

# Endotoxaemic myocardial dysfunction: the role of coronary driving pressure in subendocardial perfusion

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Sepsis is a systemic inflammatory response to infection and frequently occurs in the presence of myocardial dysfunction.<sup>1</sup> Patients with septic myocardial dysfunction have significantly higher mortality (70%) compared with patients with no cardiovascular impairment (20%).<sup>2</sup> Experiments using *in vivo* animal models, isolated hearts and cultured cells, as well as human studies, have shown that decreased contractility and impaired myocardial compliance are the primary factors in septic myocardial dysfunction.<sup>3-6</sup> Recently, the role of microcirculatory alterations in the physiopathology of organ dysfunction has also been studied.

Under normal physiological conditions, microcirculatory autoregulation is responsible for maintaining constant coronary flow over a large range of systemic blood pressures (BPs).<sup>7</sup> Pathological release of vasodilatory substances in septic patients can result in extensive coronary vasodilatation, impairing myocardial blood flow autoregulation.<sup>8</sup> The current literature focuses on coronary blood flow, lactate metabolism and oxygen extraction, aspects which reflect only macrocirculatory changes.<sup>9,10</sup> In fact, macrocirculatory coronary blood flow has been reported to increase in established septic shock.<sup>11</sup> However, the role of the microcirculation in septic myocardial dysfunction remains unclear because direct studies are lacking.<sup>11</sup> Indirect indicators, such as lactate and oxygen extraction, can be misleading in that overperfused areas can cause small but important underperfused areas to be missed. Additionally, during sepsis, endothelial signalling is disrupted, impairing precapillary arteriolar regulation and reducing blood flow.<sup>12</sup> This heterogeneity of microvascular perfusion is a hallmark of sepsis that ultimately may contribute to organ dysfunction and death.<sup>12</sup> Moreover, impaired autoregulation of myocardial microcirculation could make such microcirculatory blood flow pressure dependent.<sup>7,8</sup>

Coronary driving pressure (CDP) is defined as the difference between diastolic arterial BP and left ventricular (LV) end-diastolic pressure (LVEDP). The effect of CDP on myocardial blood flow in sepsis is unclear but CDP is known to be an important factor in several cardiac diseases.<sup>13,14</sup> A minimal

## ABSTRACT

**Objective:** To investigate the role of coronary driving pressure (CDP) in myocardial microcirculatory blood flow during sepsis. We hypothesised that in septic shock there is an impaired autoregulation of microcirculation, and blood flow is totally dependent on CDP. We analysed the effect of lipopolysaccharide (LPS)-induced shock on myocardial microcirculation, separating subendocardial and epicardial areas. We then studied the effect of CDP increases using noradrenaline (NOR) or metaraminol (Aramine [ARA]) on myocardial microcirculation and function, and we analysed the effect of volume infusion on CDP and myocardial function.

**Design and setting:** Endotoxaemia was induced in male Wistar rats by an intraperitoneal injection of LPS 10 mg/kg. Animals were divided into a control (CT) group, an LPS-injected group, and an LPS-injected group treated with saline fluid, NOR or ARA.

**Main outcome measures:** Ninety minutes later, a haemodynamic evaluation was performed. NOR or ARA were used to manage the mean arterial pressure (MAP) and CDP, and we inserted a catheter into the left ventricle to measure cardiac parameters. To measure blood flow in the myocardium and other organs, microspheres were introduced into the left ventricle using an infusion pump.

**Results:** After LPS treatment, left ventricular (LV) systolic function (dP/dt max) and diastolic function (dP/dt min) decreased by 34% and 15%, respectively, and load-independent indices (LV contractility in ejection phase and dP/dt max ÷ end-diastolic volume) were reduced. The CDP was also reduced (by 58%) in the endotoxaemic rats. Myocardial blood flow was reduced (by 80%) in animals with an MAP ≤ 65 mmHg. NOR increased the CDP (LPS, 38 mmHg [SEM, 2 mmHg]; LPS+NOR, 59 mmHg [SEM, 3 mmHg]) and microcirculatory perfusion (LPS, 2 mL/min/g tissue [SEM, 0.6 mL/min/g]; LPS+NOR, 6.2 mL/min/g [SEM, 0.8 mL/min/g]). ARA was also effective in improve microcirculation but saline volume infusion was ineffective in improving CDP or myocardial function. CDP showed a significant correlation with subendocardial blood flow.

**Conclusions:** Myocardial blood flow in the LV subendocardium and the right ventricle decreases in endotoxaemic rats. Increasing CDP improves myocardial blood flow and function. Thus, in endotoxaemia, microcirculatory blood flow is pressure dependent, suggesting that it may be beneficial to treat patients with sepsis using a higher CDP.

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CDP is required to sustain adequate tissue perfusion, taking into account that the oxygen demand increases under hyperdynamic myocardial conditions.<sup>13</sup> Sustaining mean arterial pressure (MAP) at about 65 mmHg may allow diastolic arterial pressure and CDP to decrease until they cannot maintain adequate myocardial blood flow.<sup>13</sup> The myocardium is perfused during diastole, thus increasing CDP and decreasing the heart rate (ie, increasing the period of diastole) may improve subendocardial perfusion during septic shock.

We hypothesised that, in septic shock, there is impaired autoregulation of microcirculation and that blood flow is dependent on CDP. Therefore, we hypothesised that we could protect against myocardial hypoperfusion and prevent subsequent cardiac dysfunction by increasing CDP.

### Abbreviations

ARA	Metaraminol
BP	blood pressure
CDP	coronary driving pressure
CO	cardiac output
CT	control
dP/dt max	maximum rate of LV pressure increase during isovolumetric contraction
dP/dt min	maximum rate of LV pressure decrease during isovolumetric relaxation
dP/dt@EDV	dP/dt max ÷ end-diastolic volume
Ea	arterial elasticity
Ees	LV contractility in ejection phase
EF	ejection fraction
ESPVR	end-systolic pressure–volume relationship
IP	intraperitoneal
IV	intravenous
IVRT	isovolumetric relaxation time
LPS	lipopolysaccharide
LV	left ventricular
LVEDP	LV end-diastolic pressure
LVEDV	LV end-diastolic volume
LVESV	LV end-systolic volume
LVSP	LV systolic pressure
MAP	mean arterial pressure
NOR	noradrenaline
P <sub>pulse</sub>	pulse pressure
PE	polyethylene
PET	positron emission tomography
PV	pressure–volume
PVC	pressure–volume catheter
SF	saline fluid
SV	stroke volume
SW	stroke work
<i>tau</i>	time constant of isovolumetric pressure decrease during ventricular relaxation

