Long-term Induction and Regression of Diet-Induced Atherosclerotic Lesions in Rhesus Monkeys

II. Morphometric Evaluation of Lesions by Light Microscopy in Coronary and Carotid Arteries


Abstract Atherosclerotic lesions were induced in rhesus monkeys (Macaca mulatta) by feeding them a high-saturated fatty acid and high-cholesterol diet. After 5.4 years the extent of lesions in three major coronary arteries and the right carotid artery was evaluated morphometrically by light microscopy in one group of animals (group P). The remaining animals were switched to a low-cholesterol diet that remained high in saturated fatty acids and provided the same percentage of total calories as did the atherogenic diet. Lesion regression was then evaluated in one group of monkeys 1.9 years (group R4) and in another group of monkeys 3.7 years (group R5) after withdrawal of cholesterol alone from the diet. In group P, the mean intimal thickness varied between 26 and 47 μm, maximum intimal thickness between 70 and 92 μm, and luminal reduction between 9% and 12% in the three major coronary arteries. Luminal reduction varied between 1% and 11% in right carotid artery segments. After 1.9 years of consuming the basal diet, group R4 animals were no different from group P animals with respect to morphometric measures. Total intimal and medial areas of the left anterior descending (LAD) coronary artery in groups P and R4 were also similar. In contrast, after 3.7 years of consuming the basal diet, group R5 animals showed consistently although not statistically significantly lower values than those in group P for the morphometric measures in coronary arteries and total intimal area in the LAD. Similar results were obtained for the common carotid and external carotid arteries. Thus, our study shows that long-term diet-induced lesions in coronary arteries and in common and external segments of the right carotid artery regressed only when the animals were fed the basal diet for 3.7 years. We conclude that atherosclerotic lesions induced in coronary and carotid arteries can regress toward normal to a certain extent, but they require a longer time for regression than do other arterial segments. These findings support the results of clinical trials in human subjects. (Arterioscler Thromb. 1994;14:2007-2016.)

Key Words • coronary atherosclerosis • carotid atherosclerosis • diet induction • morphometry • light microscopy • lesion regression

In humans, coronary artery atherosclerosis and its complications manifest clinically as coronary heart disease. Retarding the progression of coronary artery atherosclerosis and promoting its regression are major public health goals for decreasing morbidity and mortality from coronary heart disease.

In patients with symptomatic coronary artery disease, angiographic studies indicate that some obstructive atherosclerotic lesions in coronary arteries can regress when serum cholesterol levels are reduced by dietary, drug, surgical, or lifestyle interventions.1,2 However, although treatment was drastic in some lesions, lesions regressed only partially, many lesions did not regress at all, and some lesions even continued to progress. The average length of treatment before repeated angiography was 2 years.

Studies of experimentally induced atherosclerotic lesions in animal models have been important in assessing the feasibility and particularly the mechanisms of regression of established lesions. Investigations with nonhuman primate models have been most applicable to the human situation. In 1970, Armstrong et al3 were the first to show that diet-induced coronary atherosclerosis in rhesus monkeys regressed after changing their diet. After producing coronary atherosclerosis with a high-fat, high-cholesterol diet, these investigators then switched to diets that were either free of cholesterol and low in fat, or free of cholesterol and high in polyunsaturated fatty acids to produce lesion regression. Although both lesion size and luminal narrowing of the coronary arteries decreased, the coronary lesions did not disappear completely, nor was stenosis completely resolved even after 40 months of the regression diet. Investigators have continued to explore the features and mechanisms of atherosclerotic lesion regression in nonhuman primates.4–15 In 1974 we1 reported that rhesus monkeys fed basal monkey food supplemented with fat and cholesterol for 12 weeks developed substantial atherosclerotic lesions in the aorta but only minimal lesions in the coronary arteries. Little if any regression of these coronary lesions occurred after a

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return to 32 or 64 weeks of feeding with the basal monkey food.6

In a recent study15 we induced atherosclerotic lesions in rhesus monkeys by feeding them an atherogenic diet for 2 years; then cholesterol alone was removed from the diet for 30 or 52 weeks. Coronary lesions showed large but insignificant differences between progression and regression groups.15 However, unequivocal regression did occur in the aorta and other arterial segments.15 Examination of the data for individual animals suggested that there was appreciable regression of coronary atherosclerosis only in those monkeys that had developed the most severe lesions.16

In this report we present morphometric evaluations of the regression of atherosclerotic lesions for as long as 3.7 years in three major coronary arteries and the carotid artery in rhesus monkeys in which atherosclerotic lesions had been induced by feeding them an atherogenic diet for 5.4 years.

