In the past decade, carotid artery stent placement (CAS) has emerged as a therapeutic alternative to carotid endarterectomy for patients with carotid artery stenosis. Multicenter randomized clinical trials comparing CAS with endarterectomy are in progress, and favorable initial results have been reported. Nevertheless, most endovascular devices currently used for CAS were clinically introduced without having been proved beneficial in animal studies. This is largely because an appropriate large animal model of carotid artery stenosis has not been largely available.

The balloon injury approach has been a widely used experimental technique to simulate atherosclerosis-like lesions. This technique has been shown to induce intimal thickening in response to mechanical intimal-to-medial injury in virtually any sites within the vascular tree in any species. However, several limitations are noted in this model. Lesions usually lack advanced histologic features of “complicated atheroma,” such as calcification, intraplaque hemorrhage, and necrotic cores, in contrast to lesions encountered in clinical practice. Although extensive deep vascular injury with concomitant dietary hypercholesterolemia have been shown to yield more advanced features in addition to intimal thickening, the high rate of acute thrombotic occlusion resulting from mechanical trauma in this model remains a major drawback in view of the high expenses encountered in working with large laboratory animals.

We took a different approach in creating a novel large animal model to overcome these limitations. Dietary hypercholesterolemia and experimentally induced diabetes are both known to accelerate atherosclerosis in swine. Nevertheless, it has been demonstrated that carotid arteries are generally spared of lesions in both models. Carotid arteries in swine are quite straight at the neck and do not have major branches or curvatures that disturb the laminar blood flow pattern. Numerous studies suggest that disturbed flow patterns, particularly low or oscillatory wall shear stress, increase susceptibility to atherosclerosis, whereas vessel regions exposed to high or laminar wall shear stress remain comparatively disease-free. We therefore hypothesized that the addition of hemodynamic instability to carotid arteries in hyperlipidemic swine induces advanced atherosclerotic lesions.

In this pilot study, we dynamically altered blood flow conditions in swine carotid arteries by surgical partial ligation but maintained the animals on dietary hyperlipidemia for up to 6 months. We then evaluated this model in balloon-injured arteries to determine whether the absence of intact endothelium exacerbates or attenuates evolution of atherosclerotic plaque in this model.

**Methods**

**Animals**

Eighteen common carotid arteries in 9 healthy young Yucatan mini-pigs (S&S Farms, Ranchita, Calif) of mixed sex with an initial weight of 20–30 kg were used in this study. Surgical partial ligation was carried out in 6 untreated (group I; n = 6) and balloon-injured arteries (group II; n = 9). Three arteries were subjected to sham-operation for control (group III; n = 3). All animals were fed with a high-fat diet until sacrifice. Angiograms and histologic sections of the vessels were analyzed to evaluate both models.

**RESULTS:** Atherosclerotic changes were confirmed in 6 of 6 in group I and 6 of 9 arteries in group II, whereas all in group III remained intact. Three arteries in group II resulted in thrombotic occlusion.

**CONCLUSION:** In this series, surgical partial ligation with concomitant dietary hyperlipidemia is an appropriate experimental technique to develop advanced atherosclerotic plaques with minimal technical complications. This model showed no evidence of such benefits when applied in balloon-injured arteries.

**INTRODUCTION:** Limited availability of a large animal model of carotid atherosclerosis has limited preclinical evaluation of endovascular therapeutic devices. The present study is aimed at developing such animal models with a novel approach, emphasizing the role of hemodynamics.

**METHODS:** Using 18 carotid arteries from 9 miniswine, surgical partial ligation (approximately 80% stenosis) was carried out in untreated (group I; n = 6) and balloon-injured arteries (group II; n = 9). Three arteries were subjected to sham-operation for control (group III; n = 3). All animals were fed with a high-fat diet until sacrifice. Angiograms and histologic sections of the vessels were analyzed to evaluate both models.