**Table 1. Haemodynamic data at baseline and after IP LPS-induced endotoxaemia (N = 10)**

Parameter (mean ± SEM)	Baseline*	After LPS†
Systolic arterial pressure (mmHg)	121 ± 13	93 ± 10 <sup>‡</sup>
Diastolic arterial pressure (mmHg)	93 ± 10	55 ± 6 <sup>‡</sup>
Mean arterial pressure (mmHg)	107 ± 10	72 ± 5 <sup>‡</sup>
Heart rate (beats/minute)	386 ± 5	389 ± 16
Pulse pressure (mmHg)	28 ± 2	38 ± 3 <sup>‡</sup>
LV systolic pressure (mmHg)	120 ± 5	94 ± 8 <sup>‡</sup>
LV end-diastolic pressure (mmHg)	7 ± 1	19 ± 4 <sup>‡</sup>
dP/dt max (mmHg/second)	6256 ± 372	4122 ± 579 <sup>‡</sup>
dP/dt min (mmHg/ second)	5352 ± 155	4536 ± 579 <sup>‡</sup>
Coronary driving pressure (mmHg)	86 ± 4	36 ± 3 <sup>‡</sup>

IP = intraperitoneal. LPS = lipopolysaccharide. SEM = standard error of the mean. LV = left ventricular. dP/dt max = maximum rate of LV pressure increase during isovolumetric contraction. dP/dt min = maximum rate of LV pressure decrease during isovolumetric relaxation. \* Before intraperitoneal LPS injection. † 1.5 hours after intraperitoneal LPS injection. ‡ P < 0.05. Data show significant alterations at 1.5 hours post-LPS injection.

We investigated the role of CDP as a determinant of myocardial microcirculation in endotoxaemic hearts. We analysed blood flow in each myocardial segment (the right ventricle and the subendocardial and epicardial areas of the left ventricle). We used three study arms to compare the effects of lipopolysaccharide (LPS) on microcirculatory control and shock. The interventions in the three arms were volume infusion, elevation of CDP using noradrenaline (NOR) and elevation of CDP using metaraminol (Aramine) (ARA), a vasoactive drug without β-adrenergic action. These three arms of the study enabled us to investigate the role of volume in myocardial function and, most importantly, to show that microcirculation and function are dependent on perfusion pressure. By comparing NOR (which has β-adrenergic action) with ARA (which does not) we could separate the effects that are dependent on β-adrenergic action.

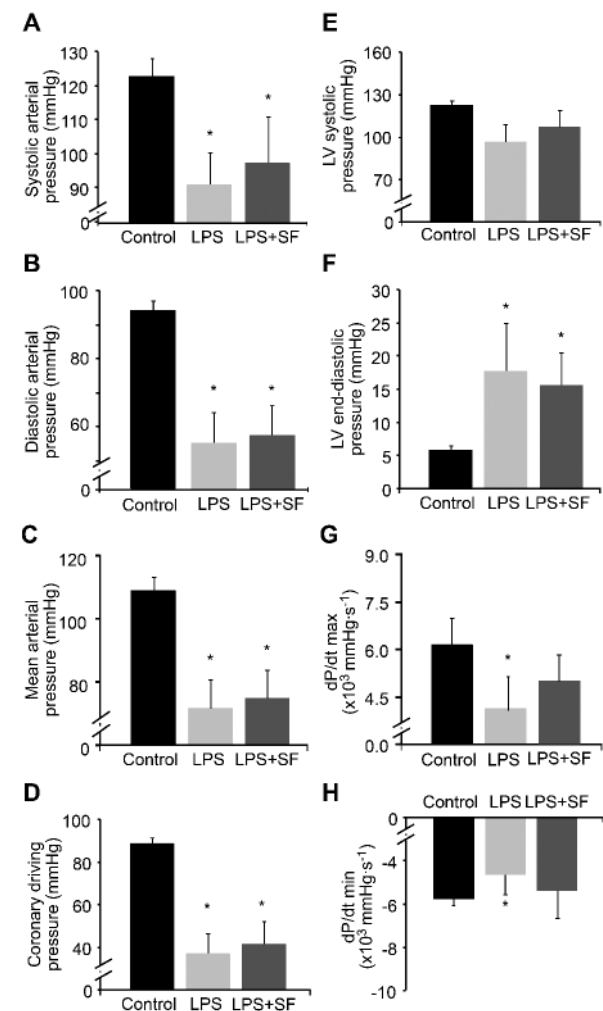
### Materials and methods

All procedures were performed in accordance with the standards of the Brazilian College of Animal Research, and the study protocol was approved by the ethics committee of the University of São Paulo Medical School. Funding was through a grant from the Foundation for Research Support of the State of São Paulo (FAPESP) (09/15530-0).

### Shock induction

Male Wistar rats (weighing 300–320 g) were used and were divided into two groups: LPS and control (CT). Endotoxaemia was induced by intraperitoneal (IP) injection of LPS 10 mg/kg (*Escherichia coli* cepa 026:B6, Sigma-Aldrich), with

**Figure 1. Effect of volume resuscitation on haemodynamic parameters in three groups of rats (N = 8). The control (baseline) group did not receive LPS. The LPS group was challenged with IP LPS 10 mg/kg. The LPS+SF group was challenged with IP LPS 10 mg/kg and received 0.9% NaCl 30 mL/kg intravenously. Compared with the control group, the LPS and LPS+SF groups showed important reductions in systolic arterial BP (A), diastolic arterial BP (B), MAP (C) and coronary driving pressure (D). LV systolic pressure was not changed by LPS (E), LV end-diastolic pressure was increased in the LPS and LPS+SF groups (F), dP/dt max was significantly reduced in the LPS group (G), and dP/dt min was reduced only in the LPS group (H). Values are means and standard errors of the mean**



LPS = lipopolysaccharide. IP = intraperitoneal. SF = saline fluid. BP = blood pressure. MAP = mean arterial pressure. LV = left ventricular. dP/dt max = maximum rate of LV pressure increase during isovolumetric contraction. dP/dt min = maximum rate of LV pressure decrease during isovolumetric relaxation. \*  $P < 0.05$  (analysis of variance followed by the Bonferroni test).

endotoxaemic shock defined as  $MAP \leq 65$  mmHg. The CT animals received IP injections of an equal volume of phosphate-buffered saline. In total, 130 rats were divided into two groups: 90 LPS rats and 40 CT rats. The 90 LPS animals were divided into four groups:

- LPS 10 mg/kg IP ( $n = 54$ )
- LPS + saline fluid (SF) 0.9% NaCl 30 mL/kg IV over 15 minutes ( $n = 8$ )
- LPS + NOR 0.3  $\mu\text{g/kg/min}$  ( $\pm 0.05$   $\mu\text{g/kg/min}$ ) into the right jugular vein to achieve an MAP of 85 mmHg ( $n = 22$ )
- LPS + ARA 1.9  $\mu\text{g/kg/min}$  ( $\pm 0.92$   $\mu\text{g/kg/min}$ ) into the right jugular vein to achieve an MAP of 85 mmHg ( $n = 6$ ).