Methods

Animals and Diets

The study was approved by the Louisiana State University Medical Center Institutional Animal Care and Use Committee. The procedures for treatment of the animals, their diets, and details of the experimental design have already been reported.17 Briefly, 80 young male rhesus monkeys (Macaca mulatta) aged 1 to 3 years and weighing 2.4 kg on average were purchased from an animal importer, quarantined for 90 days, and fed commercial monkey food (Purina Monkey Chow 25, Ralston Purina Co) ad libitum for the first 60 days and then a basal diet high in saturated fatty acids (approximately 38% of total calories provided by beef tallow and butter) and low in cholesterol (0.02 mg/kcal). After baseline lipid values were established on the basal diet, the animals were switched to an atherogenic diet containing crystalline cholesterol (USP) and 1.7% dried egg yolk powder, which were added to the aforementioned basal diet to provide a cholesterol content of 0.35 mg/kcal.

The animals were ranked on the basis of their mean steady-state serum total cholesterol concentration, which was determined by averaging 36 values between 12 and 204 weeks after beginning the atherogenic diet. The assignment of animals to "progression" and "regression" groups was done in a manner described earlier.18 In each group of animals, this assignment procedure resulted in a uniform mean and distribution of steady-state serum cholesterol concentration as measured during the "lesion induction" period, thus obviating the need to adjust for this variable in statistical analyses of differences between groups. A group of 15 animals was then selected (progression group P) and necropsied 279 weeks (approximately 5.4 years) after beginning the atherogenic diet. The remaining animals were switched to the basal diet, which remained high in saturated fatty acids and provided the same percentage of total calories as did the atherogenic diet but was low in cholesterol (only 0.02 mg/kcal versus 0.35 mg/kcal in the atherogenic diet). These animals were assigned to the regression groups. The animals in regression groups R4 (n=13) and R5 (n=14) were necropsied at 101 weeks (about 1.9 years) and 191 weeks (about 3.7 years), respectively, after switching to the basal diet.

Termination and Processing Procedures

After an overnight fast, each animal was sedated with ketamine (10 mg/kg IM), and a final blood sample was obtained. The animal was killed with an overdose of pentobarbital (65 mg/kg IV), and a complete autopsy was performed. The heart and 2 cm of the aortic arch were removed. The aortic arch was cannulated and connected by a plastic tube to a container of 2.5% glutaraldehyde, which was elevated 135 cm above the level of the heart. The glutaraldehyde was then allowed to flow freely by gravity through the coronary arteries for 30 minutes, after which time the coronary arteries had become rigid. The proximal left coronary artery was removed, cleaned, and further fixed by immersion in the same fixative for an additional 90 minutes. Consecutive blocks of the left main, main bifurcation, and proximal anterior descending coronary arteries and the proximal circumflex branch were embedded in plastic (Maraglas), cut into 1-μm-thick cross sections, and stained with toluidine blue and basic fuchs in for morphometric studies (see below). The remainder of the heart was fixed by immersion in 2.5% glutaraldehyde for an additional 90 minutes. The distal left coronary artery and the entire right coronary artery were transected at 5-mm intervals and then embedded in paraffin (Histowax). Five-micron-thick cross sections were prepared from the proximal end of each block and stained with Verhoeff-van Gieson's stain for morphometric evaluation. The morphometric data are presented as means of 5 sections of each Histowax-embedded arterial segment, ie, sections at 0, 5, 10, 15, and 20 mm from the origin of the right coronary artery; 5 sections at 5-mm intervals between 10 and 30 mm from the origin of the left circumflex branch; and 5 sections between 15 and 35 mm from the origin of the left anterior descending branch.

The proximal right carotid artery was cannulated, and the proximal vertebral arteries were clamped. The carotid artery was perfused for 60 minutes with 10% buffered formalin under a pressure of approximately 100 mm Hg. The common carotid artery, the carotid sinus, and the internal and external carotid arteries up to the cranium were excised, immersed in formalin, and processed as described above for coronary arteries. The morphometric data are presented as means of 5 sections at 5-mm intervals from the distal end of the Histowax-embedded common carotid artery; one value for 1 section at the origin of the internal carotid artery, and the mean of 2 sections near the origin of the external branch of the carotid artery at the carotid sinus.

