Cerebral response to haemodilution during cardiopulmonary bypass in dogs: the role of nitric oxide synthase

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During cardiopulmonary bypass, haemodilution is standard practice and is accompanied by increases in cerebral blood flow (CBF). We investigated if changes in cerebral vascular resistance (CVR) during cardiopulmonary bypass–haemodilution are dependent on nitric oxide synthase. The cerebral response to haemodilution in nine dogs treated with the nitric oxide synthase inhibitor, Nω-nitro-L-arginine methyl ester (L-NAME), was compared with a control group (n=8). Both groups underwent serial isovolaemic haemodilution (target packed cell volumes 0.39, 0.26, 0.19 and 0.14) using 6% dextran 70. CBF, CVR and cerebral metabolic rate for oxygen (CMRO₂) were measured. While initial CVR was different in the two groups, haemodilution-dependent reductions in CVR were equivalent and the curves describing the packed cell volume–CVR relationship were parallel in control and nitric oxide synthase inhibition groups. Our data indicate that nitric oxide synthase does not play a primary role in the cerebral response to haemodilution.

Keywords: heart, cardiopulmonary bypass; brain, blood flow; blood, haemodilution; pharmacology; L-NAME

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Haemodilution is standard practice during cardiopulmonary bypass (CPB). Under CPB and non-CPB conditions, increases in cerebral blood flow (CBF) support cerebral oxygen delivery (CDO₂) and maintain metabolic rate for oxygen (CMRO₂) over a wide range of packed cell volumes (PCV).¹⁻⁴ This increase in CBF has been attributed to both passive biophysical changes in blood rheology¹⁻⁵ and to primary regulation of CDO₂ ²⁻⁶ but a nitric oxide synthase-dependent response to haemodilution has received little attention.

Haemodilution decreases blood viscosity and thereby increases flow velocity.⁷ Furthermore, the endothelium is mechanically receptive and mediates vasodilatation to fluid mechanical changes.⁸ Increases in vascular diameter in response to changing flow velocity has been termed ‘flow-mediated’ vasodilatation, and endothelial nitric oxide synthase may play a role in this response in both cerebral⁹ and non-cerebral vasculature.⁹¹⁰ The probable mechanism for flow-mediated vasodilatation is that changes in flow velocity alter endothelial shear stress and result in opening of ion channels.¹¹ Specifically, shear-induced K⁺ and Ca²⁺ currents may trigger nitric oxide synthesis in response to flow.¹¹¹²

As there is experimental evidence that a nitric oxide synthase-dependent mechanism mediates the systemic vasodilatory response to normovolaemic haemodilution,¹³ we have investigated if decreases in cerebral vascular resistance (CVR) secondary to haemodilution depend on nitric oxide synthase. To do this, we used the nitric oxide synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME).¹⁴ Because administration of L-NAME increases systemic vascular resistance (SVR) and mean arterial pressure (MAP), comparison of a control group and a nitric oxide synthase inhibition group may be confounded by differing MAP values.¹³¹⁵¹⁶ This potential difficulty is eliminated by conducting experiments during CPB where MAP can be
controlled rigidly by changes in pump flow rate. Bypass is also an appropriate model as it constitutes the unique clinical situation where large changes in PCV occur routinely.

Materials and methods
This study follows an earlier report on CPB–haemodilution.3 In that investigation, the cerebral response to progressive CPB–haemodilution was described in eight dogs.3 In the present study, we assessed the contribution of nitric oxide synthase to the cerebral response to haemodilution; the animals of the earlier report served as our control group.

After review and approval by the Institutional Animal Care and Use Committee, we studied nine unmedicated, fasting, adult mongrel dogs, weighing 15–20 kg. The dogs were placed in a Plexiglas box, and anaesthesia was induced with 3–4% inspired halothane. After the onset of anaesthesia, peripheral venous access was obtained, pancuronium 0.1 mg kg⁻¹ administered and the trachea intubated. The lungs were ventilated mechanically using a Harvard pump set to maintain arterial carbon dioxide tension (PₐCO₂) at 4.7–5.3 kPa and arterial oxygen tension (PₐO₂) >20 kPa. Anaesthesia was maintained with fentanyl and midazolam (bolus doses of fentanyl 250 μg kg⁻¹ and midazolam 350 μg kg⁻¹, followed by infusion of fentanyl 3.0 μg kg⁻¹ min⁻¹ and midazolam 9.6 μg kg⁻¹ min⁻¹). Neuromuscular block was maintained by continuous infusion of pancuronium 0.8 μg kg⁻¹ min⁻¹.