**RESULTS:** Atherosclerotic changes were confirmed in 6 of 6 in group I and 6 of 9 arteries in group II, whereas all in group III remained intact. Three arteries in group II resulted in thrombotic occlusion. Advanced plaques with intraplaque hemorrhage and/or calcification were seen in 4 of 6 arteries in group I but none in group II. The cross-sectional area stenosis and atherosclerotic stage for plaques in group I were both significantly higher than that in groups II and III.

**CONCLUSION:** In this series, surgical partial ligation with concomitant dietary hyperlipidemia is an appropriate experimental technique to develop advanced atherosclerotic plaques with minimal technical complications. This model showed no evidence of such benefits when applied in balloon-injured arteries.
Table 1: Subgroup allocation of experimental animals

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Right Carotid</th>
<th>Left Carotid</th>
<th>Time of Harvest (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ia</td>
<td>Ia</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Ia</td>
<td>Ia</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>Ia</td>
<td>Ia</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>Ib</td>
<td>Ib</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>Ib</td>
<td>Ib</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>Ia</td>
<td>Ia</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>Ia</td>
<td>Ia</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>Iib</td>
<td>Iib</td>
<td>6</td>
</tr>
<tr>
<td>9</td>
<td>Iib</td>
<td>Iib</td>
<td>6</td>
</tr>
</tbody>
</table>

Further divided into 2 subgroups based on time points of sacrifice: Ia and Iia, 3 months after the surgical procedure; Ib and Iib, 6 months after the surgical procedure. Animals for group III were sacrificed at 6 months. Allocation of experimental animals is summarized in Table 1. All animals were fed with a high-fat, high-cholesterol diet (Test Diet; Purina, St. Louis, Mo) to induce hypercholesterolemia. The diet was started at least 14 days before the surgical procedure and continued for the duration of the study until the time of sacrifice. A blood sample was collected at the time of the surgical procedure after an 18-hour fast to document hyperlipidemia. After the surgical procedure, all animals were placed on aspirin, 81 mg daily, for a month to minimize the risk of thrombotic occlusion that surgical or endovascular procedures could possibly cause. Aspirin has been shown to inhibit platelet aggregation in swine. A low dose (81 mg) of aspirin was chosen because aspirin administration of 100 mg daily was shown to completely inhibit platelet aggregation in swine weighing 55–97 kg. Given that animals used in the present study weighed 20–30 kg, 81 mg of aspirin was considered sufficient. All animal experiments were conducted in accordance with policies set by the institutional Chancellor’s Animal Research Committee and National Institutes of Health guidelines.

**Group I (Subgroup Ia and Ib): Surgical Partial Ligation in Untreated Arteries**

Anesthesia was induced with intramuscular tiletamine and zolazepam (Telazol) followed by orotracheal intubation. One percent to 2% isoflurane was given to maintain general anesthesia for the duration of the study until the time of sacrifice. A blood sample was started at least 14 days before the surgical procedure and continued for the duration of the study until the time of sacrifice. A blood sample was collected at the time of the surgical procedure after an 18-hour fast to document hyperlipidemia. After the surgical procedure, all animals were placed on aspirin, 81 mg daily, for a month to minimize the risk of thrombotic occlusion that surgical or endovascular procedures could possibly cause. Aspirin has been shown to inhibit platelet aggregation in swine. A low dose (81 mg) of aspirin was chosen because aspirin administration of 100 mg daily was shown to completely inhibit platelet aggregation in swine weighing 55–97 kg. Given that animals used in the present study weighed 20–30 kg, 81 mg of aspirin was considered sufficient. All animal experiments were conducted in accordance with policies set by the institutional Chancellor’s Animal Research Committee and National Institutes of Health guidelines.