Morphometric Evaluation of 5-μm-Thick Histowax-Embedded Sections of Coronary and Carotid Arteries

Five-micron-thick sections stained with Verhoeff-van Gieson were projected onto the surface of a graphic digitizer for morphometry. The following measurements were obtained for each section: the area of the intima, the length (perimeter) of the internal elastic lamina (IEL), and the maximum thickness of the intima. The mean thickness of the intima was calculated for each section as the ratio of intimal area to perimeter of IEL. We also calculated the percentage of luminal area reduction as the percentage of area within the IEL (assumed to be circular) that was measured as total intimal area. Finally, the mean of these derived measures and the mean of maximum intimal thickness over the proximal 5 sections from each artery for each animal were calculated and used as measures of atherosclerosis.

Light Microscopic Evaluation of 1-μm-Thick Sections of the Maraglas-Embedded Proximal Left Coronary Artery

One-micron-thick sections were prepared from the distal part of the main branch, the main bifurcation, three consecutive blocks of the proximal left anterior descending branch, and the proximal part of the circumflex branch. The anterior wall and the flow divider wall of each coronary artery block had been marked with small incisions according to the method previously reported.19

The location, degree, and extent of lesions were determined from tracings of sequential coronary cross sections. In most cases, we prepared tracings of 5 cross sections spaced at 3-mm intervals along the length of the coronary segment. To obtain the tracings, we projected slides with the 1-μm-thick cross sections on sheets of paper by using a Leitz Neopromar...
TABLE 1. Atherosclerotic Lesions in Coronary Arteries*

<table>
<thead>
<tr>
<th>Morphometric Measures/Artery Segments</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P (15)</td>
</tr>
<tr>
<td>Mean intimal thickness, μm</td>
<td></td>
</tr>
<tr>
<td>LC</td>
<td>47.4±18.0</td>
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<tr>
<td>LAD</td>
<td>26.3±11.5</td>
</tr>
<tr>
<td>RC</td>
<td>31.6±10.7</td>
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<tr>
<td>Mean†</td>
<td>35.9±13.5</td>
</tr>
<tr>
<td>Maximum intimal thickness, μm†</td>
<td></td>
</tr>
<tr>
<td>LC</td>
<td>91.7±29.4</td>
</tr>
<tr>
<td>LAD</td>
<td>70.6±23.1</td>
</tr>
<tr>
<td>RC</td>
<td>79.3±20.7</td>
</tr>
<tr>
<td>Mean†</td>
<td>78.8±22.9</td>
</tr>
<tr>
<td>Lumen reduction, %</td>
<td></td>
</tr>
<tr>
<td>LC</td>
<td>11.9±3.9</td>
</tr>
<tr>
<td>LAD</td>
<td>8.9±3.4</td>
</tr>
<tr>
<td>RC</td>
<td>10.9±3.4</td>
</tr>
<tr>
<td>Mean†</td>
<td>11.6±3.7</td>
</tr>
</tbody>
</table>

P indicates progression group; R4 and R5, regression groups; LC, left circumflex, coronary artery; LAD, left anterior descending coronary artery; and RC, right coronary artery. Figures in parentheses are number of animals in each group. Values are mean±SE.

*Measured in 5-μm-thick sections of HistoWax-embedded tissues (see “Methods”).
†Average over all animals of the mean over all sections of the maximum thickness in a section.
‡Mean of three arteries combined.

Statistical Analyses

Standard descriptive statistics, Student's t test of the difference between means, ANOVA, and Duncan's multiple range test were done using SAS programs (The SAS Institute).

Results

This report presents the morphometric evaluations by light microscopy of diet-induced atherosclerotic lesions in three major coronary arteries and the right carotid artery from group P, the progression group fed the atherogenic diet for 5.4 years, and from groups R4 and R5, the two regression groups fed the basal diet (high in saturated fatty acids but low in cholesterol) for 1.9 and 3.7 years, respectively, after being fed the atherogenic diet for 5.4 years.

Body weights and mean steady-state serum cholesterol levels during each dietary period for each group have been reported previously. Body weights and mean steady-state serum cholesterol levels were similar among groups during any diet period.

