Flow Affects Development of Intimal Hyperplasia After Arterial Injury in Rats

Ted R. Kohler and Arkadiusz Jawien

This study examined the effects of blood flow on intimal hyperplasia after balloon catheter injury of the rat common carotid artery. Flow was altered by ligation of the opposite common carotid artery (increased flow) or of the ipsilateral internal carotid artery (decreased flow). Blood flow decreased by 35% in the low-flow group and increased by 29% in the high-flow group. Similar changes in mean velocity were observed. Cross-sectional intimal area was significantly greater in the low- than the high-flow group at 2 weeks (0.11 ± 0.01 versus 0.06 ± 0.01 mm², p < 0.01) and 4 weeks (0.17 ± 0.02 versus 0.12 ± 0.01 mm², p = 0.01) but not at 1 or 8 weeks. Smooth muscle cell proliferation rates (thymidine labeling indexes) were not different in high- and low-flow groups at 2 days and at 1 and 4 weeks. Matrix accumulation at 2 and 4 weeks was the same in both groups. Mature neointima did not respond to changes in flow; when vessel ligation was delayed until 2 months after injury, there was no effect on neointimal area. These data indicate that early neointimal hyperplasia is increased when flow is reduced, possibly because of alteration of smooth muscle cell migration.

**Methods**

**Surgery**

Male Sprague-Dawley rats weighing 350–400 g (Tyler Laboratories, Seattle, Wash.) were anesthetized with intraperitoneal ketamine (225 mg/kg body wt) and xylazine (15 mg/kg body wt). Both common carotid arteries and the left internal carotid artery were exposed through a midline neck incision. A 2F balloon catheter was advanced through the left femoral artery into the left common carotid artery. The catheter was rotated as it was passed three times through the carotid artery with the balloon distended sufficiently with saline to generate slight resistance and to minimally distend the artery. After balloon injury, the left internal carotid artery was ligated in the low-flow group and the right common carotid artery was ligated in the high-flow group (Figure 1).

**Hemodynamic Measurements**

Blood pressure was measured with a strain-gauge transducer connected via pressure tubing to a 25-gauge needle inserted into the common carotid artery. Pressures were recorded immediately before and after temporary occlusion of either the internal carotid artery or the opposite common carotid artery (increased flow) or of the ipsilateral internal carotid artery (decreased flow). There was significantly more intimal hyperplasia in the decreased-flow group. Because the endothelium was absent in the injured vessel, this finding suggests that luminal smooth muscle cells (SMCs) respond to alterations in shear and can modify the function of underlying SMCs to modify vessel wall structure.

Changes in shear rate affect arterial structure in both normal and diseased vessels. During development, arterial caliber varies with shear rate, and in mature arteries, diameter increases when flow is augmented and decreases when flow is diminished.1–8 This regulation of vessel diameter results in maintenance of normal shear stress. Shear modulates structure in diseased vessels as well. Atherosclerotic plaques, intimal hyperplasia after angioplasty, and vein graft thickening are all increased in areas of low shear.9–18 The purpose of this study was to examine the effect of shear in a well-established model of intimal hyperplasia after balloon catheter injury of the rat common carotid artery.19–21 Flow through the injured artery was altered by ligation of the opposite common carotid artery (increased flow) or of the ipsilateral internal carotid artery (decreased flow). There was significantly more intimal hyperplasia in the decreased-flow group. Because the endothelium was absent in the injured vessel, this finding suggests that luminal smooth muscle cells (SMCs) respond to alterations in shear and can modify the function of underlying SMCs to modify vessel wall structure.

From the Department of Surgery, Seattle Veterans Affairs Medical Center and the University of Washington, Seattle, Wash. Supported by the Washington Affiliate of the American Heart Association, the Medical Research Service of the Department of Veterans Affairs, grant HL-42270 from the National Institutes of Health, US Public Health Service, and a grant to A.J. from W.L. Gore Co., Inc.

