 ± 54 ml, P < 0.05).

There was a significant correlation between FMD and the APACHE II score (Pearson’s r = −0.77, P < 0.01) (Fig. 4). However, there was no correlation between PLM-induced blood flow AUC and APACHE II score (Pearson’s r = 0.11, P > 0.1), PLM-induced blood flow AUC and FMD (Pearson’s r = −0.15, P > 0.1), or PLM-induced change in blood flow and FMD (Pearson’s r = 0.004, P > 0.1) (Fig. 4).

On day 3 of hospitalization, percent FMD (6 subjects) was 3.7 ± 1.4%, which was not significantly different from the control or day 1 measurements. Because of patient discharges from the ICU, PLM was obtained for 5 subjects on day 3. Baseline femoral LBF, PLM-induced peak blood flow, change in blood flow, and blood flow AUC were 181 ± 52 ml/min, 268 ± 34 ml/min, 97 ± 26 ml/min, and 11.7 ± 25.3, respectively. There was no statistical difference in the baseline blood

<table>
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<th>Characteristics</th>
<th>Sepsis (n = 17)</th>
<th>Controls (n = 16)</th>
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<td>Age, yr</td>
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<tr>
<td>Sex, n (%)</td>
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<td>Chinese</td>
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<td>BMI, kg/m²</td>
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<td>25 ± 4</td>
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<td>Comorbidities, n (%)</td>
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<td>MAP, mmHg</td>
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<td>Heart rate, beats/min</td>
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<td>Respiratory rate, breaths/min</td>
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<td>Severe sepsis, n (%)</td>
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<td>Shock, n (%)</td>
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<td>WBC, ×10⁹/ml</td>
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<td>PaO₂, mmHg</td>
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<td>A-a gradient, mmHg</td>
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Data are presented as means ± SD. BMI, body mass index; COPD, chronic obstructive pulmonary disease; CAD, coronary artery disease; MAP, mean arterial pressure. *Significantly different from controls (P < 0.05).
Fig. 3. Passive leg movement (PLM)-induced hyperemia in patients with severe sepsis and septic shock ($n = 13$) and controls ($n = 16$) expressed as (A) the blood flow response (inset is the quantification of the response as area under the curve (AUC) for the first 40 s of PLM) and (B) the change in the blood flow (inset is the AUC analysis). Data presented as means $\pm$ SE. *Significantly different from the controls ($P < 0.05$).
flow on day 3 in the septic group compared with controls, but the PLM-induced peak blood flow, change in blood flow, and blood flow AUC were each still significantly reduced compared with controls ($P < 0.05$). There was no statistical difference in these indices of PLM measurements between days 1 and 3.

**DISCUSSION**

This study sought to assess PLM as a method to evaluate NO-mediated vascular function in subjects with severe sepsis or septic shock and compare it with the results of FMD testing. Consistent with the previously published literature, FMD was decreased in septic subjects when compared with healthy controls. Likewise, all indices of the PLM-induced hyperemic response were significantly attenuated in patients with sepsis compared with controls. Taken together, these findings support the concept that vascular function in septic patients is attenuated, and PLM appears to be a novel and feasible approach to assess NO-mediated vascular function in this patient population.

**Flow-mediated dilation.** The usefulness of FMD as a tool to measure NO-mediated vascular dysfunction in subjects with severe sepsis or septic shock and compare it with the results of FMD testing. Consistent with the previously published literature, FMD was decreased in septic subjects when compared with healthy controls. Likewise, all indices of the PLM-induced hyperemic response were significantly attenuated in patients with sepsis compared with controls. Taken together, these findings support the concept that vascular function in septic patients is attenuated, and PLM appears to be a novel and feasible approach to assess NO-mediated vascular function in this patient population.

Fig. 4. A–D: relationships between the indices of the passive leg movement (PLM) assessment, the brachial artery flow-mediated vasodilation (FMD) test, and severity of disease (APACHE II score) in patients with severe sepsis and septic shock.
inflammatory cascade in sepsis (2), but the potential link with altered FMD in this population has yet to be explored.

