

# Endotoxin Has an Indirect Vasodilatory Effect on Isolated Human Skeletal Muscle Arterioles

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**Background:** Septic shock and its effects are a major cause of mortality in the intensive care environment. The exact effect and mechanism of endotoxin has yet to be fully described. With a better understanding of this process, better clinical tools could be developed to treat these patients.

**Hypothesis:** Endotoxin has no direct effect on human skeletal muscle microvasculature and requires the release of an endothelial-derived factor to produce the vasodilation seen in gram-negative sepsis.

**Design:** Benchtop research using an isolated arteriole model with controlled exposure to endotoxin.

**Setting:** University medical center.

**Methods:** First-order arterioles (approximately 150- $\mu$ m diameter) were isolated from human cremasteric muscles and pressurized to physiologic levels before exposure to an endotoxin-rich effluent with and without an upstream conduit vessel (superficial epigastric vein). The vasodilatory effect was measured with videomicroscopy and compared with control samples.

**Main Outcome Measures:** Mean vessel diameter and percentage of loss in tone.

**Results:** When compared with controls, the isolated arteriole had no significant response when exposed to endotoxin alone (3.5% change in basal tone). When the endotoxin was allowed to pass over an upstream conduit vessel, the arteriole showed marked dilation (14.2% loss of basal tone).

**Conclusions:** This study demonstrates that endotoxin has no direct vasodilatory effect on human skeletal muscle arterioles, but it is the release of an endothelial factor from the upstream conduit vessels that produces the loss of tone in the microvasculature. Further research is ongoing to characterize the factors involved (nuclear factor- $\kappa$ B, tumor necrosis factor  $\alpha$ , and interleukin 6) for possible clinical intervention (antioxidants, cyclosporine, and nitric oxide synthase inhibitors).

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**E**VEN IN THE FACE OF SUBSTANTIAL technological advancements in the critical care setting, sepsis is still the major cause of morbidity and mortality. Septic shock syndrome is characterized by organ hypoperfusion, cardiac dysfunction, increased vascular (venule) permeability, and hypotension caused by a profound reduction in systemic vascular resistance. It is believed that the initiating event in the patient with septicemia is the release of lipopolysaccharide from the cell membranes of gram-negative organisms.

## See Invited Critique at end of article

Previous studies using an isolated arteriole model to limit confounding factors have shown that endotoxin has an indirect vasodilatory effect on skeletal muscle

arterioles in a rat model.<sup>1-3</sup> Specifically, these studies have shown that an unknown factor, released from an upstream conduit, causes a vasodilation of the peripheral vascular resistance vessels.<sup>1-4</sup>

The goals of this study are to evaluate this model in the human setting and to investigate this indirect effect of endotoxin on the peripheral microvasculature in a comparable human model. By demonstrating this indirect phenomenon in a human model, it is our hope to be able to further classify this substance and delineate the true mechanism of endotoxin-induced circulatory dysfunction.

## METHODS

Protocols were approved by the investigational review board at Eastern Virginia Medical School, Norfolk, as protocol IRB 00-08-EX-0384. Necessary patient con-

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sents were already in use that included experimentation on any discarded tissue.

Discarded cremaster muscle from inguinal surgery was used for arteriole harvesting. Harvested inferior superficial epigastric vein was used as an upstream conduit vessel. Consent was included in the standard consent form describing experimental use of discarded tissue.