Haemodynamic measurements were recorded 1.5 hours after the LPS injection. For all experimental procedures, the animals underwent general anaesthesia using a mixture of ketamine (100 mg/kg) and xylazine (50 mg/kg). We used the lowest number of animals necessary for each experiment.

### Haemodynamics

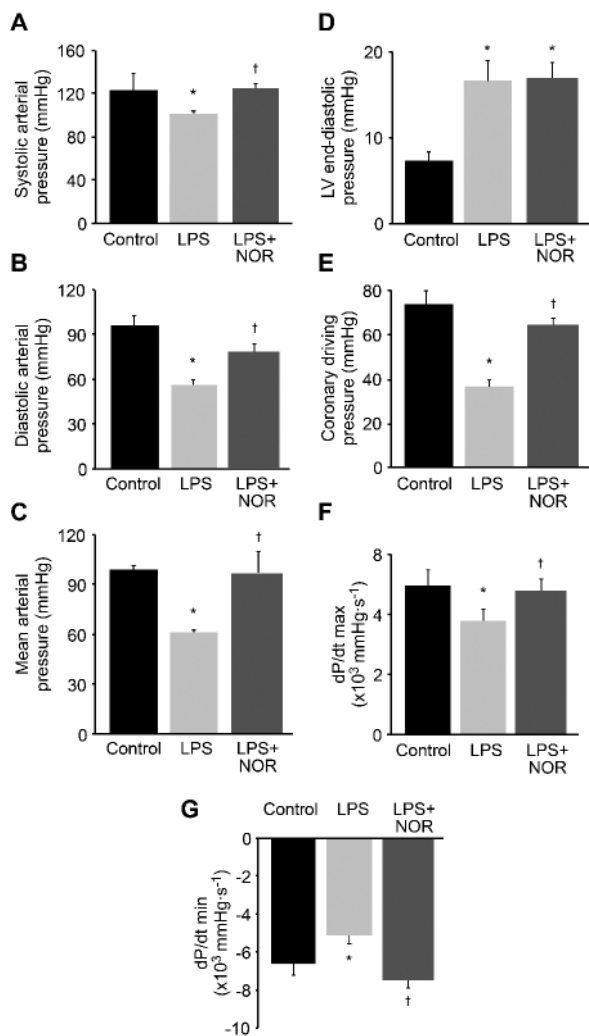
Under anaesthesia, the rats underwent simultaneous systemic and left ventricular haemodynamic measurements at 1.5 hours after LPS injection. In our study, 1.5 hours was the time when all haemodynamic parameters were altered by endotoxaemia.

A 0.01 mm catheter was introduced through the right femoral artery, which was exposed by blunt dissection, and advanced into the descending abdominal aorta. The catheter was connected to pressure transducers coupled to a calibrated preamplifier A/D converter (PowerLab 16/SP, ADInstruments). Pressure tracings were recorded and analysed using a computerised system processor (PowerLab, ADInstruments) and, on stabilisation of the animal, haemodynamic measurements were recorded.

A microtip pressure–volume (PV) catheter (PVR catheter, Millar Instruments) was inserted into the left ventricle via the right carotid artery. After stabilisation, the pressure and volume signals were continuously recorded using an MPVS-300 PV conductance system (Millar Instruments) coupled to the A/D converter. Heart rate, LV systolic pressure (LVSP), LVEDP, LV end-systolic volume (LVESV) and LV end-diastolic volume (LVEDV) were measured. Stroke volume (SV), stroke work (SW), ejection fraction (EF), and cardiac output (CO) were then calculated and corrected according to in vitro volume calibrations using a cardiac PV analysis program (PVAN version 3.2, Millar Instruments).<sup>15</sup> Pulse pressure ( $P_{\text{pulse}}$ ) was calculated as the difference between the systemic systolic and diastolic blood pressures. CDP was calculated as the difference between systemic diastolic blood pressure and LVEDP and was used as an estimate of myocardial perfusion pressure.<sup>13</sup>

Total effective arterial elasticity ( $E_a$ ), which is an integrated index of arterial load that is sensitive to resistive and pulsatile

**Figure 2. Effects of noradrenaline on haemodynamic parameters ( $N = 10$ ). The control (baseline) group did not receive LPS. The LPS group was challenged with IP LPS 10 mg/kg. The LPS+NOR group was challenged with IP LPS 10 mg/kg and received intravenous NOR to sustain an MAP of 85 mmHg. LPS reduced arterial pressures (A, B and C), increased LV end-diastolic pressure (D) and reduced coronary driving pressure (E). Treatment with NOR resulted in the recovery of all these parameters, except for LV end-diastolic pressure, and resulted in the recovery of  $dP/dt$  max (F) and  $dP/dt$  min (G). Values are means and standard errors of the mean**



IP = intraperitoneal. LPS = lipopolysaccharide. NOR = noradrenaline. MAP = mean arterial pressure. LV = left ventricular.  $dP/dt$  max = maximum rate of LV pressure increase during isovolumetric contraction.  $dP/dt$  min = maximum rate of LV pressure decrease during isovolumetric relaxation. \*  $P < 0.05$  v control. †  $P < 0.05$  v LPS (analysis of variance followed by the Bonferroni test).

loads, was calculated as the LV systolic pressure:systolic volume ratio and was used as an index of LV afterload.<sup>16</sup> The maximum rate of LV pressure increase during isovolumetric contraction ( $dP/dt$  max) was computed from the pressure signal.

The inotropic state of the left ventricle was determined by two load-independent indices of contractility. First, the ratio of  $dP/dt$  max divided by the end-diastolic volume ( $dP/dt@EDV$ ) was computed as a measure of contractility in the isovolumetric phase. Second, the slope of the end-systolic pressure–volume relationship (ESPVR) of successive pressure volume loops at a rapidly reduced preload was computed as a measure of the LV contractility during the ejection phase ( $Ees$ ).<sup>15,16</sup> Indices of diastolic function, including the maximum rate of LV pressure decrease during isovolumetric relaxation ( $dP/dt$  min), the time constant of isovolumetric pressure decrease during ventricular relaxation ( $\tau$ ), and the average  $dP/dt$  during the isovolumetric relaxation period (IVRT), were computed from the pressure–volume signal.

