Experimental Human Endotoxemia Is Associated With Depression of Load-Independent Contractility Indices*  
Prevention by the Lipid A Analogue E5531

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**Objective:** To evaluate the efficacy of a novel lipopolysaccharide (LPS) antagonist, E5531, in blocking LPS-induced cardiac responses including myocardial depression (as assessed by relatively load-independent echocardiographic indices of contractility) in a human model of experimental endotoxemia.

**Design:** Randomized, prospective, placebo-controlled, double-blind trial.

**Setting:** ICU procedure room.

**Participants:** Thirty-two healthy, male volunteers.

**Interventions:** Administration of LPS (4 ng/kg) and either a placebo or one of four sequential doses of E5531 (100 μg, 250 μg, 500 μg, or 1,000 μg) followed by volumetric echocardiography before and during 4-L saline solution infusion (3 L over 3 h, followed by 1 L over 2 h).

**Results:** In addition to the generation of a hyperdynamic circulation throughout the study period, administration of LPS resulted in a biphasic contractility response. Ejection fraction (EF), rate-corrected mean velocity of circumferential fiber shortening (Vcfc), peak systolic BP (SBP)/end-systolic volume index (ESVI) ratio, and end-systolic pressure (Pes)/ESVI ratio increased at the 3-h post-LPS assessment, compared to a control group of subjects receiving only similar amounts of saline solution (minimum p < 0.001). End-systolic myocardial wall stress (σes)/ESVI ratio, one of the most load independent of the contractility indices, was unchanged. At 5 h after endotoxin, EF, Vcfc, SBP/ESVI, Pes/ESVI, and σes/ESVI were all decreased (minimum p < 0.01), indicating myocardial depression. When present, early (3 h after LPS), apparent enhancement of myocardial contractility and later (5 h after LPS) myocardial depression were substantially blunted by administration of E5531 (minimum p < 0.025), typically in a concentration-dependent manner.

**Conclusions:** Endotoxin generates significant myocardial depression when measured using highly load-independent indices of cardiac contractility. E5531 is a potent inhibitor of the early hyperdynamic cardiovascular and later myocardial depression response seen in experimental human endotoxemia.

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**Key words:** endotoxin; experimental endotoxemia; lipid A; lipopolysaccharide antagonist; myocardial depression; sepsis

**Abbreviations:** CI = cardiac index; EDVI = end-diastolic volume index; EF = ejection fraction; ESVI = end-systolic volume index; HR = heart rate; LPS = lipopolysaccharide; MAP = mean arterial pressure; σes = end-systolic wall stress; Pes = end-systolic pressure; SBP = systolic BP; SV = stroke volume; SVR = systemic vascular resistance; TVR = total vascular resistance; Vcfc = rate-corrected mean velocity of circumferential fiber shortening

Although an overtly decreased cardiac output is uncommon, most patients with severe sepsis and septic shock exhibit a significant degree of myocardial depression.1,2 Human septic myocardial depression has been characterized by reversible reduction of preload-dependent responses (flattening of the Frank-Starling curve),3 reduced inotropic responsiveness to catecholamine stimulation,4 and by biventricular dilation and depression of the ejection fraction (EF).2 In addition, peak systolic BP (SBP)/end-systolic volume index (ESVI) ratio, a relatively load-independent index of cardiac contractility, has also been shown to be reversely depressed.5

Administration of small reference doses of lipopolysaccharide (LPS) to healthy human volunteers is a safe and well-accepted method of modeling the cardiovascular manifestations of sepsis and septic shock. Suffredini and coworkers6 examined LPS-induced myocardial dysfunction following of 4 ng/kg IV LPS administration in human volunteers moni-
tored over an 8-h period using a pulmonary artery catheter and radionuclide cineangiography. This model qualitatively mimics the hyperdynamic circulatory pattern of spontaneous septic shock with an early (1 to 3 h after LPS) increase in cardiac index (CI) and fall in systemic vascular resistance (SVR) that persists to at least 6 to 12 h.6,7 Myocardial contractility parameters including EF are typically increased initially (1 to 3 h after LPS). Despite a persistence of the hyperdynamic circulation, decreased myocardial contractility, as evidenced by a decrease in EF and ventricular dilatation (relative to volunteers given only similar amounts of saline solution), is present by 5 h after LPS. In addition, test subjects also exhibited a late decrease in peak SBP/ESVI ratio as has also been documented in human sepsis.5,6