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Received October 22, 1991; revision accepted April 29, 1992.
Morphology

At the time of death, animals were anesthetized and the carotid arteries fixed by perfusion with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) via a large cannula placed in the abdominal aorta. Pressure was maintained at 100–120 mm Hg. Evans blue dye in phosphate-buffered saline (60 mg/kg body wt) was infused intravenously 15 minutes before fixation to aid in identifying denuded (blue) regions from reendothelialized (white) regions. After 5 minutes of perfusion fixation, the entire right and left carotid arteries were retrieved, including the aortic arch, innominate artery, and carotid bifurcations. The arteries were further fixed by immersion in the same fixative. Two samples from the central blue region in the left carotid artery were processed and embedded in Epon for preparation of thin sections for TEM photomicrographs.22

Estimation of Smooth Muscle Cell and Matrix Contents of the Wall

The relative contribution of SMC and matrix accumulation to intimal thickening was assessed by morphometric analysis of thick and thin sections to determine the total volume of SMCs. This analysis used point-hit, stereological measurements from TEM photomicrographs.22 Reproducible estimates of SMC volume can be obtained by measuring SMC content at each of four quadrants around the vessel circumference.23,24 Sections from carotid arteries of 12 animals killed at 4 weeks (six each from the low- and high-flow groups) and 20 animals killed at 2 weeks (10 in each of the two flow groups) were embedded in Epon for preparation of thin sections for TEM. Photographs were taken at a magnification of ×3,000 and printed at a final magnification of ×9,000. Photographs were taken in such a manner that...
the entire intima from the lumen to the internal elastic lamina was included. A 9×12 grid (108 points, 2 cm apart) was placed over each photomicrograph, and the number of points overlying SMCs was counted. The fraction of area occupied by SMCs was then calculated as the number of points overlying SMCs divided by the total number of points counted times 100. Given the usual total number of points (approximately 5,000) and the fact that SMCs occupy approximately 40% of the volume, the expected relative error in estimating this SMC density is 2%.22 The volume of intimal SMCs per centimeter of vessel wall length (expressed as cubic millimeters per centimeter of vessel length) was calculated as the product of the intimal cross-sectional wall area (from digitized, light-microscope sections) and the fraction of area occupied by SMCs.

Effect of Flow Alteration on Mature Neointima

The effect of flow on the mature neointima was assessed in experiments in which vessels were not ligated until 2 months after injury. At 2 months, animals were anesthetized and underwent either sham operation (control) or ligation of either the left internal carotid artery (low flow) or right common carotid artery (high flow). Velocities were measured before and after these vessels were ligated. Animals were killed after an additional 3 weeks, and neointimal areas were measured in both blue (denuded) and adjacent white (reendothelialized) regions.

Statistics

Statistical comparisons of morphometric measurements and thymidine labeling indexes from the central blue region of the carotid artery were made between groups (high and low flow) within single time points with an unpaired t test (two tailed). When comparing velocities and blood pressures before and after ligation in the same animals, paired t tests were used (two tailed). Analysis of variance was used to compare intimal area of control, low-flow, and high-flow groups in the reversal experiment. Differences were considered significant at \( p < 0.05 \). Results are presented as mean±SEM. All calculations were performed with spss for the Macintosh 4.0 (SPSS Inc., Chicago, Ill.).

Results

Morphology of the Denuded Surface

All arteries examined 1 hour after balloon injury were uniformly stained blue, and their luminal surfaces were totally denuded of endothelium as observed in SEM photomicrographs. By TEM, no difference in platelet adhesion between low- and high-flow groups was evident at 1 hour or 7 days (n=3 in each group).

Hemodynamic Changes

Short-term changes. Peak systolic blood pressure did not change immediately after temporary occlusion of the internal carotid artery (107±20 mm Hg preclosure versus 108±9 mm Hg postocclusion, \( n=5 \)). Peak systolic pressure increased by 6 mm Hg immediately after temporary occlusion of the opposite common carotid artery (101±10 versus 107±8 mm Hg, \( n=5, p<0.05 \)).

Flow had a minimal influence on the overall diameter of the injured arteries, as defined by the measured perimeter of the internal elastic lamina. This parameter was unaffected by flow at all times except 2 weeks, when it was reduced by approximately 7% in the low-flow group. Luminal area, on the other hand, changed by approximately 33% (Table 2). This area was significantly greater in the high-flow group than in the low-flow group; changes in EDV and mean velocity were significant (\( p<0.01 \), Figure 4). Mean velocity increased by 24%, the same percent increase that was observed for flow.

Long-term changes. At 8 weeks, peak systolic velocity, EDV, and mean velocity were all decreased in the low-flow group compared with the high-flow group; however, only the difference in EDV was statistically significant (Figure 5).