### Cardiac blood flow

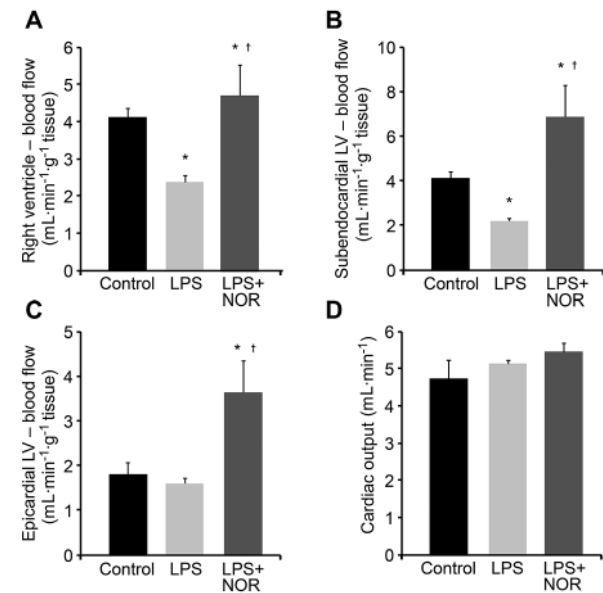
To determine cardiac blood flow, saline-filled catheters were implanted into the left ventricle and the femoral artery. A polyethylene (PE) 10 catheter was positioned in the abdominal aorta via the right femoral artery for direct measurement of the systemic arterial pressure and for blood sample collection. A PE 50 catheter was also inserted into the jugular vein to deliver the intervention drug. A third catheter (PE 50) was inserted into the left ventricle via the right carotid artery for the infusion of coloured microspheres. The femoral artery cannula was connected to a strain-gauge miniature pressure transducer (Narco Biosystems RP 1500), and blood pressure was monitored continuously.

After 20 minutes of basal arterial pressure, Dye-Trak coloured microspheres (15  $\mu$ m) (Triton Technology) were infused (150 000 yellow microspheres) to obtain blood flow measurements. Microsphere infusion and processing were performed according to a previously described method.<sup>17,18</sup> Next, the animals were euthanased with a thiopentone overdose, and we removed the hearts to determine the regional blood flow. The heart was divided into the right ventricle and the left ventricle, and the left ventricle wall was divided into the inner third area, known as the denominated subendocardial area, and the outer two-thirds area, called the epicardial area.

Reference blood samples and tissues were processed following a method previously described.<sup>17,18</sup> The microsphere concentrations in the samples were determined using a DU 640 spectrophotometer (Beckman Instruments) with a  $< 1.8$  nm slit. The absorption spectrum peaks of the yellow microspheres were obtained at 448 nm.

For each infusion, the tissue flow rates were calculated according to the following formula:  $Q_t = A_t (Q_b \div A_b)$ , where  $Q_t$  and  $Q_b$  represent flow in the sample tissue and in the

**Figure 3. Myocardial blood flow of NOR-treated endotoxaemic animals in shock ( $N = 10$ ). Myocardial blood flow was measured using the microsphere perfusion procedure. The control (baseline) group did not receive LPS. The LPS group was challenged with IP LPS 10 mg/kg. The LPS+NOR group was challenged with IP LPS 10 mg/kg and received intravenous NOR to sustain an MAP of 85 mmHg. Myocardial flow was analysed separately in the RV (A), the subendocardial LV (B) and the epicardial LV (C). The myocardial blood flow was reduced in the RV and the subendocardial LV in the LPS group compared with the control group, and the LPS+NOR group showed improvement compared with the LPS group. Cardiac output was not changed by LPS (D). Values are means and standard errors of the mean**



IP = intraperitoneal. LPS = lipopolysaccharide. NOR = noradrenaline. MAP = mean arterial pressure. RV = right ventricle. LV = left ventricle. \*  $P < 0.05$  v control. †  $P < 0.05$  v LPS (analysis of variance followed by the Bonferroni test).

reference blood, respectively, and  $A_t$  and  $A_b$  represent the peak absorbance of the tissue sample and of the reference blood, respectively.  $Q_b$  (mL/min) was calculated as follows:  $Q_b = \text{reference blood sample weight} \div 1.05 \text{ g/mL} / (\text{reference blood sample volume} \div 0.5 \text{ mL/min})$ , where 1.05 g/mL is the specific gravity of blood, and 0.5 mL/min is the withdrawal rate. The blood flow rates were divided by the tissue weights to yield mL/min/g.

### Statistical analysis

Data are expressed as means and standard errors of the mean (SEMs). A student  $t$  test and one-way repeated-measures

analysis of variance coupled with the Bonferroni method were used as post-hoc tests to compare individual groups. The Pearson correlation was used to test the following potential relationships: between CDP and the subendocardial and epicardial blood flow, and between CDP and  $dP/dt$  max and  $dP/dt$  min. Measurement of CDP is continuous and measurement of myocardial blood flow is intermittent so, to properly correlate CDP with myocardial blood flow, we used the average CDP values of a segment before a myocardial blood flow measurement. Fifteen minutes of a stabilised CDP curve, and taking into account myocardial blood flow values, meant that the CDP data was robust. Linear regression analysis was performed between CDP and subendocardial blood flow, and between subendocardial blood flow and  $dP/dt$  max@EDV and Ees. Normality and equal variance were tested in all analyses. Statistical significance was established as  $P < 0.05$ . All analyses were performed using SigmaStat statistical software, version 1 (Jandel Scientific).

## Results

### Shock model: CT v LPS

Ninety minutes after LPS injection, the baseline haemodynamics were significantly altered (Table 1). The systolic, diastolic and mean arterial blood pressures were significantly reduced (by 23%, 40% and 32%, respectively). In contrast, the  $P_{\text{pulse}}$  was increased, which is characteristic of septic patients with normal blood volumes. Additionally, the LVSP was reduced by 22% but the LVEDP was increased. Left ventricular systolic function ( $dP/dt$  max) was reduced by 34%, and  $dP/dt$  min was decreased by 15%. The CDP was also significantly reduced (58%) (Table 1).

### Effects of saline volume infusion

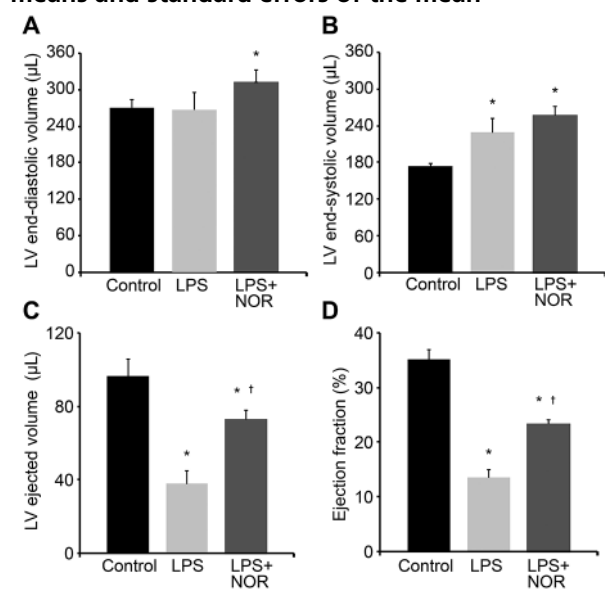
Saline volume was given to eight other LPS animals to ensure that low ventricular filling was not causing cardiac changes (Figure 1). Infusion of 30 mL/kg saline was not sufficient to increase the following parameters for LPS v LPS+SF:

- CDP: 38 mmHg (SEM, 9 mmHg) v 40 mmHg (SEM, 8 mmHg)
- $dP/dt$  max: 4012 mmHg/s (SEM, 934 mmHg/s) v 4645 mmHg/s (SEM, 483 mmHg/s)
- $dP/dt$  min: 4212 mmHg/s (SEM, 627 mmHg/s) v 4462 mmHg/s (SEM, 671 mmHg/s)
- LVEDP: 17 mmHg/s (SEM, 7 mmHg) v 15 mmHg (SEM, 6 mmHg)
- arterial blood pressure.