**Passive leg movement.** This study aimed to determine if PLM is a potentially useful tool to measure NO-mediated vascular function in sepsis. Here, all measures of the PLM-induced hyperemic response were significantly attenuated in the patients compared with controls. Although PLM was only obtained for 13 of the 17 subjects because of osteoarthritis or the inability to completely relax during the passive movement, PLM has several advantages over FMD. Like FMD, PLM is noninvasive, but it is technically easier to perform. Specifically, the femoral artery, because of the anatomy and size of the vessel, is easier to simultaneously image and insone with Doppler ultrasound than the more superficial and smaller brachial artery. Additionally, unlike FMD, PLM does not require precise measurements of arterial diameter as there is not a measurable vasodilation in the femoral artery during PLM (37). Finally, PLM closely reflects NO-mediated vascular function and is thought to be largely regulated by NO-mediated pathways. As mentioned, Trinity et al. (37) as well as Mortensen et al. (27) have both found a 70–80% reduction in LBF during PLM with the concomitant infusion of L-NMMA, a nonspecific NOS inhibitor. Even though PLM may be limited somewhat by the ability of the subject to tolerate and cooperate with passive movement of the leg, given the technical advantages of obtaining and analyzing measurements compared with FMD, PLM appears to be a more rapid and efficient method with which to assess NO-mediated vasodilatory capacity.

Somewhat surprisingly, there was no relationship between the PLM-induced blood flow response (AUC) and FMD \( (r = -0.15, P = 0.62, \text{Fig. 4C}) \) or the PLM-induced change in peak blood flow and FMD \( (r = 0.004, P > 0.1, \text{Fig. 4D}) \). Additionally, unlike FMD, there was no correlation between PLM and APACHE II scores \( (r = 0.11, P = 0.72, \text{Fig. 4B}) \). This is an interesting observation and may reflect the fact that FMD may be a less-specific marker of NO bioavailability than PLM because an FMD test likely incorporates much more than just the NO-dependent vasodilation pathway, such as EDHF or prostacyclins (31, 41). Thus the correlation between FMD and severity of illness might reflect that FMD also acts as a surrogate marker of a broader range of the inflammatory dysregulation in sepsis, whereas PLM may not.

**The role of iNOS and eNOS in sepsis.** Dysregulated vasodilation is one of the main mechanisms leading to hypotension, hypoperfusion, tissue injury, and MODS in patients with sepsis. This dysregulation of vascular function remains a potential target for treatment in septic shock (30, 34). The \( L \)-arginine NO pathway is one of the primary mechanisms by which both this dysregulation and vasodilation occur (7, 26). While both eNOS and iNOS activate the \( L \)-arginine NO pathway, they do so by different mechanisms. The action of eNOS is mediated by a calcium/calmodulin cascade triggered by the shear stress of blood flowing across the endothelium, and vascular dysfunction can be a consequence of a disruption of this pathway (33). In sepsis, vasodilation and subsequent microvascular and macrovascular complications are associated with dysregulation of iNOS (22). Specifically, through toxin-mediated upregulation of iNOS, via TNF\( \alpha \), interleukin-1, interferon, as well as other endotoxin and exotoxin mediators, vascular smooth muscle cells are saturated with NO, leading to vasodilation and a loss of vascular tone (23). This results in hypotension, which then leads to MODS (18).

While iNOS is upregulated in septic states, work carried out by Liu et al. (19) suggests that eNOS is downregulated in sepsis. Indeed, Liu et al. (19) revealed that the injection of lipopolysaccharide, a gram-negative bacteria endotoxin, into rats decreased eNOS mRNA expression in the lung, heart, and aorta. Additionally, the authors reported a parallel upregulation of iNOS mRNA that was attenuated with the addition of the glucocorticoid dexamethasone. While the exact mechanism leading to the downregulation of eNOS is not entirely clear, iNOS appears to be upregulated via a glucocorticoid sensitive pathway (19). Combined, the effects of NO saturation from iNOS upregulation and eNOS downregulation are the likely causes of the relative vascular insensitivity to shear stress and the likely mechanism behind the decreased FMD and PLM responses that were documented in this study. Of note, consistent with this proposed mechanism of vasodilation in sepsis, the baseline brachial artery diameter was significantly larger, and baseline blood flows significantly greater, in the septic group compared with the control group in the current study. However, it is currently unclear if FMD or PLM are affected by these alternate pathways of endothelial dysfunction, and this deserves further study.