For MAP measurements and blood sampling, a catheter was inserted surgically into a femoral artery. After anticoagulation with heparin to maintain a celite-activated clotting time >500 s, the sagittal sinus was exposed, isolated and cannulated, as described previously, for direct measurements of CBF from the anterior, superior and lateral portions of both hemispheres.17 Flow was recorded continuously using a flow-through electromagnetic flow probe (EP300 API, Carolina Medical Electronics, Inc., Kin, NC, USA) which was calibrated as necessary against a graduated cylinder. Brain temperature was measured with a parietal epidural thermocouple. Intracranial pressure was measured with a fibreoptic epidural sensor (LADD Industries, Burlington, VT, USA). The cranium was then closed with surgical and adhesive.

For CPB, a left-sided thoracotomy was performed. Venous drainage to the extracorporeal circuit was by a 10-mm id cannula placed in the right atrium via the right atrial appendage. Blood was circulated by centrifugal pump and a combined heat exchanger–oxygenator (Bentley Spiral Gold, Irvine, CA, USA) and returned via a cannula (4.5-mm id) into the root of the aorta. The system was primed with 750 ml of blood and 250 ml of saline. Throughout CPB, MAP was maintained at 55–70 mm Hg by altering pump flow rate. No vasoconstrictors or vasodilators were used. As CBF is determined by MAP and not by pump flow rate, alterations in CPB flow were used to overcome the effects of L-NAME and haemodilution on SVR. After the onset of CPB, when steady state was achieved, control measurements were obtained.

When CPB conditions were stable, animals (L-NAME group, n=9) were given L-NAME 40 mg kg⁻¹ i.v. This dose of L-NAME effectively blocks nitric oxide synthase.14 18 After infusion of L-NAME, animals were stabilized for 60 min and then underwent serial haemodilution. Haemodilution was achieved by removing blood from the CPB circuit and replacing it with 6% dextran 70 to reach the desired PCV. Measurements were performed at the following target PCV values: 0.39, 0.26, 0.19 and 0.14. Each level of haemodilution was maintained for at least 15 min or until CBF measurements were stable, whichever was longer. Animals in the control group (control group, n=8) underwent the same surgical procedures, and the same CPB–haemodilution management.3

Arterial and cerebral venous blood (from the sagittal sinus catheter) was obtained at each PCV value, and blood-gas concentrations were measured. CBF was measured continuously, and CDO₂ and CMRO₂ were calculated using standard formulae.

Arterial or venous oxygen content: CxO₂ = 1.34×Hb(SxO₂)+0.003 (PₓO₂)
where Hb = haemoglobin concentration; SxO₂ = oxygen saturation; and PₓO₂ = partial pressure of oxygen; x = arterial or venous.

Cerebral oxygen delivery (CDO₂): CDO₂ = CBF×CaO₂.

Cerebral metabolic rate for oxygen (CMRO₂): CMRO₂ = (CaO₂−CvO₂)×CBF ml O₂ 100 g⁻¹ min⁻¹.

Cerebral oxygen extraction ratio (OER): OER = (CaO₂−CvO₂) / CaO₂.

Arterial haemoglobin concentration and blood-gas data were monitored continuously by in line detectors (CDI 100 and CDI 400, Cardiovascular Devices, Inc., Tustin, CA, USA). At each value of PCV, blood-gas values, haemoglobin and oxyhaemoglobin concentrations were measured directly with a blood-gas analyser and CO-oximeter (IL 1306 pH/blood gas analyser and IL 482 CO-oximeter, Instrumentation Laboratory, Lexington, MA, USA). Blood oxygen content was calculated from oxyhaemoglobin concentrations and oxygen tensions measured on electrodes maintained at 37°C. The blood-gas analyser and CO-oximeter were adjusted for the use of dog blood.