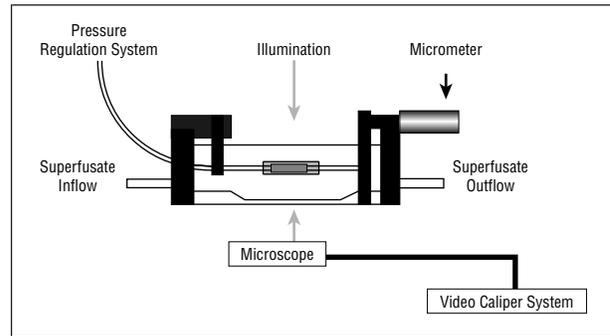
### PREPARATION AND ISOLATION OF THE ARTERIOLE

Discarded cremaster muscle and vein were harvested at the time of the operation and stored in cold (4°C) Krebs-HEPES [bicarbonate-4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (10µM) buffer solution (pH 7.4). The cremaster muscle was then flattened out and an appropriate-sized arteriole with no branch points was removed by microdissection. The dissection lasted, on average, 15 minutes. The vessel was quickly transferred to a vessel chamber (Living Systems Instrumentation, Burlington, Vt), cannulated with micropipettes, and secured with a 10-0 monofilament suture. The vessel chamber was then moved to the platform of an inverted microscope (Nikon Instech, Kanagawa, Japan) that was connected to a charged-coupled device video camera and high-resolution monitor. A calibrated pressure servomechanism (Living Systems Instrumentation) was used to gradually pressurize the vessel to 90 mm Hg in the absence of intraluminal flow (**Figure 1**). The vessel was slowly warmed to 34°C by continual infusion of warmed Krebs-HEPES buffer solution at a rate of 4 mL/min. The buffer was bubbled with a gas mixture (95% nitrogen/5% carbon dioxide) within the reservoir, resulting in a PO<sub>2</sub> of no less than 60 mm Hg at the level of the vessel. A total of 60 minutes was allowed for the vessel to achieve spontaneous basal tone under these conditions. For inclusion in the experiment, the vessel had to be free of all leaks and demonstrate spontaneous tone of a minimum amount of 12% when compared with 3µM acetylcholine-induced maximal dilation as well as transient reactivity to 1µM phenylephrine solution.

### ENDOTOXIN EXPOSURE

The harvested vein was opened up along its long axis and secured with monofilament in a small flow-through chamber connected in series to the arteriolar setup such that superfusate (buffer with or without endotoxin) flowed over the vein segment and then onto the arteriole in the tissue bath of the vessel chamber. Internal diameters of the cannulated arterioles were measured with videomicroscopy and videocalipers (Living Systems Instrumentation).

After adequate equilibration time, 2.5 µg/mL of *Salmonella enteridis* endotoxin (BACTO lipopolysaccharides; Difco Labs, Detroit, Mich) was added to the buffer for 120 minutes and vessel diameters were recorded every 15 minutes for a total of 120 minutes. The 2-hour time limit was a value observed during previous vein conduit experiments done at this facility. Group 1 (n=6) samples were a control group exposed to plain buffer only, group 2 (n=6) samples were arterioles exposed to en-



**Figure 1.** A schematic drawing of the pressurized vessel setup. Microscope (Nikon Instech, Kanagawa, Japan); micrometer, platform, and pressure servomechanism (Living Systems Instrumentation, Burlington, Vt).

dotoxin in the absence of the upstream vein, and group 3 (n=6) samples were endotoxin exposure to both vein and arteriole in series. After adequate plain buffer wash-out, reactivity to 1 µM of phenylephrine followed by 3 µM acetylcholine was confirmed, demonstrating the viability of the vessel and its inherent ability to constrict and dilate as well as its baseline maximal dilation.

### STATISTICS ANALYSIS

Results are expressed as diameter at 120 minutes and mean (SEM) percentage of tone. Basal tone was calculated as the percentage of the maximal diameter of the vessel achieved following the 3µM acetylcholine treatment described in the "Endotoxin Exposure" subsection of the "Methods" section to show endothelial independent dilation. Statistical significance was determined by analysis of variance analysis expressed as the F ratio with significance presumed at  $P < .05$ .

## RESULTS

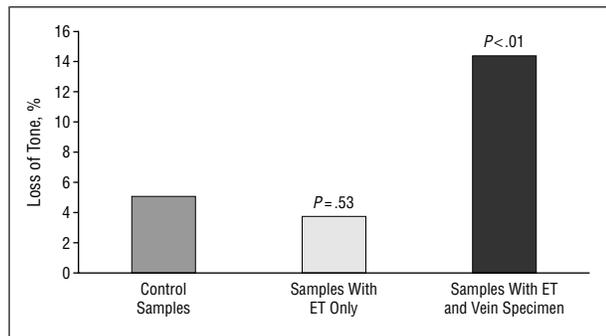
### ENDOTOXIN EXPOSURE TO ARTERIOLE ONLY

Under basal conditions, the isolated, cannulated arterioles had a mean (SEM) luminal diameter of 172 to 190 (3) µm. Addition of endotoxin (2.5 µg/mL) for 2 hours to the superfusate caused no significant loss in the basal tone (4.2% [1.3%];  $F = 1.1, P > .05$ ). Following this, application of a maximally effective concentration of the endothelial-dependent vasodilator acetylcholine (3 µM) to the isolated arteriole resulted in dilation to 214 to 246 (3) µm on which the basal tone was calculated.