**Longitudinal vascular function assessment in sepsis.** In an exploratory longitudinal component of this study, the FMD and PLM responses of a small subset of subjects were measured again on day 3 of their ICU stay. We chose 3 days as the average length of stay in the ICUs, in which we studied the patients, was \(-3\) days; thus measurements could, theoretically, be performed twice. By day 3 there was an improvement in the APACHE II score, as well as normalization of lactate acid levels in all subjects. Though the sample size was small, there was no difference in FMD on day 3 in the sepsis group compared with controls. Additionally, there was also no significant difference in the baseline femoral artery blood flow in the sepsis group on day 3 compared with controls. However, PLM-induced peak blood flow and AUC were still attenuated compared with the controls. These PLM data reveal that while baseline blood flow returned to baseline comparable to controls after 3 days, likely reflecting a normalization of cardiac output as the inflammatory response resolved, there continued to be a significant attenuation of the hyperemic response and NO-mediated vascular dysfunction. The differing findings with FMD and PLM may be due to the greater sensitivity of PLM for detecting NO-mediated vascular dysfunction in this population when compared with FMD.

**Implications.** Questions remain regarding optimal management of sepsis, especially beyond the initial 6 h of presentation, and research is ongoing regarding optimizing earlier diagnosis and treatment (17). Complicating this work is the recognition that the pathophysiology of sepsis is complex and diverse. Therefore it is possible that only subsets of the general population with this condition respond to any given treatment at any given time. Methods such as FMD or PLM show promise as tools with which to better understand sepsis and assess, in vivo, NO bioavailability and vascular function in this disease. For example, even though dysregulation of NOS is a main contributor to vasodilation in sepsis, a large randomized controlled study found that nonselective inhibition of NOS did not improve mortality in patients with sepsis (20). The reasons for
this finding remain unclear but might include use of nonselective rather than selective NOS inhibitors, dose of the medication, timing of the medication, or other confounding factors. Furthermore, proper patient selection for inclusion in studies treating sepsis remains a significant challenge. Real-time monitoring of NO-mediated vascular physiology, with FMD and PLM functioning as an in vivo NO bioassay, might offer an additional tool to overcome the aforementioned limitations and more accurately identify the subset of patients with sepsis that would be candidates for NOS inhibition. Additionally, FMD and PLM might be able to quantify the extent of disease, as well as the physiologic response to potential interventions, and could lead to more personalized management of sepsis and improved outcomes.

**Experimental considerations.** Because of the design of this study and the subject cohort, there are a few potential limitations that should be acknowledged. Given the hemodynamic instability of the septic subjects, we did not assess the effects of L-NMMA on the PLM response. However, as documented in previous studies, PLM is thought to be 70–80% NO-mediated (27, 37). Similarly, we did not administer nitrates to assess for endothelial-independent vasodilation. Furthermore, quantifying the effects of vasoactive drugs, which three of the patients were receiving, is difficult. Additionally, compared with previous studies looking at FMD in sepsis, there were no mortalities in this study compared with 33%, 17%, and 4% in the Becker et al., Wexler et al., and Vaudo et al. studies, respectively (4, 38, 39). While mortality in sepsis has decreased over the last 10 yr, this might, again, reflect the relatively small sample size assessed in the current study or could highlight differences in patient population or clinical practice between the institutes (14). The subjects in this study had a SOFA score comparable with that of Vaudo et al. (38); however, the mean APACHE II score in this study was 17, whereas in the Becker et al. and Wexler et al. studies they were both 23. The baseline comorbidity as measured by the Charlson index was comparable between each of the studies. Finally, the pathophysiology of sepsis is complex, with multiple physiologic derangements. The individual impact of these derangements on FMD and PLM is currently unknown.

**Conclusions.** As documented in previous studies, FMD is decreased in sepsis patients compared with controls matched for comorbid conditions and correlates with severity of disease. PLM appears to be a feasible and relatively simple tool with which to assess NO-mediated vascular dysfunction in patients with severe sepsis or septic shock. Further research is needed to determine the relationship between the results of PLM testing and clinical outcomes, such as organ dysfunction or mortality.

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