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# Inhibition of Nitric Oxide but Not Prostacyclin Prevents Poststenotic Dilatation in Rabbit Femoral Artery

William J. Calvo, PhD; George Hajduczuk, PhD; James A. Russell, PhD; Scott L. Diamond, PhD

**Background**—Poststenotic dilatation (PSD) occurs in a low-pressure region where recirculation eddies oscillate in size during the cardiac cycle. NO may be an important mediator of PSD.

**Methods and Results**—Femoral arteries of 7 adult male New Zealand White rabbits were stenosed bilaterally to achieve a diameter reduction of  $70.9 \pm 6.7\%$  ( $n=14$ ). At the time of stenosis, the adventitia of one of the arteries was coated with 1 mmol/L of  $N^G$ -nitro-L-arginine methyl ester (L-NAME) in 22% (wt/vol) Pluronic gel, while the contralateral vessel was coated with gel without L-NAME. In stenosed femoral arteries that were treated with gel without L-NAME, a maximum PSD of  $30.99 \pm 7.92\%$  ( $n=7$ ) was observed in polymer casts at 3 days relative to the mean proximal diameter of  $1.57 \pm 0.25$  mm at a position 12 mm upstream of each stenosis. In contrast, the vessels treated with L-NAME exhibited a maximum PSD of only  $7.16 \pm 8.81\%$  ( $n=7$ ) relative to the mean proximal diameter of  $1.55 \pm 0.16$  mm. L-NAME caused a 76.9% reduction ( $P<0.001$ ,  $n=7$ ) of PSD. Similarly,  $N^G$ -monomethyl-L-arginine 1 mmol/L and  $N^G$ -nitro-L-arginine 10  $\mu$ mol/L attenuated PSD by 57.5% ( $P<0.001$ ,  $n=6$ ) and 63.9% ( $P<0.05$ ,  $n=6$ ), respectively. Indomethacin 10  $\mu$ mol/L caused no reduction in PSD. Arterial rings obtained from the poststenotic region were more sensitive and responsive to acetylcholine than those obtained proximal to the stenosis.

**Conclusions**—NO, but not prostacyclin, is a major mediator of PSD. (*Circulation*. 1999;99:1069-1076.)

**Key Words:** endothelium ■ hemodynamics ■ stenosis

The pronounced dilatation of vessels distal to a stenosis is a dramatic response of vessel and vascular cell function to hemodynamic forces. The earliest descriptions of poststenotic dilatation (PSD) date back to 1842<sup>1</sup> and 1845 (as cited in References 2 and 3), but the mechanisms and mediators of the process are still unknown. As reviewed by Roach,<sup>4</sup> aneurysms associated with PSD can occur in humans in regions distal to the coarctation of the aorta, abdominal aorta, and pulmonary arteries. Atherosclerotic plaques of the renal, carotid, and femoral arteries can cause PSD, as can compression of arteries by abnormal anatomy of bones, muscle, or ligaments. PSD can be generated experimentally in rabbit carotid and thoracic arteries<sup>5,6</sup> and canine femoral and carotid arteries<sup>4</sup> and is reversible after removal of the stenosis.<sup>7</sup> Fenestration and fragmentation of the internal elastic lamina increase distal to a stenosis<sup>8,9</sup> within 10 days of vessel stenosis. Degenerative changes during PSD include a decrease in smooth muscle cell number density and elastin content and an increase in collagen<sup>6</sup> and collagenase activity.<sup>10</sup>

The studies by Roach<sup>4</sup> and others in the 1960s can be viewed in the context of the well-established endothelial response to hemodynamic forces (for review, see References

11 and 12). Fluid shear stress enhances, within seconds, endothelial production of NO and prostacyclin,<sup>13–15</sup> both of which are relatively short-lived species that act locally. Exposure to arterial shear stresses elevates endothelial NO synthase (eNOS) mRNA and protein<sup>16,17</sup> within a few hours and suppresses endothelin gene expression in cultured endothelium.<sup>18,19</sup> The NO production and eNOS mRNA levels are elevated during the stretching of endothelial cells,<sup>20</sup> as is endothelin production.<sup>21</sup> Yet, these studies do not fully explain the occurrence of PSD, because the region distal to the stenosis is a site of low pressure and complex hemodynamics where recirculation eddies oscillate in size as the flow reattachment point moves back and forth during the cardiac cycle.<sup>22–24</sup>

Turbulence-induced vibration had been hypothesized to cause PSD, because isolated human iliac arteries dilate when vibrated via a loudspeaker.<sup>2</sup> However, Gow et al<sup>25</sup> showed that mechanical vibration of rabbit thoracic aorta in vivo does not cause vasodilation. In addition, Ojha and Langille<sup>26</sup> conducted extensive flow visualization of model stenosed rabbit carotids and found that PSD can occur with stenoses from 50% to 60% diameter reduction in the carotid arteries, whereas no transition to turbulence is observed in corre-

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From the Bioengineering Laboratory, Department of Chemical Engineering (W.J.C.), and the Department of Physiology and Biophysics, School of Medicine (G.H., J.A.R.), State University of New York at Buffalo, and the Institute for Medicine and Engineering, Department of Chemical Engineering, University of Pennsylvania, Philadelphia, Pa (S.L.D.).