CVR was calculated as MAP minus intracranial pressure divided by CBF. Similarly, SVR was determined as the product of MAP and 80 divided by pump flow. A CBF divider into the right atrium to ensure complete caval drainage such that central venous pressure could be assumed to be negligible.

Statistical analysis
All data are presented as mean (SD). A Student’s paired t test was used to compare physiological variables before
and after treatment with l-NAME before haemodilution. Within-group differences in response to haemodilution were assessed by repeated measures analysis of variance, followed by the Student–Newman–Keuls test when necessary.

Differences between the control and l-NAME groups were assessed using unpaired $t$ tests for each PCV value. Because pretreatment with l-NAME significantly altered baseline values for CBF, CVR and SVR in that group, the curves describing the PCV–CVR and PCV–SVR relationships were assessed for each animal for these variables in both groups. Then a comparison of the mean slopes for the curves was performed between the control and l-NAME groups using Hotelling’s $T$ statistic. Regression curves fitted to the equation $y=a+b\times\ln(x)$ were generated from individual values for PCV, CVR and SVR, respectively, for each haemodilution step (Fig. 1). Statistical analysis indicated that the two PCV–CVR slopes did not differ significantly between the control and l-NAME groups.

Because prior treatment with l-NAME increased CVR, CVR in the l-NAME group was higher than that in the control group at every measurement time (Table 3). However, both groups showed equivalent reductions in CVR at each haemodilution step (Fig. 1). Statistical analysis indicated that the two PCV–CVR slopes did not differ significantly between the control and l-NAME groups.

In the control group, mean $\text{CD}_2$ and $\text{CMRO}_2$ did not change significantly as PCV was reduced from 0.39 to 0.14. In contrast, l-NAME significantly decreased $\text{CD}_2$ before haemodilution (Table 1). Subsequent haemodilution further decreased $\text{CD}_2$ in l-NAME-treated animals. The reduction in $\text{CD}_2$ in the l-NAME group resulted in a reduction in $\text{CMRO}_2$ at a PCV of 0.20 or less (Table 3).

Intracranial pressure remained stable in both groups throughout the experiment. Mean values for intracranial pressure in both groups were within the range 6–8 mm Hg (Table 3). Similarly, temperature, $P_{\text{aO}_2}$, $P_{\text{aCO}_2}$, pH and MAP did not differ within or between groups during any experimental period (Table 2).

Progressive CPB–haemodilution led to a significant decrease in SVR (Table 2), but MAP remained stable as a result of controlled increases in pump flow. The reduction in PCV from 0.39 to 0.14 decreased mean SVR in control animals by 35%. A greater reduction in SVR with haemodilution (45%) was demonstrated in the l-NAME group. When the PCV–SVR relationship was compared in control

### Table 1

<table>
<thead>
<tr>
<th>Study period</th>
<th>Group</th>
<th>PCV</th>
<th>CBF (ml 100 g$^{-1}$  min$^{-1}$)</th>
<th>$\text{CMR}_2$ (ml 100 g$^{-1}$  min$^{-1}$)</th>
<th>$\text{CD}_2$ (ml 100 g$^{-1}$  min$^{-1}$)</th>
<th>OER</th>
<th>ICP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before l-NAME</td>
<td>Control</td>
<td>1.8 (0.3)</td>
<td>34 (5)</td>
<td>3.6 (0.8)</td>
<td>6.1 (1.3)</td>
<td>0.59 (0.04)</td>
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<tr>
<td>After l-NAME</td>
<td>l-NAME</td>
<td>2.2 (0.4)*</td>
<td>26 (5)*</td>
<td>3.4 (0.6)</td>
<td>4.8 (1.1)*</td>
<td>0.72 (0.06)*</td>
<td>7 (4)</td>
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</table>