**Group II (Subgroup IIa and IIb): Surgical Partial Ligation in Balloon-Injured Arteries**

Under general anesthesia, the carotid artery was dissected and prepared for ligation after the balloon injury procedure. A 6F introducer sheath was inserted into the right or left femoral artery, followed by an intraarterial bolus injection of 100 IU/kg heparin. A 6F guiding catheter was advanced into the common carotid artery. A 6-mm angioplasty balloon was positioned into the common carotid artery over a 0.018-inch guidewire and inflated to a pressure of 10 atm. The inflated balloon catheter was moved approximately 5 cm up and down the carotid artery for 1 minute. This manipulation was repeated 3 times in the same fashion. The surgical partial ligation was then placed in the same fashion as in group I in the center of the balloon-injured site under fluoroscopic guidance. Animals were fed with a high-fat diet and sacrificed at 3 (subgroup Ila) or 6 months (subgroup Iib).

**Group III: Sham Operation (Control)**

Under general anesthesia, the carotid artery was dissected through a midline skin incision at the neck. The surgical wound was closed layer by layer without unnecessary local manipulations. Animals were sacrificed after 6-month dietary maintenance.

**Quantitative Analysis of Carotid Angiograms**

All animals underwent selective carotid angiography immediately after surgery and immediately before sacrifice to document the degree of surgical stenosis and poststenotic dilation. The degree of surgical stenosis was calculated as $1 - \frac{\text{MLD}}{\text{RLD}} \times 100$, where MLD and RLD represent minimum lumen diameter and distal referenced lumen diameter, respectively. The degree of poststenotic dilation was measured as $(\text{PLD}/\text{RLD} - 1) \times 100$, where PLD stands for poststenotic dilated lumen diameter. All parameters were measured by 2 investigators with the use of a workstation equipped with an angiography machine.

**Qualitative and Quantitative Tissue Analysis**

All animals were euthanized with pentobarbital infusion at either 3 months (subgroup Ia and Iia) or 6 months (subgroup Ib and Iib, and group III) after the surgical procedure. Carotid arteries were perfused with 1% paraformaldehyde at physiologic diastolic pressure to fix the tissue.
Angiograms

The mean degree of surgical stenosis at surgery for each group and subgroup is shown in Table 2. There was no intergroup difference. The mean plasma cholesterol level for each group and subgroup is shown in Table 2. No significant intergroup or subgroup differences were found. The mean value for all animals was 82.7 ± 76.4 mg/dL. These figures are approximately 10 times higher than those observed in swine fed with a regular diet.18

Plasma Lipid Levels

The mean degree of surgical stenosis at surgery for each group and subgroup is shown in Table 2. There was no intergroup difference. The mean plasma cholesterol level for each group and subgroup is shown in Table 2. No significant intergroup or subgroup differences were found. The mean value for all animals was 82.7 ± 76.4 mg/dL. These figures are approximately 10 times higher than those observed in swine fed with a regular diet.18

Results

All animal surgery was performed successfully. Various degrees of atherosclerotic plaque were successfully induced in group II. However, the most characteristic feature of lesions induced in group II was intimal thickening (Fig 2).

All data are presented as mean value ± SD. Statistical analysis was carried out using SPS (SSPS, Chicago, Ill). Statistical analysis was carried out using SPS (SSPS, Chicago, Ill). Analysis of variance (ANOVA) test was used for analysis of homogeneity of proportions, such as the rate of atherosclerotic change, total occlusion, and advanced atherosclerotic lesions.17 In brief, type I represents lesions with smooth muscle cell dominant intimal proliferation. All slides were photographed with a scale bar to allow computer-based morphometric analysis. Measurements were carried out on cross-sections including the most narrowed portion of the lumen. The cross-sectional area at stenosis (as %) was calculated as follows: cross-sectional area at stenosis (%) = [(actual lumen area/potential lumen area) x 100, in which potential lumen area was defined as the area within the internal elastic membrane. Immunohistochemical studies using primary antibodies potential lumen area was defined as the area within the internal elastic membrane. Immunohistochemical studies using primary antibodies to smooth muscle actin (SMA) were also used to characterize plaques.