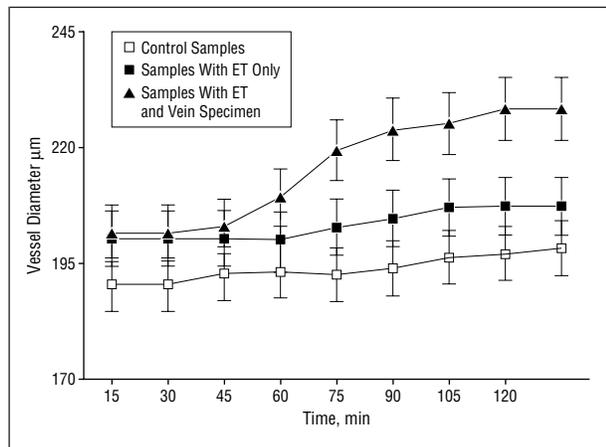
### CONDUIT VEIN SEGMENT PLACED IN SERIES WITH ISOLATED ARTERIOLE

The conduit vein was placed upstream in series with the cannulated arteriole so that the Krebs-HEPES buffer was passed over it then continued on to bathe the arteriole in effluent. After 2 hours of superfusate, the control series showed no significant loss in basal tone (5.4% [1.8%];  $F = 1.1, P > .05, n = 6$ ) (**Figure 2**).

Following the stabilization period of 1 hour, endotoxin (2.5 µg/mL) was added to the superfusate. Vessel diameter started increasing after 45 minutes to a cutoff



**Figure 2.** Percentage of loss in basal tone of each group compared with control samples. ET indicates endotoxin.



**Figure 3.** Normalized vessel diameter vs time during endotoxin (ET) exposure.

of 120 minutes. At 60 minutes, there was a 3.7% (0.8%) loss in basal tone (201-210 [3]  $\mu\text{m}$ ). This difference became statistically significant at the 2-hour mark which was chosen as a cutoff based on previous vein conduit experiments, with average diameters of 228  $\mu\text{m}$  resulting in a basal tone loss of 13.4% (1.4%) ( $F = 20.2$ ,  $P < .01$ ,  $n = 6$ ) (Figure 2 and **Figure 3**). The effect continued for longer than 20 minutes despite the removal of the vein from the system.

## COMMENT

Septic shock remains a complex process of high morbidity and mortality. Endotoxin plays a significant role in the pathogenesis of many processes associated with sepsis, particularly hypotension.<sup>5</sup> This study is an extension of the previous work in animal physiology that demonstrated an indirect effect of endotoxin on the skeletal muscle arteriolar system.<sup>1-4</sup> What has been documented in the rat model is demonstrated in a human model.

To our knowledge, this is the first successful attempt at illustrating the physiologic effects of endotoxin on the resistance vasculature of the human patient. Samples from pericardium (connective tissue by nature) along with a segment of internal mammary artery were harvested during standard coronary artery bypass grafting procedures showing no vasodilatory effect using this model. Skeletal muscle was used because a ma-

ior portion of total peripheral vasculature resistance resides in this tissue of enormous surface area. It is apparent from these data that the toxin itself does not directly induce vasodilation in the microvasculature, but rather it requires a conduit vessel to possibly release factors that, in turn, will dilate the arteriolar bed.

The isolated arteriolar setup allows for system integrity reliability and the control of outside effects. Also, it allows for a level of basal tone, albeit less than the rat model, that is impossible in studies using larger conduit vessels such as aortic rings or femoral vessel preparations requiring precontraction. In comparison to the rat model, which uses an identical method, the human vessels gain less inherent tone (18%-24% [1.9%]) compared with 35%-42% [4.3%]). This effect is likely because of the excessive handling of tissue during the hernia dissection and subsequent prolonged transport times. Overall, the average arteriole diameter is larger in this human model, secondary to the inconsistency of tissue samples and technical limitations of the dissection when compared with the rat model.

Continued research using the rat isolated vessel model is warranted to specifically identify the exact mediators that produce the profound vasodilation and pressor resistance seen in the hypotension in the endotoxic shock patient. Possible inhibitors, such as nitric oxide and nuclear factor- $\kappa\text{B}$ , are actively being investigated in this laboratory.

## CONCLUSIONS

To our knowledge, this study is the first of its kind to reproduce the results of a rat model of endotoxin's indirect effect on the peripheral microvasculature in the human. The vasodilatory factor(s) produced by the upstream conduit vessel in significant quantities is capable of eliciting arteriolar vasodilation that is reflected as loss of peripheral vascular resistance in the critical care patient afflicted with endotoxic sepsis. Further work is being carried out to isolate the responsible factors that are being released from the upstream conduit vessel.

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