### Table 2

<table>
<thead>
<tr>
<th>Study period</th>
<th>Group</th>
<th>PCV</th>
<th>SVR (dyn s cm$^{-5}$)</th>
<th>MAP (mm Hg)</th>
<th>$\dot{Q}$ (litre min$^{-1}$ m$^{-2}$)</th>
<th>Temp. (°C)</th>
<th>$P_{\text{aO}_2}$ (kPa)</th>
<th>$P_{\text{aCO}_2}$ (kPa)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Control</td>
<td>0.39 (0.05)</td>
<td>2554 (852)</td>
<td>58 (9)</td>
<td>2.0 (0.6)</td>
<td>37.6 (0.5)</td>
<td>36.1 (12.7)</td>
<td>4.5 (0.4)</td>
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<tr>
<td></td>
<td>l-NAME</td>
<td>0.40 (0.06)</td>
<td>4964 (1381)*</td>
<td>65 (6)</td>
<td>1.1 (0.3)*</td>
<td>37.7 (0.3)</td>
<td>45.1 (6.8)</td>
<td>4.4 (0.3)</td>
<td>7.38</td>
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<tr>
<td>II</td>
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<td>0.26 (0.03)$^*$</td>
<td>2373 (429)</td>
<td>64 (4)</td>
<td>2.2 (0.3)</td>
<td>37.7 (0.6)</td>
<td>39.5 (10.7)</td>
<td>4.7 (0.4)</td>
<td>7.35</td>
</tr>
<tr>
<td></td>
<td>l-NAME</td>
<td>0.27 (0.03)</td>
<td>3853 (812)$^{*\ast}$</td>
<td>65 (7)</td>
<td>1.4 (0.4)$^*$</td>
<td>37.7 (0.4)</td>
<td>43.5 (14.4)</td>
<td>4.7 (0.4)</td>
<td>7.34</td>
</tr>
<tr>
<td>III</td>
<td>Control</td>
<td>0.19 (0.02)$^{\ast\ast}$</td>
<td>2289 (555)</td>
<td>63 (3)</td>
<td>2.3 (0.6)</td>
<td>37.8 (0.4)</td>
<td>38.4 (10.1)</td>
<td>4.9 (0.4)</td>
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<tr>
<td></td>
<td>l-NAME</td>
<td>0.20 (0.02)</td>
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<td>1.5 (0.3)$^{\ast\ast}$</td>
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<td>1667 (273)$^\ast$</td>
<td>64 (5)</td>
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<td>2755 (578)$^{*\ast}$</td>
<td>64 (3)</td>
<td>1.9 (0.4)$^\ast$</td>
<td>37.7 (0.4)</td>
<td>47.3 (10.1)</td>
<td>4.7 (0.8)</td>
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Table 3 Cerebral variables in the control and L-NAME groups during progressive haemodilution (mean (SD)). PCV = packed cell volume; CVR = cerebral vascular resistance; CBF = cerebral blood flow; CMRO₂ = cerebral metabolic rate for oxygen; CDO₂ = cerebral oxygen delivery; OER = oxygen extraction ratio; ICP = intracranial pressure. *P < 0.05 control vs L-NAME group; †P < 0.05 vs study period I (within groups)