We have published a study7 demonstrating that increasing doses of E5531, a synthetic structural analog and competitive inhibitor of the toxic lipid A component of LPS, generated a dose-dependent decrease in basic clinical (subjective symptoms, temperature, heart rate [HR], mean arterial pressure [MAP]), invasive hemodynamic (cardiac output, temperature, heart rate [HR], mean arterial pressure [MAP]), inflammatory (WBC count, tumor necrosis factor-α, interleukin-6, C reactive protein) responses to experimental endotoxemia. In this study, we extend our observations from the same subject group to provide a detailed analysis of cardiovascular function (beyond that examined in the previous publication) during human endotoxia and E5531 therapy.

In these studies, LPS infusion was utilized to model the cardiovascular manifestations of severe sepsis and septic shock. Volumetric echocardiography with phonocardiography was used to confirm the presence of LPS-induced cardiovascular dysfunction (including myocardial depression) using more robust measures of afterload (end-systolic pressure [Pes] and end-systolic wall stress [σes]) and relatively load-independent measures of myocardial contractility (Pes/ESVI ratio, σes, σes/ESVI ratio, and rate-corrected mean velocity of circumferential fiber shortening [Vcfc]). In addition, the ability of E5531 to blunt these responses was evaluated. Several of the echocardiographically derived parameters examined (Pes, σes, Pes/ESVI, σes/ESVI, and Vcfc) have never been previously used to examine cardiovascular dysfunction in either clinical sepsis or human models of endotoxia.

**Materials and Methods**

This study received Institutional Review Board approval at Rush-Presbyterian-St. Luke’s Medical Center. Thirty-two healthy men aged 18 to 38 years volunteered and gave informed consent for this study. Detailed clinical research methods are available in a previous publication.7 The study had four sequential blinded dosing groups of E5531 (100 μg, 250 μg, 500 μg, or 1,000 μg) with eight participants per group. Six members in a group were randomly assigned to receive a given dose of E5531, while the other two members received a placebo, resulting in four active drug groups (n = 6 per group) and a placebo control group (n = 8). Subjects and investigators were blinded with regard to dosing of the active compound or placebo. All subjects received LPS (United States Pharmacopoeia reference standard endotoxin from Escherichia coli 0113)9 dosed at 4 ng/kg body weight.

A series of echocardiograms was performed on all subjects on 2 separate days. On the first study day, 4 L of dextrose 5% saline solution 0.9% (3 L over 3 h followed by 1 L over 2 h) was infused without dosing of either LPS or E5531. Volumetric echocardiograms were obtained prior to initiation of infusion and both 3 h (after 3 L of fluid administration) and 5 h (after 4 L of fluid administration) after infusion initiation. The data obtained from these studies were utilized to generate a control group (day 1 saline control) that represented the aggregate response of subjects to saline solution without LPS and E5531.

The next day (study day), E5531 or placebo in 500 mL of 5% dextrose solution was infused over 30 min. Half way through the infusion, LPS (4 ng/kg) was administered by IV bolus over 1 min. At the end of the E5531 or placebo infusion, a dextrose 5% saline solution 0.9% infusion was again initiated. Fluid was infused in a manner identical to day 1 (3 L over 3 h followed by 1 L over 2 h). Echocardiograms were again obtained at baseline and both 3 h and 5 h into the study.