Table 2: Mean values of parameters for each group and subgroup

<table>
<thead>
<tr>
<th>Group (Subgroup)</th>
<th>No. of Subjects</th>
<th>Time of Harvest (mo)</th>
<th>Partial Ligation</th>
<th>Balloon Injury</th>
<th>High-Fat Diet</th>
<th>Total Cholesterol (mg/dL)</th>
<th>Angiographic Stenosis at Surgery (%)</th>
<th>Poststenotic Dilatation (%)</th>
<th>Total Occlusion</th>
<th>Atherosclerotic Changes</th>
<th>Advanced Lesions</th>
<th>Plaque Stage</th>
<th>Cross-sectional Area Stenosis (%)</th>
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<tr>
<td>I (a + b)</td>
<td>6</td>
<td>3 or 6</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>944 ± 119</td>
<td>79 ± 9*</td>
<td>41 ± 15*</td>
<td>0/6</td>
<td>6/6*</td>
<td>4/6*</td>
<td>48.4 ± 16.8</td>
<td>53.4 ± 34.8</td>
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<tr>
<td>Ia</td>
<td>3</td>
<td>3</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>821 ± 200</td>
<td>83 ± 20*</td>
<td>48 ± 12*</td>
<td>0/3</td>
<td>3/3*</td>
<td>3/3*</td>
<td>5.7 ± 0.6</td>
<td>65.8 ± 31.3</td>
</tr>
<tr>
<td>Ib</td>
<td>3</td>
<td>6</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>1057 ± 125</td>
<td>75 ± 6*</td>
<td>35 ± 18*</td>
<td>0/3</td>
<td>3/3*</td>
<td>1/3*</td>
<td>4.0 ± 2.0</td>
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<tr>
<td>IIA (a + IIb)</td>
<td>9</td>
<td>3 or 6</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>740 ± 107</td>
<td>77 ± 4*</td>
<td>44 ± 10*</td>
<td>3/9</td>
<td>6/9*</td>
<td>0/0</td>
<td>2.7 ± 1.0</td>
<td>25.4 ± 16.5</td>
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<tr>
<td>IIA</td>
<td>5</td>
<td>3</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>730 ± 90</td>
<td>76 ± 5*</td>
<td>42 ± 12*</td>
<td>1/5</td>
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<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>752 ± 235</td>
<td>79 ± 3*</td>
<td>49 ± 1*</td>
<td>2/4</td>
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<td>3.0 ± 1.4</td>
<td>30.2 ± 14.4</td>
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<tr>
<td>III</td>
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<td>1057 ± 200</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0/0</td>
<td>0/0*</td>
<td>0/0</td>
<td>0.0 ± 0.0</td>
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</table>

* Indicates statistically significant difference compared with group III (control).

All slides were photographed with a scale bar to allow computer-based morphometric analysis. Measurements were carried out on cross-sections including the most narrowed portion of the lumen. The cross-sectional area at stenosis (%) was calculated as follows: cross-sectional area at stenosis (%) = [(actual lumen area/potential lumen area) x 100, in which potential lumen area was defined as the area within the internal elastic membrane. Immunohistochemical studies using primary antibodies to smooth muscle actin (SMA) were also used to characterize plaques.
group significant difference except for group III. Final angiograms performed at the time of sacrifice showed that 3 of 9 (30%) arteries in group II (1 of 5 in subgroup IIa and 2 of 4 in subgroup IIb) were occluded, whereas all arteries in group I remained patent. The rate of total occlusion for group II was higher than that for group I, but not significantly so. All arteries in groups I and II demonstrated various degrees of poststenotic dilation (Fig 1C). The mean degree of poststenotic dilation of treated arteries is shown in Table 2, where 3 occluded arteries in group II were excluded because poststenotic dilation was not documented as a result of subacute vessel occlusion. No significant differences were found between the mean degree of poststenotic dilation of group I and II at either 3 months (subgroup Ia versus IIa) or 6 months (subgroup Ib versus IIb).