Correspondence to Scott L. Diamond, Institute for Medicine and Engineering, Department of Chemical Engineering, 394 Towne Bldg, University of Pennsylvania, Philadelphia, PA 19104. E-mail sld@eniach.seas.upenn.edu

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sponding flow models. In flow models of more severe carotid stenoses of 70% diameter reduction, a very localized transition to turbulence was identified (6 to 8 tube diameters downstream) during the early deceleration phase of the cardiac cycle.<sup>26</sup> However, this position of helical flow and vortex shedding (a transition to turbulence) did not correlate with the position of maximal PSD. These studies indicate that turbulence is not strictly required for the development of PSD.

Locally elevated capacity of the vessel wall to produce and/or respond to NO may be the cause of PSD and represents a response to the complex hemodynamics within the poststenotic recirculating vortex. Our study investigated pharmacological antagonism of NO production at the site of a stenosis to modulate the progression of PSD. The stenoses of 70% reduction in diameter used in this study are not associated with turbulence and create a modest reduction in flow associated with vasoconstriction proximal to the stenosis.<sup>27</sup>

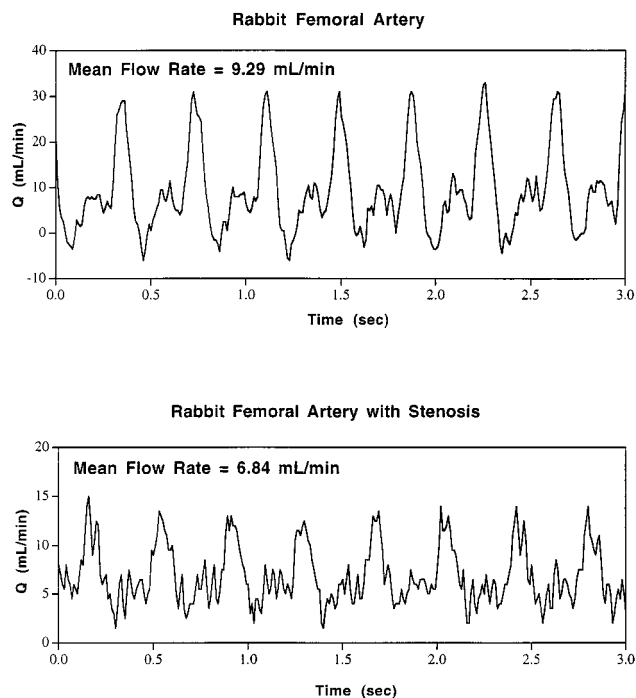
## Methods

### Animal Surgery

Male New Zealand White rabbits ( $2.1 \pm 0.3$  kg) were anesthetized with ketamine (30 to 40 mg/kg IM) and xylazine (5 to 8 mg/kg IM). The femoral arteries were exposed distal to the hip joint and carefully isolated from the femoral vein and sciatic nerve. A well-defined stenosis was produced on the left and right arteries according to the methods of Langille et al<sup>28</sup> by use of a 4-0 Tevdek (polyester fiber, nonabsorbable) suture tied snugly around the vessel and the shaft of a 25-gauge hypodermic needle (OD, 0.51 mm). The needle shaft was then removed. The L-arginine analogue *N*<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME) (Research Biochemicals International) was added after stenosis by coating the adventitia of one of the exposed femoral arteries with 1 mL of a sterile suspension of 1.0 mmol/L L-NAME in 22.5% (wt/vol) of F-127 Pluronic gel (Molecular Probes, Inc) diluted in sterile PBS. The contralateral femoral artery was treated with 1 mL of a sterile suspension of 22.5% (wt/vol) of F-127 Pluronic gel alone. In some experiments, *N*<sup>G</sup>-monomethyl-L-arginine (L-NMMA) 1 mmol/L, *N*<sup>G</sup>-nitro-L-arginine (L-NNA) 10  $\mu$ mol/L, or indomethacin 10  $\mu$ mol/L was in the gel. In experiments using unilateral stenosis without Pluronic gel, we observed that the gel was not required for the development of PSD. Placement of a sterile 2-mm transit-time ultrasonic probe (Transonics Systems, Inc) before and after the placement of the stenosis allowed measurement of volumetric flow rate (mL/min) through the femoral vessels. Time-averaged flows through rabbit femoral artery were  $\approx 10$  mL/min before stenosis. After placement of the suture, the stenoses caused an  $\approx 30\%$  reduction in mean flow (Figure 1). Rabbits were given Crystiben (sterile penicillin G benzathine and penicillin G procaine in aqueous suspension; 300 000 IU/mL) on the day of surgery (1 mL) and every 48 hours afterward. All surgeries were performed in the School of Medicine, Laboratory Animal Facility, at SUNY–Buffalo under approval by the Internal Review Board.

### Polymer Casting and Diameter Analysis

At 3 days after surgery, the rabbits were anesthetized by injection of 30 mg/kg sodium pentobarbital IV through the marginal ear vein. The midabdominal aorta was catheterized and perfused with a warm saline solution (37°C) followed by a methyl methacrylate casting compound (Batson's No. 17 corrosion casting compound, Polysciences Inc) under a constant pressure of 100 mm Hg, according to the methods of Langille<sup>27,28</sup> and Levesque.<sup>29</sup> After the compound had set for 24 hours, the abdominal aorta and the femoral branches were dissected as a unit. The remaining tissue was removed by immersion of the cast into 25% NaOH at 50°C for 12 hours. Diameters along the vessel cast were measured by calibrated light microscopy with NIH Image 1.54 software (pixel resolution of