**Hemodynamics**

Standard echocardiographic views were obtained including parasternal long- and short-axis, apical four-chamber views, and Doppler outflow across the aortic valve at each study time point. For each study, simultaneous recordings of left ventricular echocardiography, phonocardiography, carotid pulse tracings, ECG, and SBP/diastolic BP were made. A detailed methodology of hemodynamic and echocardiographic measurements and methodology is available in the on-line supplement of another publication.9 Echocardiograms, phonocardiograms, and carotid pulse tracings were read by a single, highly experienced echocardiographer blinded to the subject and study sequence. A previous study10 has demonstrated that mean changes of > 2% in end-diastolic volume, 5% in end-systolic volume, and 2% in left ventricular EF in groups of subjects of comparable size to this study represent clinically significant alterations. Accuracy of ventricular volumes was internally validated by comparing stroke volume (SVs) derived from integration of the flow velocity across the aortic valve and subtraction of the end-systolic volume from the end-diastolic volume.

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CHEST / 126 / 3 / SEPTEMBER, 2004 861
Vcfc, normalized to end-diastolic dimension and HR, was calculated by dividing the fractional shortening by the rate-corrected ejection time.\textsuperscript{11} σes was calculated as described by Grossman et al.\textsuperscript{12}

\[
σes = \frac{(1.35)(Pes)(Des)}{(4)(hes)(1 + (hes/Des))}
\]

where σes is in grams per square centimeter, Pes is in millimeters of mercury, Des is the left ventricular internal dimension (centimeters), and hes is the posterior wall thickness (centimeters), each at end-systole. The value 1.35 is a conversion factor (millimeters of mercury to grams per square centimeter), and 4 is a geometric factor that derives from conversion of radius to internal dimension. Indices of contractility used in addition to EF and Vcfc were SBP/ESVI, Pes/ESVI, and σes/ESVI.\textsuperscript{13-15}

Statistical Analysis

Hemodynamic and echocardiographic parameters at 3 h and 5 h after study initiation were indexed as a percentage of baseline values on each study day. Subject responses to LPS and increasing doses of E5531 at both time points on the second study day were analyzed by mixed-model analysis of variance to determine whether group-dependent differences existed. After a Bonferroni correction to adjust for analysis at two time points, an α score ≤ 0.025 was considered significant. If group-dependent effects were noted, data were further analyzed by linear contrast analysis to determine if stepwise, dose-dependent responses were present (α = 0.05).

In order to determine the test dose of E5531 that blocked LPS effects, cardiovascular responses to individual E5531 doses on the second study day were compared to responses from all 32 subjects at equivalent time points on the first study day when only saline solution was administered (day 1 saline solution control). Significance was assessed using a Student two-tailed t test. Use of a Bonferroni correction for multiple comparisons yielded an α = 0.01. These first study day aggregate responses to saline solution loading (without LPS/E5531) were used as the primary comparison group because our previous work has demonstrated significant cardiovascular responses to saline solution loading alone.\textsuperscript{9} For purposes of comparison, all figures include both the first study day aggregate saline solution without LPS/E5531 response for all 32 subjects (day 1 saline solution control) that was not included in the mixed-model analysis of variance, and the second study day sequential E5531 dosing groups including the placebo control group that received LPS but no E5531 (placebo control).

Additional analyses involving paired-sample t tests of hemodynamic variables at 3 h and 5 h after LPS infusion compared to pre-LPS baseline for the placebo group receiving LPS without E5531 on the second study day are shown in Table 1. Bonferroni correction for comparisons at two time points for each variable mandates an α = 0.025 for significance.

Results

During the initial design of this clinical study, two distinct data sets were prospectively defined. HR, SV, cardiac output, and TVR (described as SVR) were collected and analyzed as part of a “conventional” hemodynamic data set. These results were published along with clinical symptoms, additional physiologic variables, and inflammatory markers in a previous article.\textsuperscript{7} The current article examines a distinct, prospectively defined group of “advanced” hemodynamic variables including ventricular volumes and additional indexes of cardiac contractility and afterload. The constitutive elements of these “advanced” hemodynamic parameters were collected and analyzed independently of the previously published data.\textsuperscript{7}

Vital Signs

The placebo control group (LPS without E5531) experienced a monophasic increase in HR (maximal at 3 h after LPS) and decrease in MAP (significant only at 5 h after LPS) [Table 1, Fig 1, top, A, and middle, B]. Pulse pressure was increased substan-