Effect of Flow on Vessel Diameter and Intimal Hyperplasia

Flow in the left common carotid artery decreased by 35% immediately after ligation of the internal carotid artery and increased by 29% immediately after ligation of the opposite common carotid artery (\( p<0.01 \), Table 1). Placement of the flow probe was cumbersome and required extensive dissection and manipulation of the carotid artery. Therefore, these measurements were abandoned after the magnitude of the flow changes was established and were determined to correlate well with the simpler technique of mean velocity measurements (see below).

Representative left common carotid artery velocity waveforms before and after vessel ligation are shown in Figure 2. In the low-flow group, peak systolic velocity, end-diastolic velocity (EDV), and mean velocity decreased significantly (Figure 3). The 29% decrease in mean velocity was consistent with the 35% decrease in measured flow. Velocities increased in the high-flow group; changes in EDV and mean velocity were significant (\( p<0.01 \), Figure 4). Mean velocity increased by 24%, the same percent increase that was observed for flow.

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Effect of Flow on Smooth Muscle Proliferation

Thymidine labeling indexes in the intima and media were not significantly different in low- versus high-flow groups at 48 hours or at 1 or 4 weeks (Table 3).
Effect of Flow on Smooth Muscle Cell and Matrix Contents in the Wall

At both 2 and 4 weeks, the percentage of the neointima occupied by SMCs was also the same in low- and high-flow groups (Table 4). These data indicate that the amount of matrix produced by individual SMCs was not affected by flow and that the total volume of SMCs was greater in the low-flow than the high-flow group (Table 4). In previous work, we have noted that most of the SMC proliferation occurs in the region of the intima that is adjacent to the lumen and that the cells in this region produce less matrix than those in deeper regions of the neointima. This was again observed in the current studies in both high- and low-flow groups at both 2 and 4 weeks (data not shown).

Effect of Flow on Mature Neointima

Velocities before and after vessel ligation at 8 weeks are shown in Figure 8. Significant increases were observed in all velocity parameters in the high-flow group and in EDV in the low-flow group. Neointimal areas were essentially the same in control, low-, and high-flow groups in both blue and white (reendothelialized) regions 3 weeks after vessel ligation (Figure 9).

Discussion

This study shows that alteration of flow, and therefore shear, affects intimal thickening after balloon injury in the rat carotid artery. The fact that significant differences in intimal thickening were observed even though flow was changed by only 20–40% suggests that alterations in flow rate that are within the physiological range can have important effects on wall thickening after injury. Furthermore, because the endothelium was absent in this model, this study demonstrates that luminal SMCs are affected by changes in flow and can influence wall structure, presumably by altering the function of underlying SMCs.
Increased Blood Flow Is Associated With Decreased Intimal Thickening

Alteration in blood flow probably affects wall structure because of changes in shear. During development, artery caliber varies with shear rate, and when flow is increased in mature arteries (for example, by creation of an arteriovenous fistula), the vessel diameter increases until shear stress is normalized. Conversely, vessels can constrict in response to decreased flow. Langille and O'Donnell documented a 21% decrease in the diameter of the rabbit common carotid artery 2 weeks after flow was reduced by 70%. This diameter reduction was not reversed by the smooth muscle relaxant papaverine and therefore was thought to reflect a structural modification of the wall. This arterial response to blood flow reduction was abolished when the endothelium was removed, suggesting that the endothelium is essential for the compensatory arterial response to long-term changes in blood flow. There is evidence that shear may modulate structure in diseased vessels as well. Glagov et al and Zarins et al found that human coronary arteries dilate as atherosclerotic plaque accumulates, perhaps in response to increased blood velocity and shear. Fatty streaks in cholesterol-fed animals, atherosclerotic plaques in humans, and wall thickening in vein grafts all tend to occur in areas of low fluid shear. It is not known how flow velocity regulates vessel wall structure, although the endothelium is commonly thought to regulate wall structure and function by transducing the effects of shear into a biochemical signal. Langille and O'Donnell have pointed out that it is unlikely that the SMCs within the wall can directly sense changes in shear stress, as even high shear rates cause <1% shear strain (deformation of the wall).