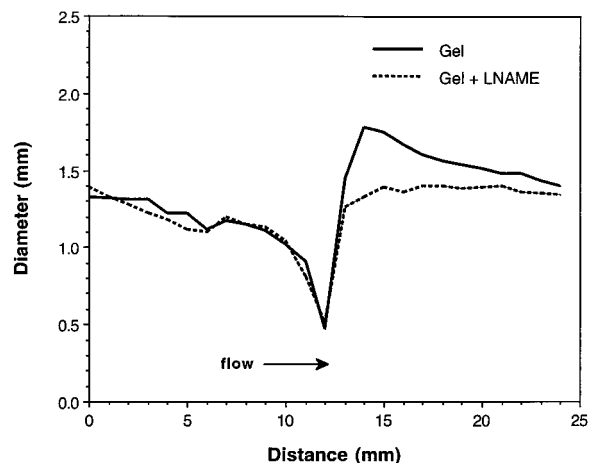


**Figure 1.** Blood flow rate (mL/min) in rabbit femoral artery before (top) and after (bottom) 75% diameter reduction stenosis.

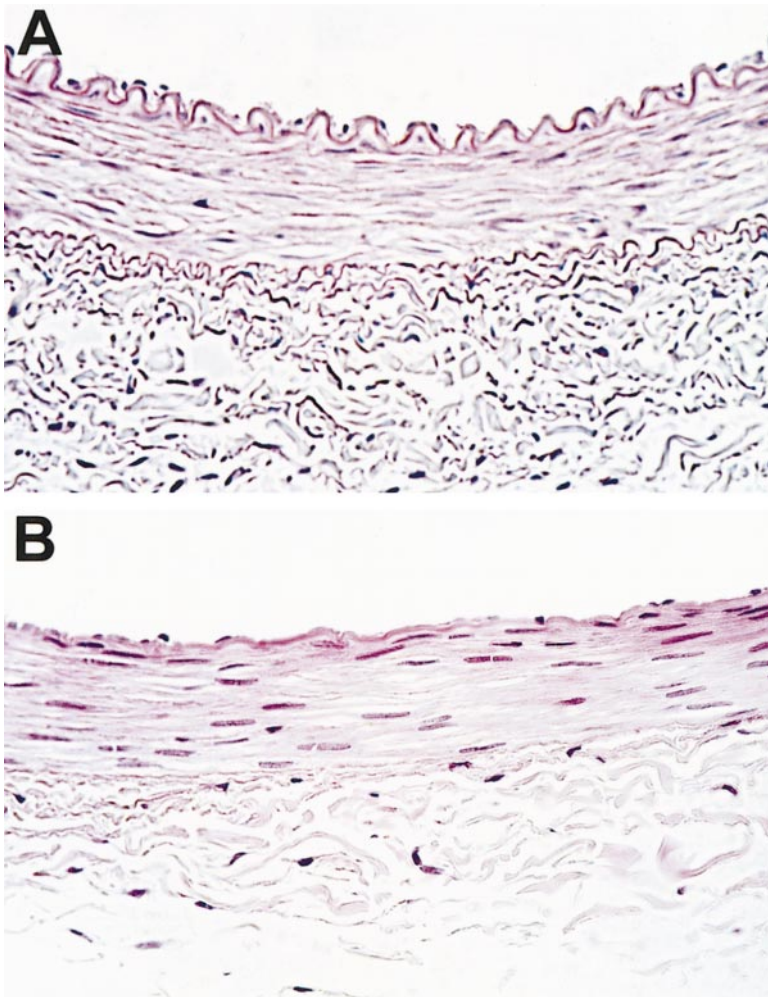
$\pm 0.01$  mm). Recent studies by Moore et al<sup>30</sup> demonstrated excellent geometric fidelity of the above casting technique compared with in vivo determination by MRI of the geometry of the aortoiliac bifurcation in New Zealand White rabbits. Diameter data from vessel casts obtained from  $n=5$  to 7 animals were then averaged by aligning the position of the stenosis. The percent stenosis was calculated as the diameter of the stenosis ( $D_{\text{stenosis}}$ ) relative to  $D_{\text{prox}}$  at 12.0 mm proximal to the stenosis by Equation 1:

$$(1) \quad \% \text{ stenosis} = \left( \frac{D_{\text{prox}} - D_{\text{stenosis}}}{D_{\text{prox}}} \right) \times 100.$$

Similarly, the maximum percent PSD (max % PSD) was calculated for the maximum distal diameter ( $D_{\text{distal}}^{\text{max}}$ ) relative to the diameter ( $D_{\text{prox}}$ ) at 12.0 mm proximal to the stenosis by Equation 2:



**Figure 2.** Diameters from polymer casts of pressure-fixed rabbit femoral arteries, each with a stenosis in place for 3 days, where one femoral artery was coated with gel only (solid line) and the other coated with gel containing 1 mmol/L L-NAME (dashed line). Flow was from left to right.



**Figure 3.** Cross sections are shown for rabbit femoral artery obtained proximal (A) and distal (B) to stenosis after 3 days of stenosis.

$$(2) \quad \max \% \text{ PSD} = \left( \frac{D_{\text{distal}}^{\max} - D_{\text{prox}}}{D_{\text{prox}}} \right) \times 100.$$

The mean value of the max % PSD of casts (determined by Equation 2) does not necessarily correspond to the % PSD observed in averaged aligned cast diameter data sets because the exact position of maximal PSD of each cast varied slightly.