Qualitative Tissue Analysis
Cross-sections stained with hematoxylin-eosin demonstrated various degrees of atherosclerosis in 6 of 6 (100%) and 6 of 9 (67%) arteries for group I and II, respectively. They were more prominent proximal to the surgical stenosis. Three control arteries in group III remained intact although the animals received a high-fat diet for 6 months. Three of 9 arteries (33%) of group II were occluded by organized thrombus, most probably related to the balloon injury to the arterial intima.

All atherosclerotic lesions in group I and II contained intimal fibromuscular proliferation with large collections of foamy macrophages. Histologic features of more advanced atherosclerosis, such as foci of necrosis, calcium deposition, and/or intraplaque hemorrhage were observed in 4 of 6 (66.7%) arteries in group I (3 of 3 in subgroup Ia and 1 of 3 in subgroup Ib). None of specimens from group II (subgroups IIa and IIb) demonstrated these features.

The histologic features observed in group I were organized in layers, closely resembling human atherosclerotic plaque (Fig 3). Foamy macrophages were within the bottom of plaque, often with an overlying fibromuscular cap (Fig 3A, -B) and foci of necrosis in their central portions (Fig 3C). SMA staining showed immunoreactive smooth muscle cells and myofibroblasts immediately beneath the luminal aspect of the plaque (Fig 3B). Calcium deposition, most often found deep within plaques, was also clearly visualized (Fig 3D). EVG staining exhibited abundant collagen fibers and partially destroyed internal elastic membranes (Fig 3E). In 3 of 6 (50.0%) arteries in group I (2 of 3 in subgroup Ia and 1 of 3 in subgroup Ib), even the occurrence of intraplaque hemorrhage corresponding to the AHA criteria type 6 (ie, the most advanced [and often symptomatic] stage of atherosclerosis) was found (Fig 3A). Such lesions were not found in any specimens from group II. Lesions were eccentric in all (100%) arteries in group I, whereas only 2 of 9 arteries (22%) in group II showed such eccentricity. All these observations suggest that histologic features of atherosclerotic plaques in group I are quite comparable with advanced atherosclerotic lesions encountered in clinical practice.

The mean plaque stage scores for each group and subgroup are shown in Table 2 and Fig 4. Intersubgroup differences were significant between Ia versus IIa and la versus III. In summary, surgical partial ligation without balloon injury developed significantly more advanced plaques than that with superimposed balloon injury at 3 months.

Quantitative Tissue Analysis
The mean degree of cross-sectional area stenosis (%) is shown in Table 2 and Fig 4. Although the area stenosis for subgroups Ia and Ib seems to be higher than for IIa and IIb, respectively, no statistically significant difference was found. Although the plaque stage score for subgroup Ia was higher than that for subgroup Ib, the difference was not statistically significant. When subgroups (Ia and Ib, IIa, and IIb) are considered together, interaction between time points and subgroups was ruled out, and the mean degree of area stenosis for group I was significantly higher than that for group III. There was no statistically significant difference between groups II versus III. These observations suggest that surgical partial ligation without balloon injury developed significantly more massive plaques than in control swine whereas that with superimposed balloon injury did not.
Discussion

Surgical Partial Ligation Model without Balloon Injury
In this series, all arteries for group I (surgical partial ligation without balloon injury) demonstrated various degrees of atherosclerosis in the portion proximal to the surgical stenosis. More importantly, 4 of 6 arteries developed advanced plaque histologically characterized by calcification and/or intraplaque hemorrhage. In contrast, all 6-month sham-operated arteries (group III) remained free of atheroma. The surgical partial ligation model without balloon injury has several advantages over currently used models.