We have documented in another animal model that intimal thickening is inversely related to shear stress. In baboons, high-porosity polytetrafluoroethylene grafts placed in the arterial circulation develop an endothelium-lined neointima by both ingrowth of endothelium from the cut arterial edges and ingrowth of capillaries through the graft matrix. SMCS appear underneath the endothelium and proliferate to form a thickened intima. Therefore, this system provides a model for studying the effect of flow on intimal thickening in the presence of an intact endothelium. Increased blood flow created by a distal arteriovenous fistula was associated with decreased neointimal thickening. Furthermore, hyperplasia was stimulated in the mature neointima when normal blood flow was restored by ligation of the fistula. The fistula flow created in these experiments was greatly in excess of normal flow rates. In other studies using this model, neointimal area was reduced when blood flow was merely doubled, suggesting that this phenomenon is also important at physiological levels of blood flow.

Balloon Injury Model of Intimal Hyperplasia

Human lesions of intimal hyperplasia after carotid endarterectomy, balloon angioplasty, atherectomy, and vein grafting consist of a thickened intima with abundant SMCs and matrix (collagen, elastin, and proteoglycans). These lesions are very similar to those found in the rat carotid artery after balloon catheter injury. This model has been extensively used to study intimal hyperplasia. Damage to the media and endothelium is a prominent feature in this model, as it is in the development of the human lesions mentioned above. Endothelial denudation is followed immediately by platelet adherence to the exposed, thrombogenic subendothelium. Platelets then spread and release their granules, which contain vasoactive and thrombogenic factors as well as growth factors (platelet-derived growth factor [PDGF], transforming growth factor–β, and epidermal growth factor). 

### Table 2. Morphometry

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>IEL perimeter (mm)</th>
<th>Luminal area (mm²)</th>
<th>Intimal area (mm²)</th>
<th>Medial area (mm²)</th>
</tr>
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<tbody>
<tr>
<td>2 Weeks</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Low flow</td>
<td>16</td>
<td>2.23±0.07</td>
<td>0.21±0.02</td>
<td>0.11±0.01</td>
<td>0.09±0.01</td>
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<td>0.31±0.02</td>
<td>0.06±0.01</td>
<td>0.09±0.01</td>
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<tr>
<td>p value</td>
<td></td>
<td>0.06</td>
<td>0.002</td>
<td>0.001</td>
<td>0.98</td>
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<tr>
<td>4 Weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low flow</td>
<td>11</td>
<td>2.51±0.06</td>
<td>0.22±0.02</td>
<td>0.17±0.02</td>
<td>0.10±0.01</td>
</tr>
<tr>
<td>High flow</td>
<td>11</td>
<td>2.51±0.06</td>
<td>0.28±0.02</td>
<td>0.12±0.01</td>
<td>0.10±0.01</td>
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<td>0.99</td>
<td>0.048</td>
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<td>0.52</td>
</tr>
<tr>
<td>8 Weeks</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Low flow</td>
<td>5</td>
<td>2.13±0.11</td>
<td>0.24±0.05</td>
<td>0.13±0.02</td>
<td>0.09±0.01</td>
</tr>
<tr>
<td>High flow</td>
<td>5</td>
<td>2.16±0.07</td>
<td>0.27±0.03</td>
<td>0.11±0.01</td>
<td>0.09±0.01</td>
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<tr>
<td>p value</td>
<td></td>
<td>0.83</td>
<td>0.66</td>
<td>0.43</td>
<td>0.32</td>
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</tbody>
</table>

n, number of arteries in each group; IEL, internal elastic lamina. Values are expressed as mean±SEM.
dermal growth factor). This is a limited process; by 24 hours the denuded wall is no longer thrombogenic. The endothelium grows in from the adjacent, uninjured artery to cover denuded regions, but this process is limited to approximately 10 mm in the rat because endothelial growth stops after 6 weeks. The middle of the artery remains without endothelium. When intravenous Evans blue dye is administered before the animals are killed, regions of endothelial resurfacing at the proximal and distal ends of the injured artery exclude the dye and remain white, whereas chronically denuded areas in the mid section absorb the dye and are blue.

Between 20% and 40% of SMCs in the media synchronously respond to the injury by proliferating. Recent work suggests that endogenous basic fibroblast growth factor released from damaged medial SMCs may be the major mitogen initiating this growth.49-57 SMC proliferation is greatest between 2 and 7 days after injury. Both proliferating and nonproliferating SMCs migrate across the internal elastic lamina during this time, forming a neointima. PDGF is a major stimulus for this migration (see below). The neointima thickens because of continued SMC proliferation and matrix production, whereas the media does not thicken. SMC proliferation ceases by 12 weeks throughout the wall, except near the surface of denuded regions.21 The SMC content of the wall does not change after 2 weeks, but intimal thickening doubles between 2 and 12 weeks because of continued accumulation of extracellular matrix.