### Computational Fluid Dynamics

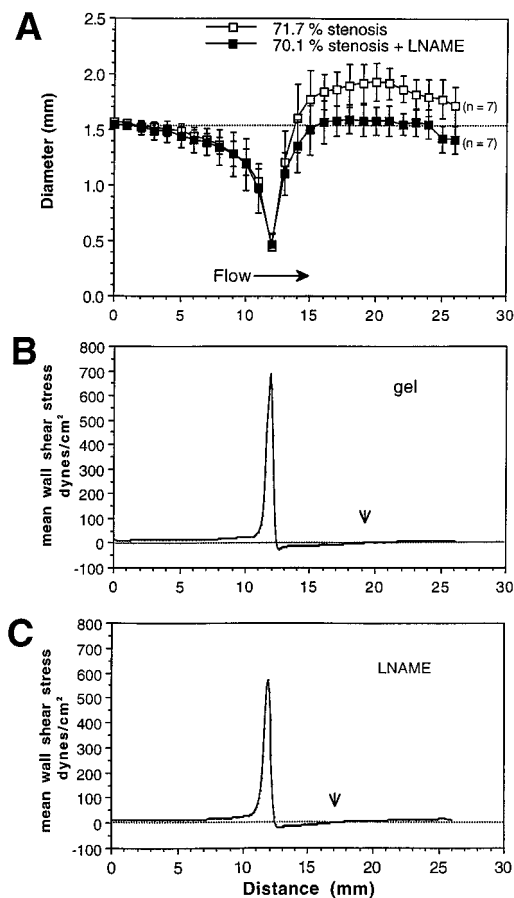
The velocity field and mean wall shear stress for cast geometries were obtained by Galerkin finite-element method (FIDAP 7.0, Fluid Dynamics International) solution of the Navier-Stokes equation for steady, laminar flow of a Newtonian fluid equivalent to blood (viscosity, 0.035 poise) through the 2-dimensional axisymmetric cast geometry as described by Strony et al.<sup>31</sup> Assuming blood to behave as a Newtonian fluid results in wall shear stresses that have been shown to be accurate to  $\approx 10\%$  compared with a more complex constitutive equation for shear thinning behavior.<sup>31</sup> Meshes were refined to  $>14\,500$  quadrilateral elements, with increased mesh resolution at the stenosis and near the wall to eliminate spurious numerical oscillations in the velocity field. Fully developed parabolic flow was used as the inlet condition, corresponding to a mean volumetric flow of 7 mL/min (as observed experimentally in Figure 1, bottom), and the no-slip boundary condition was applied at the wall.

### Vascular Ring Studies

For ring studies, the femoral arteries were dissected from anesthetized rabbits that had 3-day unilateral stenosis. The arteries were

placed in room-temperature Krebs-Ringer solution (in mmol/L: NaCl 118, KCl 4.7,  $\text{CaCl}_2$  2.5,  $\text{KH}_2\text{PO}_4$  1.2,  $\text{MgSO}_4$  1.2,  $\text{NaHCO}_3$  25.5, glucose 5.6). Dissected arteries were cut into rings 2 to 3 mm wide (3 to 4 mg each), mounted on stainless steel hooks, and placed in water-jacketed organ baths maintained at  $37^\circ\text{C}$  as previously described.<sup>32</sup> Arteries were bathed in 6 mL of Krebs-Ringer solution aerated with a mixture of 94%  $\text{O}_2$  and 6%  $\text{CO}_2$  to obtain a pH of 7.4, a  $\text{PCO}_2$  of 38 mm Hg, and a  $\text{Po}_2 > 500$  mm Hg. Continuous isometric force readings were obtained with a force-displacement transducer (Statham UC 2). All rings were allowed to equilibrate for 15 minutes in the Krebs-Ringer solution. Rings were then placed at their optimal length by repeated stretching in small increments over the next 20 minutes until resting tone remained stable at 0.8 g. This procedure places each vessel at its optimal length.<sup>33</sup> Rings were precontracted with an  $\text{EC}_{50}$  concentration of the  $\alpha_1$ -adrenergic receptor agonist phenylephrine. When the contraction had reached a plateau, acetylcholine  $10^{-8}$  to  $3 \times 10^{-5}$  mol/L was added to the bathing solution in a cumulative manner to induce endothelium-dependent vascular relaxation by activating NOS in the endothelium. Before exposure to phenylephrine, all tissues were incubated with  $10^{-6}$  mol/L propranolol for 5 minutes to prevent any potential stimulation of  $\beta$ -adrenergic receptors and with  $10^{-5}$  mol/L indomethacin to block production of prostaglandins. Data were expressed as mean  $\pm$  SEM. Statistical comparisons for the vascular reactivity studies were performed on the concentration-response curves by use of ANOVA with Student-Newman-Keuls test for post hoc testing of multiple comparisons. The 50% inhibitory concentrations ( $\text{IC}_{50}$ ) for acetylcholine inhibition of constriction were obtained from the concentration-response curves in a similar manner. Significance was accepted at a value of  $P < 0.05$ .





**Figure 4.** Average diameter of rabbit femoral artery stenosed for 3 days in absence ( $\square$ ) or presence ( $\blacksquare$ ) of adventitial administration of L-NAME 1 mmol/L (A). Vessel geometries were used for 2-dimensional computational fluid dynamic analysis of time-averaged wall shear stress (dynes/cm<sup>2</sup>) for mean blood flow rate of 7 mL/min (B). Mean positions of flow reattachment points are indicated (arrows) for vessels stenosed in absence (B) or presence (C) of adventitial L-NAME. Flow was from left to right.