### Table 1—Comparison of 3-h and 5-h Post-LPS Hemodynamic Values to Baseline (Pre-LPS) in the Placebo (LPS Only) Group*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline (Pre-LPS)</th>
<th>3-h Post-LPS</th>
<th>5-h Post-LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>61 ± 3</td>
<td>105 ± 5 (&lt;0.001)</td>
<td>100 ± 4 (&lt;0.0001)</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>82 ± 2</td>
<td>81 ± 3 (NS)</td>
<td>70 ± 3 (&lt;0.0001)</td>
</tr>
<tr>
<td>Pulse pressure, mm Hg</td>
<td>67 ± 3</td>
<td>67 ± 3 (&lt;0.0001)</td>
<td>56 ± 3 (0.0241)</td>
</tr>
<tr>
<td>CI, L/min/m²</td>
<td>2.9 ± 0.2</td>
<td>5.3 ± 0.3 (&lt;0.0001)</td>
<td>4.4 ± 0.2 (&lt;0.0001)</td>
</tr>
<tr>
<td>SV index, mL/min/m²</td>
<td>48 ± 2</td>
<td>50 ± 2 (0.0044)</td>
<td>45 ± 1 (0.0069)</td>
</tr>
<tr>
<td>EDVI, mL/m²</td>
<td>68 ± 1</td>
<td>63 ± 1 (0.0003)</td>
<td>64 ± 1 (0.0002)</td>
</tr>
<tr>
<td>ESVI, mL/m²</td>
<td>21 ± 1</td>
<td>13 ± 1 (&lt;0.0001)</td>
<td>21 ± 2 (NS)</td>
</tr>
<tr>
<td>TVR, dynes/cm⁻²/m²</td>
<td>1,286 ± 81</td>
<td>697 ± 55 (&lt;0.0001)</td>
<td>726 ± 47 (&lt;0.0001)</td>
</tr>
<tr>
<td>Pes, mm Hg</td>
<td>96 ± 4</td>
<td>76 ± 5 (0.0060)</td>
<td>72 ± 6 (0.0030)</td>
</tr>
<tr>
<td>σes, g/cm²</td>
<td>49 ± 4</td>
<td>27 ± 3 (&lt;0.0001)</td>
<td>36 ± 3 (0.0008)</td>
</tr>
<tr>
<td>EF, %</td>
<td>69 ± 2</td>
<td>79 ± 2 (&lt;0.0001)</td>
<td>68 ± 2 (NS)</td>
</tr>
<tr>
<td>Vcfc, circ/s</td>
<td>1.16 ± 0.03</td>
<td>1.51 ± 0.04 (&lt;0.0001)</td>
<td>1.15 ± 0.05 (NS)</td>
</tr>
<tr>
<td>SBP/ESVI, mm Hg/mL/m²</td>
<td>5.7 ± 0.5</td>
<td>10.7 ± 0.9 (0.0001)</td>
<td>5.5 ± 0.5 (NS)</td>
</tr>
<tr>
<td>Pes/ESVI, mm Hg/mL/m²</td>
<td>4.7 ± 0.4</td>
<td>6.1 ± 0.6 (0.0012)</td>
<td>3.7 ± 0.6 (0.0218)</td>
</tr>
<tr>
<td>σes/ESVI, g/cm²/mL/m²</td>
<td>2.3 ± 0.1</td>
<td>2.1 ± 0.2 (NS)</td>
<td>1.8 ± 0.1 (0.0160)</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SE (paired-sample p value). NS = not significant.
all doses of E5531 appeared to block the response only at 5 h (Fig 1, stepwise, concentration-dependent attenuation of LPS-induced alteration in vital signs as previously described. Increasing doses of E5531 generated a dose-related inhibition of LPS-mediated decreases in MAP engendered by LPS (MAP not significantly different from saline solution control) [Fig 1, middle, B]. At the 3-h assessment, no individual treatment group exhibited a response different than the saline control group. A significant widening of pulse pressure was seen at both time points in the placebo control, despite the fact that the 3-h post-LPS MAP response was not significantly different from baseline or saline solution control (Table 1, Fig 1, middle, b, and bottom, c). E5531 attenuated the increase in pulse pressure in a step-wise manner at the 3-h time point with doses of E5531 ≥ 250 μg/kg associated with values similar to the saline solution control (Fig 1, bottom, C). At the 5-h time point, group-dependent differences in responses were not significant despite the fact that the placebo group had a significantly widened pulse pressure while the E5531 dosing group responses were similar to the saline solution control (Fig 1, bottom, C).