<table>
<thead>
<tr>
<th>Study period</th>
<th>Group</th>
<th>PCV (mm Hg ml⁻¹ 100 g min)</th>
<th>CVR (ml 100 g⁻¹ min⁻¹)</th>
<th>CBF (ml 100 g⁻¹ min⁻¹)</th>
<th>CMRO₂ (ml O₂ 100 g⁻¹ min⁻¹)</th>
<th>CDO₂ (ml O₂ 100 g⁻¹ min⁻¹)</th>
<th>OER</th>
<th>ICP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>0.39 (0.05)</td>
<td>1.6 (0.6)</td>
<td>33 (10)</td>
<td>3.9 (0.9)</td>
<td>5.8 (1.5)</td>
<td>0.69 (0.07)</td>
<td>8 (6)</td>
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<tr>
<td></td>
<td>L-NAME</td>
<td>0.40 (0.06)</td>
<td>2.2 (0.4)*</td>
<td>26 (5)</td>
<td>3.4 (0.6)</td>
<td>4.8 (1.1)</td>
<td>0.72 (0.06)</td>
<td>7 (4)</td>
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<tr>
<td>II</td>
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<td>0.26 (0.03)*</td>
<td>1.4 (0.3)</td>
<td>43 (11)</td>
<td>3.9 (0.9)</td>
<td>5.2 (0.8)</td>
<td>0.75 (0.06)</td>
<td>7 (4)</td>
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<tr>
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<td>1.8 (0.4)*</td>
<td>34 (7)*</td>
<td>3.3 (0.7)</td>
<td>4.4 (1.1)*</td>
<td>0.77 (0.06)</td>
<td>7 (4)</td>
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<td>Control</td>
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<td>54 (13)*</td>
<td>3.6 (0.7)</td>
<td>4.9 (1.0)</td>
<td>0.76 (0.09)</td>
<td>6 (3)</td>
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<tr>
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<td>0.20 (0.02)*</td>
<td>1.6 (0.4)*</td>
<td>39 (10)*</td>
<td>3.0 (0.8)*</td>
<td>3.9 (1.1)*</td>
<td>0.78 (0.05)</td>
<td>7 (4)</td>
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<td>IV</td>
<td>Control</td>
<td>0.14 (0.01)*</td>
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<td>67 (20)*</td>
<td>3.6 (1.0)</td>
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<td>0.75 (0.09)</td>
<td>6 (4)</td>
</tr>
<tr>
<td></td>
<td>L-NAME</td>
<td>0.14 (0.01)*</td>
<td>1.4 (0.5)*</td>
<td>44 (13)*</td>
<td>2.6 (0.8)*</td>
<td>3.2 (1.0)*</td>
<td>0.81 (0.05)</td>
<td>8 (2)</td>
</tr>
</tbody>
</table>

Fig 1 Cerebral vascular resistance (CVR) vs packed cell volume (PCV) during progressive haemodilution (mean (SD)). Regression curves were generated from 32 individual values for CVR and PCV in the control group and from 36 individual values for CVR and PCV in the L-NAME group.

and L-NAME groups, the relationship demonstrated a significantly steeper slope in the L-NAME group (Fig. 2).

**Discussion**

The cerebrovascular response to haemodilution is still not fully understood. Reductions in PCV increase CBF, but the role of active and passive mechanisms in this effect is debated. It can be argued from the Hagen–Poiseuille equation that the increases in flow with haemodilution are passive and secondary to a reduction in blood viscosity.¹⁻⁵ While this effect is generally agreed upon, it can also be argued that active mechanisms contribute to changes in flow. Even outside of the hypoxic range, there are data to support the argument that increases in CBF with haemodilution are substantially the result of regulation of CDO₂.²⁻⁶ In addition, more recent observations on the interaction of nitric oxide and haemoglobin¹⁹ provide another mechanism by which a change in PCV might alter CBF. In addition to the possible action of haemoglobin in scavenging nitric oxide, it has been suggested that nitric oxide-dependent mechanisms mediate flow increases seen with haemodilution.¹³

One argument in particular can be made. There is good evidence that nitric oxide plays an integral role in flow-mediated vasodilatation in several organ beds.⁸⁻¹⁰ Rubanyi, Romero and Vanhoutte¹⁰ perfused canine femoral artery segments intraluminally and found that increasing flow velocity resulted in vasodilatation. This effect was abolished when the femoral artery endothelium was denuded.¹⁰ Caputo, Todgui and Levy²¹ demonstrated the same effect in carotid arteries. In coronary artery rings in bioassay, Kuo, Chilian and Davis²² showed that flow-mediated dilatation was abolished by either removal of endothelium or pretreatment with the nitric oxide synthase inhibitor, N⁵'-monomethyl-L-arginine (L-NMMA). In rabbit cerebral arteries, flow-mediated vasodilatation was also blunted by exposure of the artery to L-NAME.⁹

The probable mechanism for flow-mediated vasodilatation is that changes in flow velocity alter endothelial shear stress and result in the opening of ion channels.¹¹ The endothelium is capable of mechanotransduction whereby changes in shear stress at the endothelium can activate nitric oxide synthesis via K⁺ or Ca²⁺ channel activation.¹¹¹² This argument is interesting because it provides a way in which passive fluid mechanical changes in blood rheology...
may be integrated with an active process to regulate flow. While this argument is appealing, the net effect of haemodilution on endothelial shear stress is unclear because shear is a function of both flow velocity and fluid viscosity. Increasing flow velocity increases shear while decreasing viscosity reduces shear.