## Results

After 3 days, the stenosis of the femoral artery without L-NAME caused a pronounced dilatation at positions distal to the stenosis, as seen in the polymer cast, which was blocked by adventitial application of L-NAME (Figure 2). In this experiment, oxygen delivery due to an arterial flow was quite large, and any putative hypoxic dilatation would be much less than the observed PSD.<sup>34</sup> Furthermore, in large arteries, L-NAME would not be expected to completely antagonize dilatation due to hypoxia,<sup>35</sup> because intrinsic mechanisms during hypoxia would predominate.<sup>36</sup> Histology of the vessel wall after 3 days of stenosis is consistent with proximal vasoconstriction (Figure 3A) and PSD (Figure 3B).

The average diameters for aligned casts of stenosed femoral arteries in the absence or presence of L-NAME after 3 days of bilateral stenosis are shown in Figure 4A. In gel-treated femoral arteries (no L-NAME), the position of maximal PSD was  $6.1 \pm 2.8$  mm ( $n=7$ ) distal to the stenosis, corresponding to  $\approx 4$  vessel diameters downstream of the stenosis. In each of the 7 rabbits, L-NAME treatment of one of the stenosed femoral arteries attenuated the development

of a large PSD compared with the contralateral stenosed vessels (Table). The average percent stenosis for the 14 stenoses was  $70.9 \pm 6.7\%$  (Table). In gel-treated femoral arteries (no L-NAME), the max % PSD ranged from 17.9% to 39.7% (mean,  $30.99 \pm 7.92\%$  max % PSD,  $n=7$ ) after 3 days for  $71.7 \pm 7.01\%$  stenosis (diameter reduction) of rabbit femoral artery. In contrast, L-NAME-treated femoral arteries displayed significantly less PSD, ranging from 0% to 25.0% max % PSD (mean,  $7.16 \pm 8.81\%$  max % PSD,  $n=7$ ). Thus, 1 mmol/L of L-NAME caused a 76.9% reduction ( $P<0.001$ ) in PSD. The mean diameter at a position of 12 mm proximal to the stenosis was  $1.57 \pm 0.25$  mm for gel-treated vessels, compared with  $1.55 \pm 0.16$  mm for L-NAME-treated vessels. Consistent with the observations in the rabbit and canine carotid artery during chronic decreased flow,<sup>27,37</sup> we have observed proximal vasoconstriction ( $n=8$ ) in formalin-fixed or polymer-casted rabbit femoral arteries that had a stenosis relative to unstenosed contralateral sham-operated controls (data not shown).

Computational fluid dynamic analysis of the average vessel geometries shown in Figure 4A was conducted at a mean steady flow rate of 7.0 mL/min (see Figure 1) through each geometry. The wall shear stress reached peak values of  $\approx 600$  to 700 dynes/cm<sup>2</sup> in the throat of the stenosis, as expected for converging flows.<sup>31</sup> A prominent poststenotic vortex was observed in the simulations. The time-averaged position of flow reattachment was predicted to occur at 7 mm and 5 mm distal to the stenosis for gel-treated and L-NAME-treated vessel geometries, respectively (Figure 4B and 4C). This predicted position of reattachment corresponded well with the position of max % PSD of  $6.1 \pm 2.8$  mm observed in the casts of the no-L-NAME group. The spatial wall shear stress gradient as determined by computational fluid dynamic analysis at the position of flow reattachment  $[(\partial \tau_w / \partial x)|_{\tau_w=0}]$  was  $+29.7 \pm 0.3$  and  $+50.4 \pm 0.5$  dynes  $\cdot$  cm<sup>-2</sup>  $\cdot$  cm<sup>-1</sup> in gel-treated and L-NAME-treated vessel geometries, respectively, suggesting that the PSD response caused a  $>40\%$  reduction ( $P<0.001$ ) of the time-averaged shear stress gradient at the site of flow reattachment. In both gel- and L-NAME-treated stenosed vessels, the mean wall shear stress at positions between the stenosis and the reattachment point were elevated to 15 to 25 dynes/cm<sup>2</sup> relative to the far upstream shear stress of  $\approx 13$  to 15 dynes/cm<sup>2</sup>.

For 6 rabbits with bilateral stenosis with one of the stenosed femoral arteries treated with L-NMMA, the L-NMMA caused a 57.5% reduction ( $P<0.001$ ,  $n=6$ ) in PSD, from  $27.3 \pm 5.6\%$  to  $11.6 \pm 3.1\%$  average max % PSD (Figure 5A). For 6 rabbits with bilateral stenosis with one of the stenosed femoral arteries treated with L-NNA, the L-NNA caused a 63.9% reduction ( $n=6$ ,  $P<0.05$ ) in PSD, from  $24.7 \pm 17.2\%$  to  $8.93 \pm 10.1\%$  average max % PSD (Figure 5B).

A possibility exists that L-NAME may attenuate endothelial production of prostacyclin in the presence of flow<sup>38–40</sup> or antagonize muscarinic receptors.<sup>41</sup> We used adventitial delivery of 10  $\mu$ mol/L indomethacin in Pluronic gel to inhibit cyclooxygenase activity in a stenosed vessel (Figure 6A). Indomethacin had no effect on the development of PSD. The average max % PSD for indomethacin-treated stenoses was

**Demonstration of Prevention of PSD by Local Adventitial Administration of L-NAME 1 mmol/L Relative to Gel-Treated Stenosed Vessels Without L-NAME**