First, no mechanical injury is necessary in this model. Maintained intact endothelium allows in vivo evaluation of endothelial function exposed to hemodynamic instability. Healthy endothelium plays a pivotal role in vascular homeostasis, not only by mediating vasomotion\(^\text{19}\) but also by suppressing thrombosis,\(^\text{20}\) vascular inflammation,\(^\text{21}\) and proliferation\(^\text{22}\) in response to blood flow dynamics. Intensive studies of the in vitro endothelial response to fluid shear stress have been performed using cultured endothelial cells plated within a flow chamber. They demonstrated that a high magnitude of wall shear stress induces a quiescent, antiproliferative, antioxidant, and antithrombotic “atheroprotective” phenotype of endothelium.\(^\text{23}\) In disturbed flow conditions, endothelial cells monitoring flow conditions switch their gene expression profiles to “atherogenic” (ie, a thrombogenic, inflammatory, and proliferative phenotype).\(^\text{23}\) Molecular analysis of vascular regions susceptible to plaque formation in animals have revealed that disturbed blood flow patterns prime the endothelium to respond to humoral factors, such as oxidative stress, through upregulation of the proinflammatory transcription factor nuclear factor \(\kappa B\).\(^\text{24,25}\) It is thus proposed that atherosclerosis is evoked if systemic factors are superimposed onto these already-primed endothelial cells.\(^\text{24,25}\)

Second, advanced atherosclerotic lesions can be induced with high-fat diet, with minimal complications such as thrombotic occlusion. Lesions induced in this model elicit histologic features quite comparable with those in human carotid artery: intraplaque hemorrhage, calcification, and a fibrous cap. The balloon injury model is a widely used experimental model that readily induces atherosclerosis-like lesions regardless of animal species. Recchia et al reported that high-fat diet feeding with extensive balloon injury yields advanced carotid plaques.\(^\text{10}\) Formed lesions are comparable with those in humans except that luminal narrowing is mostly dependent on massive thrombus. Nevertheless, they also stated that 19% of carotid arteries were excluded from further studies as a result of subacute total occlusion, which remains a limitation of this method, especially in using expensive large animals. This technical complication has been reported to occur at a rate of 10%–50%,\(^\text{7,10,26,27}\) presumably depending on the details of balloon procedures as well as anticoagulant regimens used. On the other hand, the surgical partial ligation model without balloon injury experienced no thrombotic occlusion in our series.

Third, hemodynamic force seems to play an important role as a source of atherogenesis in this model. Several lines of evidence have shown that hemodynamics play an important role in atherosclerosis. Numerous studies have shown that

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Fig 3. Histologic features of various atheromatous plaques in carotid arteries in group I. All sections are from 1 animal sacrificed at 6 months after the surgical procedure.

A, Low magnification micrograph (H-E-stained section) shows a large eccentric plaque in the portion proximal to the surgical stenosis featuring a fibromuscular cap, intra- and extracellular lipid deposition, and massive intraplaque hemorrhage (white arrowheads). Scale bar, 1 mm.

B, anti-SMA immunostaining clearly showing a fibromuscular cap covering a large collection of foamy macrophages at the bottom. Note that smooth muscle cells are scattered throughout the plaque and line its luminal surface (at the top). Scale bar, 100 \(\mu\)m.

C, Base of plaque immunostained with anti-SMA showing necrotic portion. Scale bar, 100 \(\mu\)m.

D, Anti-SMA immunostaining clearly showing calcification at the bottom of plaque (black arrowhead).

E, EVG-stained section of plaque shows abundant collagen deposition as well as focally defective internal elastic lamina (arrows). Scale bar, 100 \(\mu\)m. H-E, hematoxylin and eosin; SMA, smooth muscle actin; EVG, Van Gieson’s elastic.
atherosclerosis-susceptible sites, such as the carotid bifurcation, are consistently exposed to oscillatory or low wall shear stress. In support of these retrospective observations, Zarin et al. showed that the surgical creation of a tight coarctation in thoracic aorta in cynomolgus monkeys induced intimal thickening in the proximal portion of the coarctation, whereas the stenosis channel as well as the distal portion was free of lesions. Sawchuk et al. reported that asymmetric stenosis surgically created in thoracic aorta in hyperlipidemic swine developed intimal thickening with abundant foamy macrophages, showing the positive correlation between low wall shear stress and lesion thickness. There is no doubt that systemic risk factors for atherosclerosis, such as hypercholesterolemia, hypertension, diabetes, and smoking, also play major roles in the initiation and progression of this disease. Nevertheless, these observations (including our study) suggest that hemodynamic force is at least responsible for localization of atherosclerotic plaque within the vascular tree. Further studies focused on hemodynamics are needed in this model.