Table 1 shows the mean and maximum intimal thickness and percent luminal reduction in the three major branches of coronary arteries in groups P, R4, and R5 and the mean for the three coronary arteries combined, as measured in 5-μm-thick sections cut from HistoWax-embedded arteries. In group P, mean intimal thickness varied between 26 and 47 μm, maximum intimal thickness between 71 and 92 μm, and luminal reduction between 9% and 12%. Group R4 animals, which were fed the regression diet for 1.9 years, were not different from group P animals in terms of mean and maximum intimal thickness and percent luminal reduction in the three coronary arteries. Group R5, on the other hand, showed 35% to 40% lower values than group P for all three measures in the three coronary arteries, but the differences were not statistically significant.

Table 2 shows mean total area of the intima and media as measured by light microscopy in 1-μm-thick

TABLE 2. Total Intimal and Medial Areas of the Left Anterior Descending Coronary Artery Measured in 1-μm-Thick Sections

<table>
<thead>
<tr>
<th>Groups</th>
<th>P (15)</th>
<th>R4 (13)</th>
<th>R5 (13)*</th>
</tr>
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<tbody>
<tr>
<td>Total intimal area</td>
<td>0.38±0.19</td>
<td>0.37±0.12</td>
<td>0.13±0.04</td>
</tr>
<tr>
<td>Total medial area</td>
<td>0.31±0.03</td>
<td>0.30±0.03</td>
<td>0.37±0.03</td>
</tr>
</tbody>
</table>

P indicates progression group; R4 and R5, regression groups. Figures in parentheses are number of animals in each group.

*One-micron-thick cross sections were prepared from only 13 of the 14 animals in this group.
Maraglas-embedded sections of the proximal left anterior descending coronary artery. The mean total intimal area in group R5 was about 66\% lower than that in group P, but the difference was not statistically significant. The mean total medial area in groups R4 and R5 was similar to that in group P.

Figs 1 and 2 show values for each lesion measure in each animal in groups P, R4, and R5. Mean and maximum intimal thickness and percent luminal reduction in nearly 50\% of group R4 animals were above those of group P, whereas values for 12 of 14 animals in group R5 were below those of group P (Fig 1). Also, the total intimal areas in nearly 40\% of group R4 animals were higher than those in group P, whereas 12 of 14 animals in group R5 had lower total intimal areas than those in group P (Fig 2). The total medial areas in individual animals in groups R4 and R5 were scattered uniformly around the group P mean (Fig 2).

Table 3 shows morphometric evaluations of different segments of the right carotid artery, ie, the common, internal, and external carotid arteries. In group P, mean intimal thickness varied between 6 and 43 \( \mu \)m, maximum intimal thickness between 31 and 103 \( \mu \)m, and luminal reduction between 1\% and 11\%. There were no differences between groups P and R4. However, the means of the three measures in group R5 animals were lower than those in group P by about 32\% in the common carotid and 13\% in the external carotid artery. The lesion measures in the internal carotid artery were higher in group R5 than in group P. None of the differences between groups P and R5 were statistically significant.

The upper portion of Fig 3 compares the mean and maximum intimal thickness and percent luminal reduction in the three coronary arteries combined for the progression groups in this 5-year study and our previous 2-year study.\textsuperscript{15} The mean intimal thickness and percent luminal reduction are significantly greater in the present study than in the 2-year study, whereas maximum intimal thickness is similar in the two studies. The lower portion of Fig 3 compares the three parameters in the 1-year regression group from our earlier 2-year study and the 1.9- and 3.7-year regression groups (R4 and R5, respectively) in the present 5-year study. The 3.7-year regression group (group R5) in the present study showed consistent decreases in the three morphometric measures, although the decreases were not statistically significant.

### Light Microscopic Composition of Coronary Artery Lesions in Groups P, R4, and R5

Five-micron-thick sections cut from Histowax-embedded, perfusion-fixed coronary arteries that were stained with Verhoeff–van Gieson were used for evaluation of the microscopic composition of lesions, which are described below.

#### Progression Group P

The left panels of Fig 4 show lesions induced in the left circumflex coronary artery in a monkey from group P that was fed the atherogenic diet for 5.4 years. There is concentric intimal thickening, with lipid in the deeper intimal layers. Lipid cores are also present in the deep intimal layer.

#### Regression Group R4

The middle panels of Fig 4 show that lesions are still present in the right coronary artery in a monkey from group R4 that was fed the regression diet for 1.9 years after being fed the atherogenic diet for 5.4 years. Intimal thickness, intimal lipid, and overall intimal cellularity are decreased compared with those in group P. However, medial lipid is present focally, and the internal elastic lamina is fragmented.