Rabbit, Vessel Treatment	Proximal Diameter, mm	Stenosis Diameter, mm	Percent Stenosis, %	Maximal Distal Diameter, mm	Percent PSD, %
<b>A</b>					
Gel	2.03±0.01	0.74±0.01	63.5	2.52±0.01	24.1
L-NAME	1.82±0.01	0.55±0.01	69.8	1.82±0.01	0.0
<b>B</b>					
Gel	1.65±0.01	0.33±0.01	80.0	2.29±0.01	38.8
L-NAME	1.54±0.01	0.40±0.01	74.2	1.73±0.01	12.3
<b>C</b>					
Gel	1.63±0.01	0.31±0.01	81.0	2.10±0.01	28.8
L-NAME	1.62±0.01	0.39±0.01	75.9	1.67±0.01	3.1
<b>D</b>					
Gel	1.62±0.01	0.43±0.01	73.5	1.91±0.01	17.9
L-NAME	1.62±0.01	0.47±0.01	71.0	1.65±0.01	1.9
<b>E</b>					
Gel	1.39±0.01	0.39±0.01	71.9	1.86±0.01	33.8
L-NAME	1.57±0.01	0.35±0.01	77.7	1.61±0.01	2.5
<b>F</b>					
Gel	1.33±0.01	0.47±0.01	64.7	1.78±0.01	33.8
L-NAME	1.39±0.01	0.51±0.01	63.3	1.40±0.01	5.3
<b>G</b>					
Gel	1.31±0.01	0.43±0.01	67.2	1.83±0.01	39.7
L-NAME	1.32±0.01	0.54±0.01	59.1	1.65±0.01	25.0
<b>Mean±SD (n=7)</b>					
Gel	1.57±0.25	0.44±0.14	71.7±7.01	2.04±0.28	30.99±7.92
L-NAME	1.55±0.16	0.46±0.08	70.1±6.79	1.65±0.13*	7.16±8.81*
				( <i>P</i> <0.005)	( <i>P</i> <0.001)

Proximal diameters were taken at a position of 12.0 mm upstream of the site of the stenosis. The average percent stenosis based on diameter for all arteries relative to their proximal diameters was 70.9±6.7% (n=14).

\**P* value relative to mean value for gel-treated control vessel.

26.4±11.5% (n=5), compared with 23.6±9.5% (n=5) for contralateral stenoses without indomethacin treatment. When both L-NAME and indomethacin were applied adventitially to a stenosed femoral artery (Figure 6B), an 11.8±8.15% (n=5) average max % PSD occurred, whereas the contralateral stenosed vessel treated only with indomethacin displayed a 29.4±8.75% (n=5) average max % PSD. Thus, L-NAME caused a 59.9% reduction in the formation of PSD when both vessels were simultaneously treated with indomethacin.

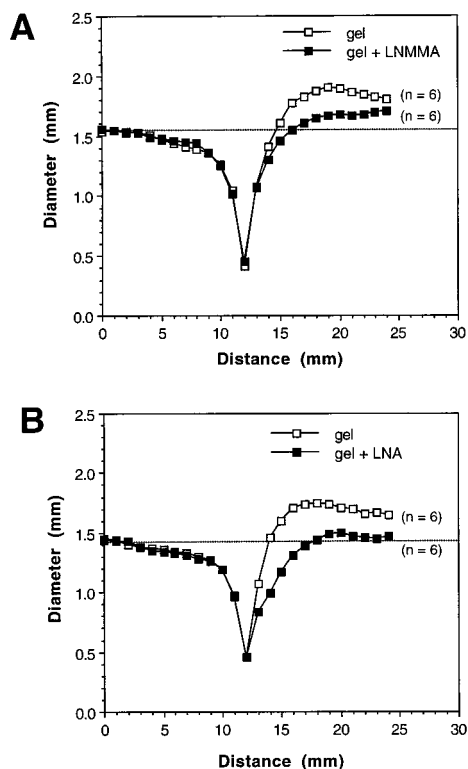
Over all experiments, stenosis of rabbit femoral artery produced 26.9±10.6% max % PSD (n=24) that was dramatically and significantly reduced in the 19 stenosed vessels treated with L-arginine analogues (L-NAME, L-NMMA, or L-NNA) and additionally in the 5 vessels treated with L-NAME plus indomethacin but was not reduced in any of the 10 vessels treated with indomethacin alone (Figure 7).

In control vessel rings and rings taken proximal (2 to 12 mm) and distal (2 to 12 mm) to the stenosis, acetylcholine at concentrations from 10<sup>-8</sup> to 3×10<sup>-6</sup> mol/L caused relaxation (Figure 8) and at higher concentrations of >10<sup>-5</sup> mol/L caused mild contraction, by stimulating the muscarinic receptors on vascular smooth muscle cells.

In rings distal to the stenosis, 10<sup>-6</sup> mol/L acetylcholine was sufficient to produce nearly 90% of maximal vasorelaxation (n=8), which significantly exceeded (*P*<0.01) the 35% and 45% relaxations achieved in proximal rings (n=9) and control rings (n=6), respectively. The 50% inhibitory concentration (IC<sub>50</sub>) from the acetylcholine dose-response curves in Figure 8 was left-shifted (*P*<0.05) for PSD segments (IC<sub>50</sub>=87±19 nmol/L, n=8) compared with proximal rings (IC<sub>50</sub>=256±60 nmol/L, n=9) or control rings (IC<sub>50</sub>=324±96 nmol/L, n=6). In addition, the poststenotic segments displayed relaxations of greater magnitude (at all doses of acetylcholine) than proximal or control rings, consistent with elevated capacity to produce and/or respond to NO in the poststenotic region. Control and proximal vessel rings precontracted to their EC<sub>50</sub> with phenylephrine achieved similar tensions of 1015±262 g/g tissue (n=6) and 1480±204 g/g tissue (n=9), respectively (*P*<0.001). However, distal vessel rings exhibited a greater EC<sub>50</sub> tension of 1625±159 g/g tissue (*P*<0.05).