Therefore, we sought to test the hypothesis that nitric oxide-dependent mechanisms play an important role in the changes in CVR demonstrated with haemodilution.

As has been reported previously, we found that exposure to L-NAME increased CVR and reduced CBF but that CMRO₂ was preserved. In addition, as has been well described, haemodilution resulted in large increases in CBF and preservation of CD₀₂ and CMRO₂. However, the increases in CBF in the control group were greater than those in the L-NAME group at the lowest PCV values. This led us to believe initially that a nitric oxide-dependent mechanism was of primary importance for the increases in flow seen with a reduction in PCV. However, recognition of the baseline reduction in CBF with nitric oxide synthase inhibition and examination of the changes in CVR with progressive haemodilution provided important information. Because CVR is determined from CBF, MAP and intracranial pressure, it represents the integrated effect of haemodilution and nitric oxide synthase inhibition on the cerebral vasculature. When the PCV–CVR curves were analysed, the control and L-NAME groups showed parallel curves (Fig. 1). The curves differed only in the offset in CVR that resulted from L-NAME treatment before haemodilution. This argues strongly against a nitric oxide synthase-dependent mechanism causing the increases in CBF demonstrated with haemodilution.

Although this was designed as a study of cerebral physiology, our data regarding the relationship between haemodilution and SVR also support this conclusion. Figure 2 shows that the reduction in SVR with haemodilution was greater in the L-NAME than in the control group. If nitric oxide mediated the systemic vascular response to haemodilution, the opposite effect would have been expected. This observation, in common with that of the changes in CVR, argues against an important role of nitric oxide in the flow increases associated with haemodilution.

A criticism of our study is that the investigation in the control group was performed before that of the L-NAME group. Our study arose from an attempt to examine further the physiology underlying the earlier report. The two studies were of the same design and it did not seem justifiable to sacrifice additional animals to repeat a study of CBF changes under control conditions. We are aware that this potentially weakens our results. However, our laboratory has considerable experience in studies of this type and we would not expect differences in the results if the control group was repeated.

This investigation may have been strengthened if nitric oxide synthase inhibition by L-NAME was assessed. However, L-NAME is one of the most widely used nitric oxide synthase inhibitors and its action has been investigated extensively. The amount of L-NAME administered was the same as used by other investigators in the same species, and the duration of inhibition of nitric oxide has been found to be at least 2 h. L-NAME has also been used successfully for experiments on CPB. If the effect of L-NAME was abolished towards the end of the experiments, the curve describing the PCV–CVR relationship would not be parallel at the lower PCV values, and in the L-NAME group, CVR would have decreased markedly with loss of nitric oxide synthase inhibition. But this was not the case and the L-NAME and control groups showed equivalent reductions in CVR at each haemodilution step (Fig. 1).

The study could also be criticized for our use of progressive haemodilution. However, we found previously that it was impractical to randomly evaluate different PCV values in a large animal CPB study. Randomization of PCV would have doubled or tripled the requirement for dogs serving as blood donors. Furthermore, if one considers the challenge, for example, of sequentially changing PCV during CPB from 0.15 to 0.40 to 0.10 to 0.27, one realizes the logistical and physiological difficulties that would be experienced with this design. Finally, as an attempt to follow up on a previous report, it was essential that we followed the same design.