Cardiac Performance/Ventricular Volumes

CI, as previously described, was elevated at both 3 h and 5 h after LPS administration in the placebo group (Table 1, Fig 2, top, left, A). SV index demonstrated a biphasic response to LPS administration, with an initial increase followed by a later decrease relative to baseline values (Table 1). However, relative to the saline solution control, SV index was modestly depressed initially and decreased more substantially by 5 h after LPS (Fig 2, top, right, B). End-diastolic volume index (EDVI) was decreased at both the 3-h and 5-h time points (both compared to baseline values and relative to the increases seen in the saline solution control), primarily due to the concurrent and substantial increase in HR (Table 1, Fig 2, bottom, left, C). ESVI was similarly but more substantially decreased at the 3-h time point, but was not different than baseline or the saline solution control by 5 h after LPS (Table 1, Fig 2, bottom, right, D).

E5531 again generated a stepwise, dose-dependent attenuation of the hyperdynamic circulatory response (increased CI) to LPS (Fig 2, top, left, A). The 500 μg and 1,000 μg doses of E5531 completely blocked this LPS-induced augmentation of CI at 3 h, but maximum doses (1,000 μg) blocked only incompletely by 5 h (relative to the saline solution control). Stepwise, dose-dependent attenuation was also seen with respect to SV index responses at both time points (Fig 2, top, right, B). However, in this case, all doses of E5531 resulted in SV index responses similar to the saline solution control. Only the placebo groups had SV index responses that were significantly less than the saline solution controls. Similarly, E5531 demonstrated marked dose-dependent attenuation of LPS-mediated decreases in
EDVI. Protection was statistically complete at doses \( \geq 250 \mu g \) at 3 h, but was inconsistent at doses between 250 and 1,000 \( \mu g \) at 5 h (Fig 2, bottom, left, C). Stepwise, dose-dependent attenuation of loss of ESVI induced by LPS was also present at 3 h with complete abrogation of volume loss at doses \( \geq 500 \mu g \). Group-(and dose-) dependent differences in response were not seen at 5 h even though a significant decrease in ESVI was seen in some treatment groups relative to the saline solution control.

**Afterload Parameters**

Measures of afterload were also altered by LPS. As has been previously described, TVR dropped almost 50% from baseline values in the placebo group at the 3-h post-LPS point, and remained at that approximate level at the 5-h mark (Table 1, Fig 3, top, A).\(^7\) Pes and \( \sigma_{es} \) also fell substantially from baseline values at similar time points (Table 1, Fig 3, middle, B, and bottom, C). These three measures of afterload were also reduced relative to the saline solution control group at 3 h and 5 h after LPS administration (Fig 3). Increasing doses of E5531 generated stepwise blockade of the response for each of TVR, Pes, and \( \sigma_{es} \). Except for the 5-h point for TVR, higher doses of E5531 abrogated the response such that no significant differences existed between the response in the saline solution control and the groups receiving higher E5531 doses (Fig 3).

**Ventricular Contractility Indices**

Indices of ventricular contractility (EF, Vcfc, SBP/ESVI, Pes/ESVI, \( \sigma_{es}/ \sigma_{es} \)) in the placebo control group demonstrated a biphasic response to LPS. Contractility indices were generally increased at 3 h but decreased by 5 h after LPS when measured in comparison to either baseline or to saline solution control (Table 1, Fig 4). The major exception was \( \sigma_{es}/ \sigma_{es} \), which was unchanged from both baseline and saline solution control at the 3-h post-LPS time point. All other contractility indices (EF, Vcfc, SBP/ESVI, and Pes/ESVI) were increased compared to the saline solution control at 3 h. By 5 h after LPS, all contractility indices were depressed relative to the saline solution control (even though there was no