### Effects of NOR on CDP

Figure 2 shows haemodynamic parameters measured in endotoxaemic rats before and after IV NOR infusions. NOR elevated arterial pressure and CDP (LPS, 38 mmHg [SEM, 2 mmHg];

**Figure 4. LV volumes and cardiac EFs of NOR-treated endotoxaemic animals in shock ( $N = 10$ ). The control (baseline) group did not receive LPS. The LPS group was challenged with IP LPS 10 mg/kg. The LPS+NOR group was challenged with IP LPS 10 mg/kg and received intravenous NOR to sustain an MAP of 85 mmHg. LPS did not change the LV end-diastolic volume but NOR did (A). The LV end-systolic volume was increased in the LPS group compared with the control group (B) and the LPS+NOR group showed recovery. LPS reduced the LV ejected volume (C) and EF (D) and NOR treatment improved these values. Values are means and standard errors of the mean**



LV = left ventricular. EF = ejection fraction. NOR = noradrenaline. LPS = lipopolysaccharide. IP = intraperitoneal. MAP = mean arterial pressure. \*  $P < 0.05$  v control. †  $P < 0.05$  v LPS (analysis of variance followed by the Bonferroni test).

LPS+NOR, 59 mmHg [SEM, 3 mmHg];  $P < 0.05$ ), and dP/dt max recovered with NOR administration (LPS, 4038 mmHg/s [SEM, 424 mmHg/s]; LPS+NOR, 6952 mmHg/s [SEM, 636 mmHg/s];  $P < 0.05$ ). Interestingly, dP/dt min also recovered with NOR administration (LPS, 4867 mmHg/s [SEM, 562 mmHg/s]; LPS+NOR, 6824 mmHg/s [SEM, 423 mmHg/s];  $P < 0.05$ ). Myocardial blood flow was reduced in animals with an MAP of about 65 mmHg or less. The changes in cardiac perfusion in the endotoxaemic rats compared with the CT group revealed lower blood flow, particularly in the right ventricle (LPS, 0.8 mL/min/g tissue [SEM, 0.2 mL/min/g]; CT, 2.7 mL/min/g [SEM, 0.3 mL/min/g];  $P < 0.05$ ), and the subendocardial region of the left ventricle (CT, 2.5 mL/min/g [SEM, 0.4 mL/min/g]; LPS, 0.7 mL/min/g [SEM, 0.2 mL/min/g];  $P < 0.05$ ). In contrast, the same measurements in the epicardial region of the left ventricle did not reveal any differences (Figure 3).

After receiving NOR, the endotoxaemic animals showed a marked recovery of myocardial blood flow (Figure 3), with flow in the right ventricular subendocardial region measured at 4.72 mL/min/g (SEM, 0.37 mL/min/g;  $P < 0.05$ ) and flow in the LV subendocardial region measured at 6.85 mL/min/g (SEM, 1.62 mL/min/g;  $P < 0.05$ ). This showed that the subendocardial regions were especially sensitive to NOR treatment.

Figure 4 shows the results for the LV chamber size, ejected volume and EF. NOR was effective in improving the LV ejected volume (LPS, 36 µL [SEM, 14 µL]; LPS+NOR, 74 µL [SEM, 12 µL];  $P < 0.05$ ) and EF (LPS, 15% [SEM, 2%]; LPS+NOR, 22% [SEM, 1%];  $P < 0.05$ ).

Load-dependent indices of LV function were assessed, including LV EF (Figure 4), LV SW (CT, 2806 mmHg/µL [SEM, 103 mmHg/µL]; LPS, 19053 mmHg/µL [SEM, 302 mmHg/µL];  $P < 0.05$ ) (Figure 5), and dP/dt max (Figure 2). All these indices were significantly reduced after LPS injection.

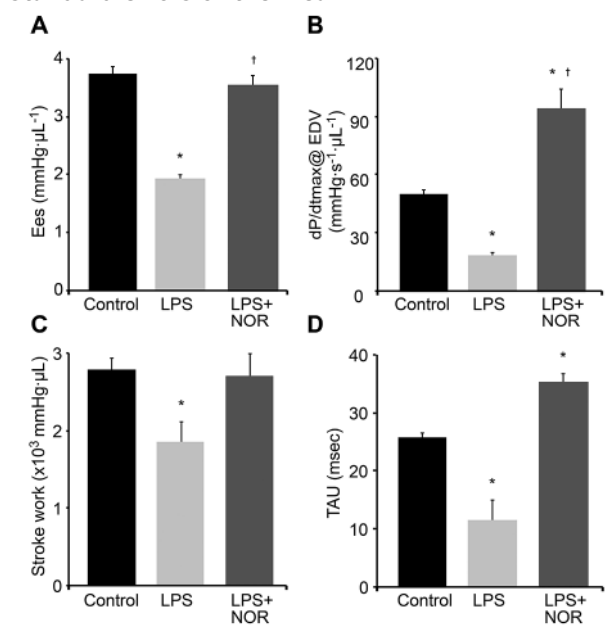
#### Effects of ARA on CDP

We also measured haemodynamic parameters and cardiac function in ARA-treated endotoxaemic animals (Figure 6). The data confirmed that even a non- $\beta$ -adrenergic agent improved cardiac function, which was dependent on CDP. ARA specifically increased the arterial pressure and CDP (LPS, 42 mmHg [SEM, 2 mmHg]; LPS+ARA, 72 mmHg [SEM, 3 mmHg];  $P < 0.05$ ). Recovery occurred with ARA for dP/dt max (LPS, 4738 mmHg/s [SEM, 536 mmHg/s]; LPS+ARA, 8122 mmHg/s [SEM, 536 mmHg/s];  $P < 0.05$ ) and interestingly, dP/dt min also recovered with ARA administration (LPS, 4844 mmHg/s [SEM, 232 mmHg/s]; LPS+ARA, 7562 mmHg/s [SEM, 562 mmHg/s];  $P < 0.05$ ).

LV loading conditions were evaluated by measuring LVEDP and LVEDV, which are indicators of the LV preload, and by computing the effective Ea indices of the LV afterload. The LV preload significantly increased 1.5 hours after the LPS injection at the expense of increased LVEDP (CT, 7 mmHg [SEM, 1 mmHg]; LPS, 19 mmHg [SEM, 4 mmHg];  $P < 0.05$ ), without changing the LVEDV (LPS, 280 µL [SEM, 42 µL]; LPS+NOR, 275 µL [SEM, 50 µL]) (Figure 4). No significant increase was noted for Ea after LPS injection (CT, 1.71 mmHg/µL [SEM, 0.75 mmHg/µL]; LPS, 1.90 mmHg/µL [SEM, 1.60 mmHg/µL]).