### Regression Group R5

The right panels of Fig 4 show that lesions are present in the left circumflex coronary artery in a monkey from group R5 that was fed the regression diet for 3.7 years after being fed the atherogenic diet for 5.4 years. Clearly, the intimal thickness is much less than that in groups P and R4. Despite decreased intimal thickness, some residual intimal lipid is present, and medial lipid remains.

### Quantitative and Semiquantitative Light Microscopic Evaluation of 1-\( \mu \)-Thick Sections of Maraglas-Embedded Coronary Artery Lesions (Table 4 and Figures 5 and 6)

#### Progression Group P

The intima varied in thickness along the length of the proximal coronary arteries in each of the 15 monkeys in the progression group. Both thick and thin intimal segments were present in every animal. In three animals (20\%), neither the thick nor thin intimal segments nor the media or adventitia contained lipid. In these animals, the thickest intimal segments were thinner than the underlying media and thinner than the intimas of animals with lipid accumulation. In general, the circumferential and longitudinal extents of thick segments were also less than those of the lipid-containing thickenings in the remaining 12 animals. The thick intimal segments consisted of intimal smooth muscle cells and normal extracellular matrix and were similar to the focal intimal thickening found in normocholesterolemic animals of many species. The mean serum cholesterol level of the three animals with only this type of intimal thickening was 304 mg/dL over the 5.4-year period of the atherogenic diet.

The mean serum cholesterol level of the 12 monkeys in which the thick intimal segments contained lipid averaged 463 mg/dL. The degree of maximum intimal thickening and the amount of lipid varied greatly. In the 6 animals with the thickest intimal segments, the intima was four to five times the thickness of the media in an adjacent, nondiseased location; the intimal lipid grade ranged from 64 to 200, and the mean cholesterol level was 504 mg/dL. In the remaining 6 animals with lipid accumulation, the intima was as much as 2.5 times the thickness of the media, the lipid grade ranged from 8 to 90, and the mean serum cholesterol level was 421 mg/dL.

The lipid in the intima was both intracellular and extracellular and involved 50\% to 100\% of the intimal thickness. The density of the accumulated lipid also varied. Intracellular lipid was mainly within macrophage foam cells. Macrophage foam cells were more numerous and larger than lipid-containing smooth muscle cells.

Of the 12 animals with intimal lipid, 9 also had lipid in the media. The amount of lipid in the media was proportional to that in the adjacent intima but always less than that in the intima. In most cases the media was thickened by the accumulated lipid, and many structural...
Fig 1. Graphs showing distribution of the mean and maximum intimal thickness and percent lumen reduction in the circumflex (LC, top) and left anterior descending (LAD, middle) branches of the left coronary artery and the right coronary artery (RC, bottom) of animals in groups P, R4, and R5. Mean±SE is shown by the x and vertical bars, respectively.
smooth muscle cells appeared atrophic or dead. In only one case was the media thinned and at one point, completely absent, with the intimal lesion extending as far as the adventitia. In contrast to that in the intima, most lipid in the media was extracellular and formed pools. Of the 9 animals with medial lipid, 7 also had lipid in the adventitia in the form of macrophage foam cells around the vasa vasorum.

**Regression Group R4**

Of the 13 monkeys in this group, 4 (31%) did not have lipid in the intima, media, or adventitia, nor did they have structural damage or changes that might indicate that lipid had been present earlier and then regressed. Each of these animals had localized segments in which the intima was thicker than elsewhere. The thickness, architecture, and composition of the thicker segments were the same as those in the 3 animals in group P that lacked lipid. The mean serum cholesterol level of these 4 monkeys was 291 mg/dL while on the atherogenic diet.

In the 9 monkeys with intimal lipid, maximal intimal thickness and the amount of lipid varied greatly. In the 5 animals with the thickest intimal segments, the intima was four to seven times the thickness of the media, intimal lipid grade ranged from 40 to 168 (Table 4 and Fig 6), and mean serum cholesterol level was 508 mg/dL during the atherogenic diet period. In the remaining 4 animals with lipid, the thickest intimal segments ranged from one to three times the thickness of the media, the lipid grade ranged from 5 to 48, and the mean serum cholesterol level was 464 mg/dL.