## Discussion

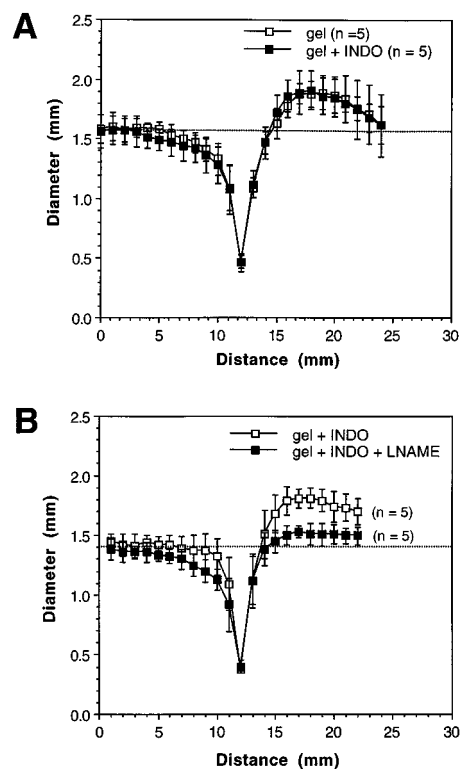
We have shown that inhibition of NO production by L-arginine analogues significantly attenuated the develop-



**Figure 5.** PSD occurred in rabbit femoral arteries with stenosis after 3 days ( $\square$ ), but PSD development was attenuated in contralateral stenosed femoral artery treated ( $\blacksquare$ ) with adventitial administration of Pluronic gel containing (A) 1 mmol/L L-NMMA or (B) 10  $\mu$ mol/L L-NNA. Flow was from left to right. SDs ( $\pm 0.2$  mm) of each mean value are not shown for clarity.

ment of PSD. To the best of our knowledge, this is the first report of pharmacological antagonism of PSD. Furthermore, the enhanced vascular reactivity of vessel rings to acetylcholine in the poststenotic region is consistent with an important role for endothelium-produced NO as a mediator of PSD. In the present study, large-scale turbulence throughout the flow field is not expected, because the upstream, time-averaged Reynolds numbers were  $<100$ , based on the mean velocity. Turbulence would be expected to occur in this geometry at a Reynolds number of 300 to 400.<sup>42</sup> In light of several reports<sup>12–14,16,17</sup> of steady unidirectional laminar shear stress inducing NO production and elevating eNOS gene expression levels in cultured endothelial cells, elevated wall shear stress or rapid changes in direction and magnitude of wall shear stress are the likely initiators of PSD. This linkage was suggested in earlier experimental studies by Ojha et al.<sup>43</sup>

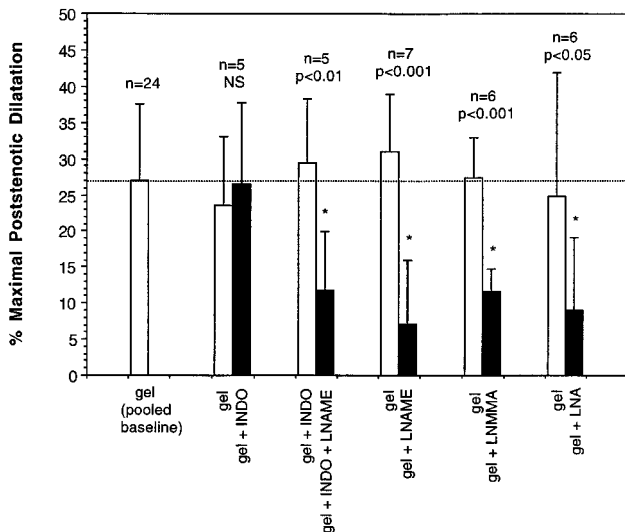
Placement of a long stenosis ( $>4$  mm) in conjunction with a thrombogenic stimulus (thrombin, endothelial denudation, or electric current) has been a model for thrombosis.<sup>44</sup> The short length ( $<1$  mm) of the stenosis in the present study was not sufficient for shear-induced platelet activation or arterial thrombosis.<sup>31</sup> We have not observed thrombosis at the site of the stenosis or proximal or distal to the stenosis even out to 14 days. The vascular casts also provided no indication of thrombosis, because filling defects were never observed. Langille et al.<sup>28</sup> observed extremely low endothelial replication rates downstream of the stenosis during the first week



**Figure 6.** Inhibition of cyclooxygenase activity with adventitiously applied indomethacin 10  $\mu$ mol/L did not prevent generation of PSD in rabbit femoral artery. After 3 days, PSD occurred in stenosed femoral artery treated with adventitiously applied Pluronic gel ( $\square$ ) and in contralateral stenosed femoral artery treated with adventitiously applied Pluronic gel containing 10  $\mu$ mol/L indomethacin ( $\blacksquare$ ) (A). Similarly, after 3 days, PSD was prominent in stenosed femoral artery treated with adventitiously applied Pluronic gel containing 10  $\mu$ mol/L indomethacin ( $\square$ ) but was substantially reduced in matched contralateral stenosed femoral artery treated with adventitiously applied Pluronic gel containing 10  $\mu$ mol/L indomethacin and 1.0 mmol/L L-NAME ( $\blacksquare$ ) (B). Flow was from left to right.