**Effects of Flow on the Balloon-Injured Artery**

Vessel diameter was essentially unchanged by flow in this model. The only significant change was a slight reduction in diameter in the low-flow group at 2 weeks. The general failure of the vessel diameter to change in

<table>
<thead>
<tr>
<th></th>
<th>Low Flow</th>
<th>High Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intima</td>
<td>0.6±0.2</td>
<td>0.7±0.3</td>
</tr>
<tr>
<td>Media</td>
<td>0.2±0.2</td>
<td>0.1±0.1</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM (n). Differences between high- and low-flow groups are not significant.

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**FIGURE 6.** Histological cross sections of arteries from high-flow (panel A) and low-flow (panel B) groups at 2 weeks. Lumen is at the top. Internal elastic lamina is indicated by arrowheads.

**FIGURE 7.** Line plot of cross-sectional intimal areas (mean±SEM, n=5 in both groups at 1 and 8 weeks; n=16 in the low-flow [••••••••] group and n=13 in the high-flow [••••••••••] group at 2 weeks; and n=11 in both groups at 4 weeks). Significant differences (p<0.01) are marked with an asterisk.

**TABLE 3.** Thymidine Indexes (Percentage of Cells Labeled)

<table>
<thead>
<tr>
<th></th>
<th>Low Flow</th>
<th>High Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>48 Hours</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intima</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Media</td>
<td>10.1±3.1 (7)</td>
<td>14.1±3.6 (9)</td>
</tr>
<tr>
<td>1 Week</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intima</td>
<td>36.5±3.7 (5)</td>
<td>32.6±6.5 (5)</td>
</tr>
<tr>
<td>Media</td>
<td>3.34±0.72 (5)</td>
<td>2.49±0.96 (5)</td>
</tr>
<tr>
<td>2 Weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intima</td>
<td>4.6±1.3 (6)</td>
<td>14.6±5.0 (8)</td>
</tr>
<tr>
<td>Media</td>
<td>0.2±0.1 (6)</td>
<td>1.1±0.9 (8)</td>
</tr>
<tr>
<td>4 Weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intima</td>
<td>0.6±0.2</td>
<td>0.7±0.3</td>
</tr>
<tr>
<td>Media</td>
<td>0.2±0.2</td>
<td>0.1±0.1</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM (n). Differences between high- and low-flow groups are not significant.
response to flow may reflect an altered ability of injured vessels to adjust their overall diameter. There was a significant reduction in cross-sectional intimal area in the high-flow group compared with the low-flow group at 2 and 4 weeks. As a result of this reduction in intimal area, luminal area was significantly greater in the high-flow group than in the low-flow group at these times. Suppression of intimal thickening in the high-flow group may be caused by increased shear. The resulting increase in luminal area results in decreased shear at the luminal surface and therefore counteracts the effect of increased flow. This response is similar to the increase in caliber observed in both mature and diseased arteries in response to increased shear. It fits with the theory that arteries adjust their luminal area in response to flow changes to maintain a constant shear force at the wall. By 8 weeks, luminal and intimal areas in low- and high-flow groups were no longer significantly different, perhaps because of a gradual normalization of flow in the two groups. This is suggested by the finding that only the difference in EDV was significant at 8 weeks.

As mentioned above, intimal thickening results from medial SMC proliferation followed by migration of these cells into the intima, where further proliferation and matrix production occur. It is important to determine which of these processes is affected by flow. Evaluation of SMC content at 2 and 4 weeks by TEM demonstrated that flow alteration did not affect the amount of matrix made by individual SMCs. The SMC content of the neointima was greater in the thicker neointima found in the low-flow group. This means that the observed differences in neointimal area were due to differences in SMC content. This may have occurred because of either reduced SMC migration into the neointima or reduced SMC proliferation in high-flow versus low-flow groups. The finding that rates of SMC proliferation were not affected by flow indicates that migration was affected more than SMC growth. In this respect, the effect of increasing flow is similar to that of blocking PDGF. Ferns et al found decreased intimal area after balloon injury in rats treated with antibody to PDGF. They, too, found that SMC proliferation was not affected and concluded that the primary role of PDGF is stimulation of migration rather than proliferation. Neither we nor Ferns et al noted differences between the medias of high- and low-flow arteries. Depopulation of the media because of massive migration of SMCs into the media does not occur in this model, and therefore, differences in rates of SMC migration are usually not apparent by inspection of the media. Relatively small changes in the rate of migration of SMCs out of the media into the intima can cause detectable changes in intimal area, as they are amplified by proliferation and matrix production by SMCs that enter the intima. Some laboratories have quantified rates of migration by SEM to count the number of cells that appear on the denuded