Two load-independent indices of LV contractility were computed. First, Ees (the slope of the ESPVR) provides information regarding LV contractility during the ejection phase of the cardiac cycle. Second, dP/dt max@EDV provides a measurement of LV contractility during the isovolumetric contraction phase of the cardiac cycle. As shown in Figure 7, the Ees was significantly decreased compared with its value in the CT group at 1.5 h after LPS injection. The mean Ees values were: CT, 3.8 mmHg/µL (SEM, 0.1 mmHg/µL); LPS, 2 mmHg/µL (SEM, 0.1 mmHg/µL); NOR+LPS, 3.5 mmHg/µL (SEM, 0.2 mmHg/µL) (Figure 5); and ARA+LPS, 3.6 mmHg/µL (SEM, 0.27 mmHg/µL) (Figure 7). Beyond the Ees, dP/

**Figure 5. Cardiac load-independent indexes of NOR-treated endotoxaemic animals in shock (N = 10). The control (baseline) group did not receive LPS. The LPS group was challenged with IP LPS 10 mg/kg. The LPS+NOR group was challenged with IP LPS 10 mg/kg and received intravenous NOR to sustain an MAP of 85 mmHg. LPS reduced Ees (A) and dP/dt max@EDV (B) compared with the control group, and treatment with NOR resulted in the recovery of both parameters. Stroke work was reduced in the LPS group compared with the CT and LPS+NOR groups (C).  $\tau$  was also reduced in the LPS group and relaxation was restored after treatment with NOR (D). Values are means and standard errors of the mean**



NOR = noradrenaline. LPS = lipopolysaccharide. IP = intraperitoneal. MAP = mean arterial pressure. LV = left ventricular. Ees = LV contractility during ejection phase. dP/dt max = maximum rate of LV pressure increase during isovolumetric contraction. EDV = end-diastolic volume. dP/dt max@EDV = dP/dt max divided by EDV.  $\tau$  = time constant of isovolumetric pressure decrease during ventricular relaxation. \*  $P < 0.05$  v control. †  $P < 0.05$  v LPS (analysis of variance followed by the Bonferroni test).

dt max@EDV was significantly decreased (Figure 7). The mean dP/dt max@EDV values were: CT, 46.2 mmHg/µL (SEM, 2.2 mmHg/µL); LPS, 17 mmHg/µL (SEM, 2.1 mmHg/µL); NOR+LPS, 94.6 mmHg/µL (SEM, 11.2 mmHg/µL) (Figure 5); and ARA+LPS, 63.3 mmHg/µL (SEM, 4.2 mmHg/µL) (Figure 7). The load-independent indices confirmed cardiac dysfunction and the use of NOR and ARA enabled the recovery of myocardial capabilities.

The indices of time constants during relaxation showed that the mean  $\tau$  values were: CT, 14.5 ms (SEM, 0.6 ms);

LPS, 13 ms (SEM, 1.1 ms); LPS+NOR, 39.7 ms (SEM, 1.7 ms) (Figure 5); and LPS+ARA, 19.2 ms (SEM, 0.89 ms) (Figure 7).

### Interactions of CDP on myocardial blood flow and contractility

We noted an important relationship between the CDP and blood flow. In the CT animals, there was a positive correlation between the CDP and subendocardial blood flow ( $\rho = 0.73$ ). The correlation between the CDP and epicardial blood flow was  $\rho = 0.38$ , and between the CDP and the right ventricle was  $\rho = 0.48$ . In the animals that received LPS, there was a correlation only between the CDP and the subendocardial blood flow ( $\rho = 0.77$ ) and right ventricular blood flow ( $\rho = 0.94$ ). Furthermore, the subendocardial blood flow was positively correlated with dP/dt max and dP/dt min. The regression between the CDP and the subendocardial blood flow was  $R^2 = 0.67$  (Figure 8) and exponential regression was  $R^2 = 0.81$ . Additionally, the subendocardial blood flow showed a linear regression for dP/dt max@EDV ( $R^2 = 0.81$ ) and Ees ( $R^2 = 0.60$ ) (Figure 8). Continuous measurements were taken and an average of values after 15 minutes of stabilisation was used, which resulted in more robust analytical data. The average value was taken before myocardial blood flow measurement.

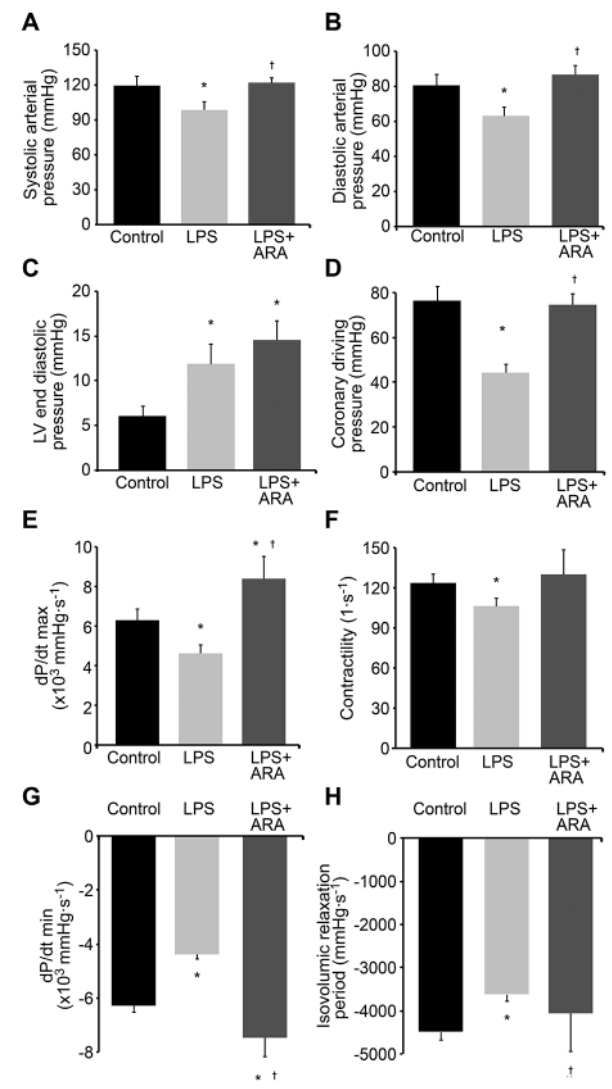
### Discussion

We showed that myocardial blood flow in endotoxaemic shock is CDP-dependent. Blood flow reduction occurred in the right and left ventricles but, in the left ventricle, only the subendocardial microcirculation was affected. We also showed that cardiac function was related to subendocardial blood flow and CDP. A therapeutic intervention increasing CDP effectively increased the myocardial blood flow and cardiac function.