Surgical Partial Ligation with Balloon Injury

Although all arteries for group II (surgical partial ligation with balloon injury) showed atherosclerotic changes except for 3 occluded arteries, no specimens showed evidence of advanced atherosclerotic lesions observed in group I. Neointimal proliferation was the most characteristic histologic finding in group II. A high rate of total occlusion (33%) was also noted despite 1-month administration of aspirin. The absence of advanced plaque in group II should be analyzed further because both balloon injury and surgical partial ligation have been shown to develop advanced plaque when applied individually.

Histologic composition of plaque developed by balloon injury can be affected not only by systemic conditions, such as hyperlipidemia and diabetes, but also by the details of balloon procedures. For example, regular diet feeding has been shown to yield solely intimal hyperplasia. It has been demonstrated that the magnitude of intimal proliferation is affected by the degree of vessel injury caused by balloon overinflation, mechanical characteristics of used balloon catheters, and shear forces caused by balloon catheter withdrawal. These factors may be responsible for the lack of advanced plaque in our series. In addition, intact endothelium may be essential to initiate the atherosclerotic process caused by surgical partial ligation. As described earlier, it was shown that endothelium has to be primed by disturbed flow to respond to humoral factors. Further studies, including molecular approach analyses, are needed to elucidate the mechanisms underlying pathogenetic factors in this model.

In addition, a high rate of total occlusion in our series may be attributed to the anticoagulant regimen used. Although low-dose aspirin inhibits platelet aggregation in swine, higher doses of aspirin or continuous injection of heparin may be effective in preventing thrombosis. Nevertheless, more probably, reduced blood flow by surgical partial ligation may have contributed to high thrombogenicity in this model, as shown in the previous study on balloon-injured, partially ligated femoral arteries in rabbits.

Limitations of the Study and Proposed Model

We acknowledge several limitations of the current study and proposed animal model. First, a relatively small number of used animals and the exploratory nature of this study may decrease its reliability, though statistical significance was found between group I versus II and group I versus III for several parameters. In addition, a high rate of thrombotic occlusion in group II may have been related to the small number of animals allocated into this group as a result of budgetary limitations. The use of a larger number of animals allocated into group II may have improved the complication rate and led to the development of advanced plaque in some animals. Second, we did not test balloon injury without surgical partial ligation because of the limited number of available animals. Although abundant literature exists on this technique, direct comparison in the same animal would help to characterize the difference between plaques formed in these 2 models. Third, a tight surgical stenosis artificially created should per se be distinguished from adjacent atherosclerotic stenosis. This surgical stenosis is mechanical rather than biological, consisting of an artificial luminal narrowing to cause hemodynamic instability. Nevertheless, this can be eliminated by the use of ab-
sorbable suture. Finally, hemodynamic measurements or molecular analysis were not presented in the current report. Further studies are forthcoming with particular attention to these aspects to elucidate mechanisms governing atherogene-
sis in this model.

Conclusions
Surgical partial ligation with concomitant dietary hyperlipid-
emia is an appropriate experimental technique to develop ad-
vanced atherosclerotic plaque in untreated carotid arteries in
swine with minimal technical complications. This model
showed no evidence of such benefits when applied in balloon-
injured arteries.

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Rigler Center for their invaluable assistance in performing the
experiments. We also thank James W. Sayre, PhD, for his great
assistance in statistical analysis.

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shear stress induce platelet-derived growth factor-A expression in baboon
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