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**Figure 2.** CI (top, left, A), SVI (systolic volume index) (top, right, B), EDVI (bottom, left, C), and ESVI (bottom, right, D) at 3 h and 5 h after LPS administration normalized to baseline values. *\( p < 0.01 \), †\( p < 0.001 \), ‡\( p < 0.0001 \) (each compared to day 1 saline solution control); \( p \) values above each group of time point data bars refer to group-dependent (top, regular font) and concentration-dependent (bottom, italics) effects. Day 1 saline solution control refers to aggregate responses of all subjects to volume loading (without LPS or E5531) at given time point on the first study day. See Figure 1 legend for expansion of abbreviation.
difference compared to their own baseline value for some parameters) [Table 1, Fig 4]. At the 3-h time point, significant group-dependent improvement in most contractility parameters suggested protection by E5531; the apparent lack of group-dependent effect as assessed by Pes/ESVI is referable to the absence of any significant LPS-mediated effect in the first place with respect to this contractility index. Stepwise concentration-dependent attenuation of increased contractility was noted for EF, Vcfc, and SBP/ESVI (with Pes/ESVI showing a strong trend in that direction) at 3 h. Increasing doses of E5531 completely prevented the early cardiac stimulatory effects of LPS in each of these four contractility indices. At 5 h after LPS, significant group-dependent differences in response existed, indicating prevention of myocardial depression by E5531. Concentration-dependence of responses also achieved statistical significance for each parameter except SBP/ESVI.

**Discussion**

In this study, experimental human endotoxemia in association with aggressive saline solution loading (3 L in 3 h followed by an additional 1 L over 2 h) has been shown to produce a hyperdynamic circulatory response that includes marked tachycardia, increased cardiac output, and widened pulse pressure, as well as substantial decreases in afterload indices including TVR, Pes, and σes (some elements of which were noted in our previous study). As has been previously described by others, there was modest decrease in BP by 5 h after LPS. SV was decreased relative to the saline solution control at both assessed time points as a consequence of the marked tachycardia. LPS was also associated with significant decreases in both EDVI and ESVI (both compared to the saline solution control and to baseline values), likely also primarily as a consequence of the marked tachycardia engendered by LPS.

Although most indices of cardiac contractility (except σes/ESVI) demonstrated an early increase at 3 h followed by a later decrease 5 h after LPS (relative to the saline solution control), a hyperdynamic circulatory state existed throughout the study period. The demonstration in this study of a later phase (5 h after LPS) of decreased contractility using more robust, ostensibly load-independent indices of contractility including Vcfc, Pes/ESVI, and σes/ESVI confirms elements of previous observations using radionuclide cineangiography. However, σes/ESVI (the most robust of the contractility indices with respect to loading variations in normal volunteers), while confirming the 5-h post-LPS decrease in cardiac contractility, failed to support an earlier 3-h post-LPS increase as described in previous studies. This may indicate that other indices remain sufficiently load sensitive to be affected by the reduction in afterload associated with volume loading (decreased viscosity) or LPS-induced vasodilatation. All these effects were either partially or completely abrogated by the administration of increasing doses of E5531 (in a dose-dependent manner in most cases).

The cardiovascular responses to LPS and their blockade by E5531 in this study are complex, and there are several potential interactions between basic...
cardiovascular parameters that may need to be considered, particularly with respect to the contractility indices. For example, despite the fact that SV index and ventricular volumes are typically decreased in subjects receiving LPS (relative to the saline solution control), the marked increase in HR yields a CI that is substantially increased (Fig 2). The increased ESVI at the 5-h point that is the exception to this observation is probably reflects the decreased EF at that point. Similarly, endotoxin-induced decreases in TVR may be reflected in the early (3 h after LPS) decreases in ESVI (Fig 2, 3).