### Myocardial perfusion in endotoxaemia

Several authors have presented data on normal macrocirculatory coronary flow and coronary sinus lactate in sepsis and have argued that septic myocardial depression cannot be attributed to myocardial ischaemia.<sup>19</sup> However, microcirculatory cardiac perfusion has not been directly assessed.<sup>4,20</sup> Microcirculatory abnormalities in sepsis are characterised by overperfused and underperfused areas in the same tissues or organs,<sup>21</sup> therefore, measurements involving macrocirculation are not useful for detecting regional hypoperfusion. The current paradigm dominating the literature states that microcirculatory blood flow is not determined by arterial pressure.<sup>22</sup> Our study had the power to detect differences in the subendocardial and epicardial areas of the heart. In particular, we showed reduced blood flow in the LV subendocardium and the right ventricle during sepsis. Perfusion of the subendocardium occurs during diastole,<sup>7</sup> therefore microcirculatory myocardial blood flow becomes dependent

**Figure 6. Effects of ARA on haemodynamic parameters (N = 10). The control (baseline) group did not receive LPS. The LPS group was challenged with IP LPS 10 mg/kg. The LPS+ARA group was challenged with IP LPS 10 mg/kg and received intravenous ARA to sustain an MAP of 85 mmHg. LPS reduced arterial pressure (A and B), increased LV end-diastolic volume (C) and reduced coronary driving pressure (D); ARA recovered all these parameters. Values are means and standard errors of the mean**



ARA = Aramine (metaraminol). LPS = lipopolysaccharide. IP = intraperitoneal. MAP = mean arterial pressure. LV = left ventricular. dP/dt max = maximum rate of LV pressure increase during isovolumetric contraction. dP/dt min = maximum rate of LV pressure decrease during isovolumetric relaxation. \*  $P < 0.05$  v control. †  $P < 0.05$  v LPS (analysis of variance followed by the Bonferroni test).

The epicardial area represents two-thirds of the myocardial tissue and thus may misrepresent changes in subendocardial blood flow. In healthy humans, myocardial perfusion has been measured using positron emission tomography (PET) scans, which showed that global measurement of flow was not able to show differences between the LV subendocardial and epicardial areas.<sup>23</sup> However, PET is not a suitable bedside method for patients in a critical condition. Doctors need a practical tool to measure and follow the treatment of patients with septic shock. We showed a strong correlation between subendocardial microcirculation and CDP, the gradient pressure that propels blood through the myocardial tissue during diastole.

The reduced CDP was associated with subsequent development of LV systolic and diastolic dysfunction. Consistent with our results, several investigators have reported a reduced LV inotropic state in endotoxaemic animals. Exposure of rats to 10 mg/kg of endotoxin resulted in a reduced Ees 6 hours after LPS treatment, which we attribute to a marked decrease in myocardial oxygenation.<sup>24-26</sup> Our data showed reduced contractility of endotoxaemic hearts based on load-dependent indices (SW and dP/dt max), load-independent indices (Ees and dP/dt max@EDV), and LV relaxation ( $\tau$ , IRVT and dP/dt min). Our results on load-independent indices also show that cardiac dysfunction is not related to volume.

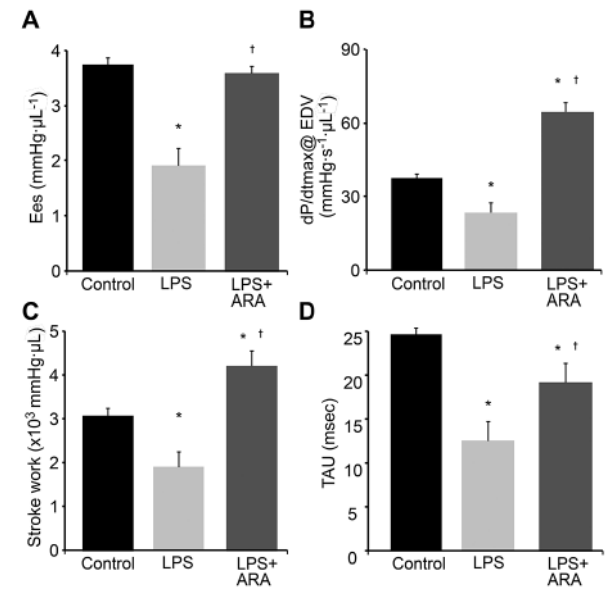
The importance of achieving an adequate circulating volume to maintain cardiac output has been elucidated in previous clinical studies.<sup>27</sup> Our haemodynamic data, from saline-treated endotoxaemic animals, showed no improvement in LVEDP, arterial BP, CDP or myocardial contractility or relaxation compared with animals that did not receive any fluid. In comparison, in the clinical scenario, some differences are relevant, for example, patients arrive in septic shock and are usually dehydrated. Our study showed elevated LVEDP in LPS animals, excluding a possible extracellular volume reduction. The fluid administration, therefore, was limited to one separate subset of animals, and not used in combination with vasopressor drugs. This approach had the advantage of enabling us to study the independent effects of fluid and vasopressor drugs but it could be considered a limitation in comparison with clinical studies.

The use of NOR and ARA proved that CDP could be managed to increase myocardial perfusion and improve cardiac function. Additionally, the effects of NOR and ARA on improving dP/dt min and  $\tau$  could not be attributed to any  $\beta$ -adrenergic action but were related to increased myocardial tissue perfusion. Our results are consistent with the literature, in that NOR provides a benefit only when the microcirculation is impaired.<sup>28</sup> The increase in CDP with NOR correlated with increased myocardial tissue microcirculation. Other experimental studies support our findings, showing severely disturbed regulation of oxygenation in the heart resulting in contractile dysfunction.<sup>4,20</sup>

on CDP and heart rate, especially in septic shock, because microcirculatory autoregulation is lost.



**Figure 7. Cardiac load-independent indexes of ARA-treated endotoxaemic animals in shock (N = 10). The control (baseline) group did not receive LPS. The LPS group was challenged with IP LPS 10 mg/kg. The LPS+ARA group was challenged with IP LPS 10 mg/kg and received intravenous ARA to sustain an MAP of 85 mmHg. LPS reduced Ees (A) and dP/dtmax@EDV (B) compared with the control group, and ARA resulted in the recovery of both of these parameters. Stroke work was reduced in the LPS group compared with the control and LPS+ARA groups (C). *tau* was reduced in the LPS group (D) and relaxation was restored by treatment with ARA. Values are means and standard errors of the mean**