A criticism that could be raised is that we conducted these studies during CPB. We chose to do this for three reasons. First, only during CPB are large, acute changes in PCV ‘normally’ experienced and therefore of practical relevance. Second, during CPB the potential effects of haemodilution or L-NAME treatment on the heart are eliminated. Specifically, the physiological changes we measured are not limited by the ability of the heart to respond to progressively lower PCV values. Third, and most importantly, systemic treatment with nitric oxide synthase inhibitors, and L-NAME specifically, causes a significant increase in arterial pressure unless measures are taken to prevent this. Studies which compare control and nitric oxide synthase inhibitor treatment are confounded by increases in MAP in the treatment group. We wished to avoid this potentially confounding variable. This is readily done during CPB where the perfusion pressure can be controlled accurately.

It can also be argued that CPB is not a good model because it may induce an inflammatory response in which cytokines increase nitric oxide release via expression of inducible nitric oxide synthase (iNOS). We believe this is of minor relevance because iNOS, requiring protein synthesis, needs more than 2 h to be expressed in brain tissue. In practice, the most substantial changes attributable to CPB are secondary to changes in temperature and acute haemodilution, and if temperature and PCV are maintained at non-CPB levels, the cerebral physiological changes with CPB are difficult to identify. Qualitatively and quantitatively, our data suggest that the CPB model is appropriate. Our CBF and CMRO₂ values and the relative
changes with haemodilution resemble those reported under non-bypass conditions.\textsuperscript{1,4} The same was true of the changes in CVR we documented with L-NAME treatment.\textsuperscript{15} The absolute values and changes in CVR we described agreed with those of McPherson, Koehler and Traystman\textsuperscript{18} reported previously in dogs under non-CPB conditions.

Although nitric oxide-dependent mechanisms do not appear to play a primary role in the flow changes demonstrated with haemodilution per se, the brain is less tolerant of haemodilution when nitric oxide synthesis is inhibited. This is reflected in the requirement for a greater PCV when nitric oxide synthase is inhibited. We reported previously that the critical PCV for the brain during normothermic CPB was approximately 0.15.\textsuperscript{3} At haemoglobin concentrations less than this, CMR O\textsubscript{2} became delivery dependent. In our L-NAME group, critical PCV was reached at a PCV greater than 0.20. However, this does not demonstrate that nitric oxide mediates the flow response to haemodilution. This greater critical PCV is probably observed for at least three reasons. First, nitric oxide synthase inhibition had a cerebrovasoconstrictive effect in that group before haemodilution. Hence the group treated with L-NAME may have had a reduced reserve in its ability to regulate CBF. Second, endothelial nitric oxide synthase is probably neuroprotective in studies of ischaemia.\textsuperscript{30} Therefore, nitric oxide synthase inhibition may have rendered that group more susceptible to anaemic hypoxia.

A third mechanism may also cause a lower tolerance for haemodilution in the L-NAME group. Recent reports from Stamler and colleagues suggest that oxyhaemoglobin serves to transport nitric oxide to the microvasculature where nitric oxide may be fundamental in regulating tissue oxygen delivery.\textsuperscript{19} This mechanism might also be impaired by treatment with the nitric oxide synthase inhibitor L-NAME. This combination of mechanisms probably accounts for the greater critical PCV, and the reduced CBF and CMR O\textsubscript{2} relative to the control group after the greatest degrees of haemodilution. These haemodilution-independent roles of nitric oxide synthase may have led us to conclude initially that nitric oxide synthase mediated the cerebral flow response to haemodilution.\textsuperscript{23}

Finally, our data do not provide evidence that haemodilution-associated increases in CBF are purely passive; that is, viscosity-mediated. Nitric oxide synthase independent mechanisms may also be active. However, these data showed that in a canine CPB model, nitric oxide synthase did not play a primary role in increases in CBF seen with haemodilution.

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References

11. Olesen SP, Clapham DE, Davies PF. Haemodynamic shear stress activates a K\textsuperscript{+} current in vascular endothelial cells. Nature 1988; 331: 168–70
17. Michenfelder JD, Milde JH. The relationship among canine brain temperature, metabolism, and function during hypothermia. Anesthesiology 1991; 75: 130–6
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23 Cook DJ. Nitric oxide is a mediator of the cerebral vascular response to hemodilution. *FASEB J* 1996; 10: A543