![Figure 8](http://atvb.ahajournals.org/)

**Figure 8.** Histogram of left common carotid artery velocities before (■) and after (□) vessel ligation at 8 weeks in the experiment on the effects of flow on mature intima (n=10 for all parameters except low-flow mean velocities, where n=8). Significant differences (p<0.05) are marked with an asterisk.

![Figure 9](http://atvb.ahajournals.org/)

**Figure 9.** Histogram of neointimal areas from blue regions (■) and white regions (□) of the left common carotid artery in control, low-, and high-flow groups from the experiment on the effects of flow on mature neointima. Differences were not significant.

### Table 4. Composition of Intima

<table>
<thead>
<tr>
<th></th>
<th>Percentage area occupied by SMCs</th>
<th>Intimal area (mm²)</th>
<th>SMC volume (mm³/cm vessel)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Weeks</td>
<td>Low flow</td>
<td>10</td>
<td>43.2±1.9</td>
</tr>
<tr>
<td></td>
<td>High flow</td>
<td>10</td>
<td>47.4±3.1</td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td>0.27</td>
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</tr>
<tr>
<td>4 Weeks</td>
<td>Low flow</td>
<td>6</td>
<td>26.9±1.6</td>
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<tr>
<td></td>
<td>High flow</td>
<td>6</td>
<td>30.6±1.3</td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td>0.11</td>
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</tbody>
</table>

SMCs, smooth muscle cells. Values are expressed as mean±SEM. n, number of arteries in each group.
surface soon after denudation, but this was not attempted in our work or in the studies by Ferns et al.55 Support for the importance of platelet factors in SMC migration comes from work of Fingerle et al.58 and Jawien et al.57 Fingerle et al studied thrombocytopenic rats and found inhibition of intimal thickening after balloon injury despite normal rates of SMC proliferation. Jawien et al found that infusion of the BB homodimer isofrom of PDGF into rats after balloon injury stimulated migration of medial SMCs into the intimal far more than it stimulated their proliferation. We have no evidence that platelet activity at the vessel wall is 970 Arteriosclerosis Vol 12, No 8 August 1992 no evidence that platelet activity at the vessel wall is stimulated migration of medial SMCs into the intimal soon after denudation, but this was not at- tempted in our work or in the studies by Ferns et al.55

It is possible that SMCs play an important role in regulation of their own growth and migration. These cells produce PDGF as well as receptors for this growth hormone. Studies by Majesky and co-workers have demonstrated that within 6 hours of balloon injury, SMCs in the injured artery increase PDGF-A–chain expression. The pattern of gene expression for PDGF and its receptors changes after 2 weeks. At this time, luminal SMCs produce different amounts of growth factor and receptor than do cells in the underlying carotid artery. Periluminal cells produce large amounts of PDGF-A and the β-receptor for PDGF. SMC proliferation is also highest in this region.21 These data suggest that the control of growth factor production is different for luminal SMCs than for underlying cells, perhaps because of influences of factors at the blood–vessel interface, which in turn may be influenced by shear. Thus, regulation of growth factor production by luminal SMCs in response to flow alteration may be responsible for the differences in intimal hyperplasia in low- and high-flow groups seen in our model. Because all current methods of clinical revascularization involve some degree of arterial denudation and medial injury, better understanding of the function of the SMCs that line the lumen of these denuded segments may allow better control of intimal hyperplasia and restenosis.

Acknowledgments
The authors thank Alexander W. Clowes, MD, for his advice and support and for reviewing the manuscript. We gratefully acknowledge the technical assistance of Selina Vergel, Richard Lee, and Lolan Chan.

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Flow affects development of intimal hyperplasia after arterial injury in rats.

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doi: 10.1161/01.ATV.12.8.963

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