after placement of the stenosis. Furthermore, they observed (by scanning electron microscopy) no indication of injury or denudation of the endothelium at or near the coarctation. Importantly, removal of endothelium is generally associated with vasoconstriction,<sup>45</sup> not vasodilation, through the loss of basal endothelial NO production as well as platelet adhesion and consequent release of serotonin and thromboxane  $A_2$ .<sup>46</sup>

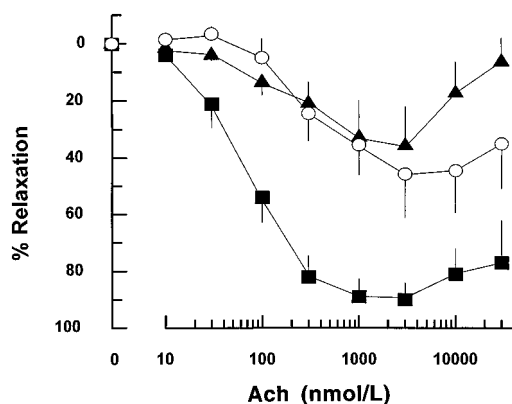
In an earlier study to address the role of NO in PSD,<sup>26</sup> the placement of L-NAME 0.1 g/L in the drinking water of rabbits did cause a blockade of acetylcholine-induced vasodilation, as measured by carotid resistance, but had no effect on the development of PSD. As the authors noted, however, L-NAME has been shown to be an antagonist of the cholinergic pathway.<sup>41</sup> It is possible that in these earlier experiments, the systemic blockade of NO production was not sufficient at the site of the stenosis to block hemodynamically released NO. A higher level of 0.5 g/L L-NAME in drinking water has been shown to attenuate vascular remodeling in rabbit carotid aorta in response to elevated flows created by an arteriovenous fistula.<sup>47</sup> With adventitial delivery or oral delivery of an L-arginine analogue, the exact level of the free agent within the target endothelium is not known. However,



**Figure 7.** Inhibition of NO but not indomethacin (INDO) attenuated development of maximal PSD in rabbit femoral artery after 3 days. Max % PSD was significantly reduced by L-NAME 1 mmol/L, L-NMMA 1 mmol/L, and L-NNA 10  $\mu$ mol/L, whereas INDO 10  $\mu$ mol/L had no inhibitory effect. Probability values were obtained by 2-tailed *t* test for comparisons between inhibitor-treated vessels (solid bars) and matched contralateral stenosed femoral arteries (open bars) for *n* animals in each comparison. \**P* < 0.02 (INDO+L-NAME) or *P* < 0.004 (L-NAME, L-NMMA, or L-NNA) by Bonferroni correction for multiple comparisons with pooled max % PSD response (*n* = 24, far left) of vessels treated with gel without any inhibitor.

any nonlocal downstream effects of L-NAME diluted in the arterial flow (a concentration that we estimate by a steady-state wall diffusion/boundary layer flow model to be quite low) would be expected to be minimal, because systemic dosing with low-level L-NAME is insufficient to block PSD.<sup>26</sup>

The experiments with indomethacin suggest that the attenuation of PSD by L-NAME or the other NO inhibitors was not due to a nonspecific inhibition of prostacyclin production.<sup>38</sup> Our observation that L-NNA (which does not inhibit the muscarinic receptor<sup>41</sup>) significantly attenuated the development of PSD supports the role of NO as the mediator of PSD.



**Figure 8.** Acetylcholine-mediated relaxation of phenylephrine-constricted vessel rings is enhanced in poststenotic region (■; *n* = 8) relative to rings obtained proximal to stenosis (▲; *n* = 9) or from contralateral sham-operated control femoral arteries (○; *n* = 6).

The induction of smooth muscle cell inducible NOS (iNOS) and release of NO (or peroxynitrite) by the altered mechanics of the stenosed vessel remain important avenues of investigation. Interestingly, the proximal region is exposed to greater distending forces due to pulsatile pressure and wave reflection, whereas the distal region experiences damped pulsatility due to the stenosis. In preliminary experiments, we have not detected elevated levels of iNOS antigen in the media of the vessel. Also, we observed that the distal PSD segments develop increased tone in response to phenylephrine and increased dilatory response to acetylcholine. In contrast, when iNOS is induced,<sup>48,49</sup> rabbit vessels display decreased response to both phenylephrine and acetylcholine.

In addition, the persistent lack of smooth muscle cell contraction in the poststenotic region may make the noncellular structural elements of the vessel wall more susceptible to distension and circumferential stresses. It may be possible that excess NO (potentially via peroxynitrite) has a role in preaneurysmal processes such as the breakdown of the internal elastic lamina.

The results presented in this study also suggest that the 3-day stenosis model resulted in a functional change of vessel reactivity. An increased responsiveness of the PSD region to a receptor-mediated release of endothelial NO was observed. Similar findings have been observed in vessels exposed to chronic increases in blood flow and shear stress.<sup>50</sup> This is consistent with our calculations of increased shear stresses in the distal segment of the vessel. Miller and Burnett<sup>50</sup> found that both tonic and receptor-stimulated production of NO was enhanced in arterial blood vessels of the arteriovenous fistula canine model.

Although the hemodynamic origin of PSD is generally undisputed, the precise mechanisms have been controversial and the mediators unknown. The present study indicates that the major molecular mediator of PSD is NO, not prostacyclin. Further experimental and computational studies will help evaluate the hemodynamic regulation of NO and NO-derived species during the development of PSD.

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