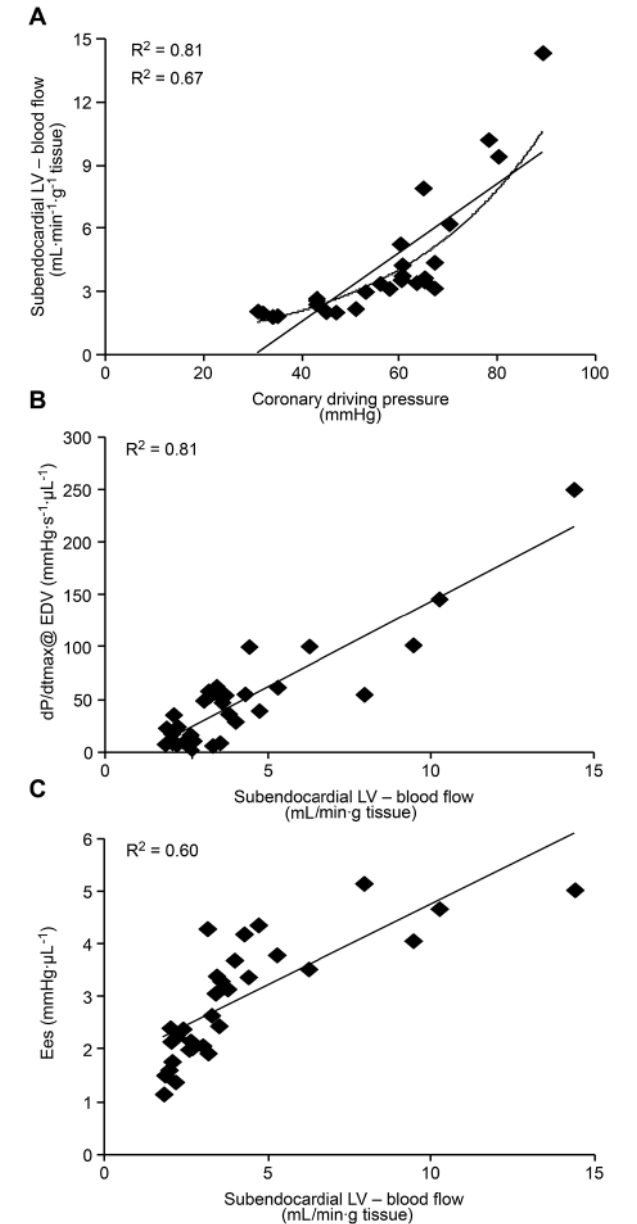


ARA = Aramine (metaraminol). LPS = lipopolysaccharide. IP = intraperitoneal. MAP = mean arterial pressure. LV = left ventricular. dP/dt max = maximum rate of LV pressure increase during isovolumetric contraction. EDV = end-diastolic volume. dP/dt max@EDV = dP/dt max divided by EDV. Ees = LV contractility during ejection phase. *tau* = time constant of isovolumetric pressure decrease during ventricular relaxation. \*  $P < 0.05$  v control. †  $P < 0.05$  v LPS (analysis of variance followed by the Bonferroni test).

**Strengths and limitations**

Other investigators have used techniques such as laser Doppler flowmetry for the coronary artery, and other methods that measure macrocirculatory flow.<sup>29</sup> An important strength in our methodology was that we used microspheres that remained trapped in the microvessels so we could measure the actual blood flow in the entire microcirculation.<sup>18</sup> In contrast, methods such as in vivo microscopy of microcirculation and Doppler flowmetry cannot be applied in the heart because these methods require a static organ. These methods are also limited to the surface of the heart and to a small part or segment. The

**Figure 8. Relationships between coronary driving pressure and subendocardial blood flow (A); subendocardial blood flow and dP/dt max@EDV (B); and subendocardial blood flow and Ees (C). Statistical analysis was with linear and exponential regression**



dP/dt max = maximum rate of LV pressure increase during isovolumetric contraction. EDV = end-diastolic volume. dP/dt max@EDV = dP/dt max divided by EDV. Ees = LV contractility during ejection phase. LV = left ventricular.

size of the microspheres we used was 15 µm, and the microcirculatory vessels have diameters of 10–100 µm.<sup>30</sup> We divided the myocardial tissue into subendocardial and epicardial areas before extraction and quantification of the microspheres

trapped in the tissue. Another strength of our methodology included the ability to obtain continuous measurements of cardiac pressure and volume, enabling us to analyse cardiac contractility and relaxation online.

It was almost impossible to sustain anaesthetised rats receiving NOR and ARA for long periods. Simulating shock in small animals needs extreme treatment, so our LPS model of septic shock was of limited use. Another limitation of our method was that use of microspheres precludes continuous blood flow measurements, and euthanasing the rats was necessary for tissue preparation to measure microspheres. We did not exclude other mechanisms previously described; we showed LV subendocardial microcirculation impairment and confirmed the need to measure blood flow separately in the epicardium and subendocardium. We have not described the blood flow of other organs but these measurements were performed and we showed that NOR did not cause any harm to them. In order to study the direct and exclusive effects of vasoactive drugs, we measured the effect of volume infusion and vasoactive drugs in a separate set of animals.

### CDP as a target in patients

Our study had potentially important clinical implications. We showed perfusion impairment in ventricular microcirculation causing endotoxaemic myocardial dysfunction, and this alteration was dependent on CDP. From a clinical perspective, CDP is a useful tool for directing haemodynamic therapy at the bedside. There are specific groups of patients who may benefit from high blood pressure, such as patients with low CDP and septic heart dysfunction.<sup>14</sup> We stress that CDP is most dependent on diastolic arterial pressure, and not directly related to MAP. In septic shock, vasodilatation leads to a profound decrease in diastolic arterial pressure, and the nervous autonomic reflex will increase systolic arterial pressure, therefore CDP and myocardial perfusion do not directly follow the changes in MAP. One study has shown that diastolic arterial pressure and CDP are independently associated with 28-day mortality in patients with cardiogenic shock.<sup>14</sup> In our study, shock in rats was defined as an MAP of  $\leq 65$  mmHg,<sup>31</sup> which is associated with myocardial hypoperfusion. Some authors have studied different systemic arterial pressures in patients with septic shock (MAPs of 65 mmHg, 75 mmHg and 85 mmHg), with no clinical risks detected at higher NOR doses.<sup>32</sup> One study also confirmed that an MAP of 85 mmHg did not produce any serious side effect and was associated with a similar mortality to lower blood pressure.<sup>33</sup> In our interpretation, these findings, together with ours, suggest that a higher blood pressure should be targeted particularly in patients who present with low CDP.<sup>33,34</sup> Our data show an improvement in myocardial contractility and especially relaxation with a higher MAP, which is indicative of a better ratio of additional cardiac work relative to increased oxygen delivery.

### Conclusions

Haemodynamic alterations in endotoxaemic shock, including increases in LVEDP and the volume of the ventricular chambers, compromise subendocardial perfusion. We found that subendocardial blood flow decreased in endotoxaemic rats in a CDP-dependent manner. Subendocardial perfusion compromise is implicated in reduced myocardial contractility and relaxation. We propose that CDP may be a useful bedside tool to ensure adequate vasoactive drug infusion, ensuring sufficient subendocardial blood flow.

### Competing interests

None declared.

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