One of the most interesting aspect of our data relates to the demonstration that LPS generates significant alterations of advanced afterload (Pes, es) and the more robust, ostensibly load-independent contractility indices (Pes/ESVI, Vcfc, es/ESVI). We have recently demonstrated that saline solution loading alone can alter several contractility indices due to an unanticipated decrease in blood viscosity (an often ignored constituent of vascular resistance). Further, LPS could potentially induce a significant decrease in afterload as a consequence of vasodilation (reflected by the previously mentioned decrease in TVR, Pes, and es at both 3 h and 5 h after LPS administration). Although the early tachycardia would suggest that sympathetic tone is increased at the 3-h post-LPS time point, the concurrent decrease in afterload may partially explain the apparent increase in cardiac contractility as measured by indices that are not completely load independent. The demonstration that es/ESVI, a parameter that is among the most robust of the load-independent contractility indices, was unchanged at the earlier 3-h post-LPS time point (while other indices are elevated) suggests the possibility that the observed early contractility increase is an artifact of decreased afterload. Dose-dependent abrogation of the early increased contractility response does not necessarily imply a true LPS-induced contractility effect since E5531 would also block vasodilatory/afterload responses. However, the later 5-h post-LPS myocardial response likely represents true myocardial depression since it occurs in the context of a decreased afterload (which would tend to increase any afterload sensitive contractility indices).

These data clearly confirm that endotoxin administration produces a hyperdynamic circulatory state similar to that seen in sepsis. Through the novel application of more robust echocardiographic load-independent indices of contractility, this study also confirms endotoxin-induced myocardial depression at the 5-h post-LPS time point while calling into question the validity of the apparent increased contractility noted at 3 h after LPS. The dose-dependent attenuation of early hemodynamic responses and the amelioration of later myocardial depression by E5531 reinforces the causal relationship of circulat-
ing endotoxin to such phenomenon during human Gram-negative septic shock.

With respect to depressed contractility indices at 5 h, multivariate regression demonstrates that significant group dependent differences (i.e., differences among responses depending on the dose of E5531) exist even though stepwise dose dependence across the limited range of E5531 (100 to 1,000 μg) doses tested is not always noticeable. The question of why a concentration-dependent attenuation of the response to LPS may be less obvious with respect to blockade of myocardial depression at 5 h, but is clearly present with respect to the increased apparent contractility at 3 h is unclear. Since the apparent contractility increase at 3 h is probably an artifact of endotoxin-induced reduction of afterload while the depression at 5 h appears to represent true myocardial dysfunction, one possibility is that the vascular effects of endotoxin are easier to block than the myocardial depressant effects so that blockade of the latter is substantially complete at the lowest doses of E5531. Another possibility is that E5531 delays the kinetics of the depressant response so that the maximal effect is missed at the 5-h time point. Unfortunately, the data available from this study do not allow differentiation between these and other possibilities.

This study does have some important strengths and limitations. Among the strengths are the ability to examine advanced measures of afterload (particularly σes) and contractility (including Pes/ESVI, Vcf, and σes/ESVI) in serial examinations in a human model of disease. None of these indices have previously been examined in a human model of endotoxemia. Foremost among the limitations is the fact that any static echocardiographic assessment represents an approximation of cardiac contractility as assessed by “gold-standard” techniques that cannot be easily performed in humans. These “gold standard” techniques include the σes: velocity of fiber shortening relation, a measure that requires multiple serial echo assessments under varied afterload states and end-systolic elastance, a contractility parameter that can only be obtained invasively. As such techniques are not logistically possible or appropriate in experimental or clinical human studies of disease, the echocardiographic measures utilized are the best available substitutes.

This study confirms that myocardial depression coexists with a hyperdynamic circulatory state (as seen in human septic shock) within hours of induction of experimental endotoxemia in humans. Our analysis using increasingly load-independent indexes of contractility suggests that the increase in myocardial contractility in the early (3 h after LPS) phase of the endotoxin response may be, at least in part, an artifact of decreased afterload. However, the study strongly supports the presence of decreased myocardial contractility in the subsequent phase of the response 5 h after endotoxin administration. E5531, a competitive lipid A analog, with potent anti-inflammatory effects in vitro, animal, and human endotoxin models has the ability to block cardiovascular responses including myocardial depression during experimental human endotoxemia.

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