Overall in group R4 animals, intimal lipid was less than that in group P and more than three times as much intimal area was occupied by extracellular lipid than by lipid-laden cells. Extracellular lipid was either finely scattered among intimal cells and matrix or aggregated in dense pools that displaced cells and matrix. Lesions contained fewer macrophage foam cells, more smooth muscle cells, and more calcium and collagen than did the lesions of group P animals. Five of the 13 animals had a trace to substantial lipid accumulation in the media. The animals with lipid in the media were among those that had lipid in the intima.

### TABLE 3. Atherosclerotic Lesions in the Carotid Artery*

<table>
<thead>
<tr>
<th>Morphometric Measures/Artery Segments</th>
<th>Groups</th>
<th>P (15)</th>
<th>R4 (13)</th>
<th>R5 (14)</th>
</tr>
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<tbody>
<tr>
<td><strong>Mean intimal thickness, μm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td></td>
<td>22.1±8.4</td>
<td>17.8±6.2</td>
<td>14.3±2.8</td>
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<tr>
<td>IC</td>
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<td>8.2±2.2</td>
<td>8.0±2.9</td>
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<tr>
<td>EC</td>
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<td>42.9±12.2</td>
<td>39.3±12.2</td>
<td>36.7±8.4</td>
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<tr>
<td><strong>Maximum intimal thickness, μm†</strong></td>
<td></td>
<td>67.8±17.4</td>
<td>56.1±16.0</td>
<td>47.5±6.8</td>
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<tr>
<td>CC</td>
<td></td>
<td>30.9±9.7</td>
<td>30.7±14.5</td>
<td>35.2±10.4</td>
</tr>
<tr>
<td>IC</td>
<td></td>
<td>103.1±24.5</td>
<td>81.7±19.3</td>
<td>94.2±17.8</td>
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<tr>
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<td>8.7±2.1</td>
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<td><strong>Lumen reduction, %</strong></td>
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<tr>
<td>CC</td>
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<td>3.9±1.4</td>
<td>3.1±1.0</td>
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<td>10.6±3.3</td>
<td>10.5±3.5</td>
<td>8.7±2.1</td>
</tr>
</tbody>
</table>

P indicates progression group; R4 and R5, regression groups; CC, common carotid artery; IC, internal carotid artery; and EC, external carotid artery. Figures in parentheses are number of animals in each group. Values are mean±SE.

*Measured in 5-μm-thick sections of Histowax-embedded tissues (see “Methods”).
†Average over all animals of the mean over all sections of the maximum thickness in a section.

By one-way ANOVA, the three morphometric measures are significantly different (P < .05) between the arterial segments in all groups.

By Duncan’s multiple range test, all three morphometric measures in the EC are significantly higher (P < .05) than in the IC in all groups.
Regression Group R5

Of the 13 monkeys in this group, 3 (23%) did not have lipid in the intima, media, or adventitia, nor did they have structural damage or changes that might indicate that lipid had been present earlier and then disappeared. The thickest intimal segments measured between one quarter and one half the thickness of the media. Intimal composition and structure were the same as those of monkeys without lipid in groups P and R4. The mean serum cholesterol level of the 3 monkeys was 270 mg/dL while they consumed the atherogenic diet.

Most of the 10 animals with intimal lipid had lower grades of intimal thickness and lipid accumulation than did the animals in groups P and R4. There was only one animal with a grade of 3+ for intimal thickness (Fig 5) and of 4+ for intimal lipid. However, the architecture and composition of the lesion resembled residual lesions in groups R4 and R5 but differed from lesions in group P animals.

Overall in group R5 animals, the amount of intimal lipid was less than that in group R4 animals. Most residual intimal lipid was extracellular. There were fewer macrophage foam cells and more smooth muscle cells with lipid and collagen than in group R4. The amount of calcium was similar to that in group R4. Seven of the 13 animals had between a trace and substantial amounts of accumulated lipid in the media. The animals with lipid in the media were among those that also had lipid in the intima.

Discussion

This is the only study in any animal model in which atherosclerotic lesions were induced by feeding a high-saturated fatty acid, high-cholesterol, atherogenic diet, resembling the average nutrient composition of the American diet, for more than 5 years. The mean intimal thickness and percent luminal reduction in coronary arteries in the present 5-year study were greater than those in our previous 2-year study (Fig 3, top half), an observation suggesting that extended feeding of the atherogenic diet induced more lesions in the coronary arteries. In contrast, in the thoracic and abdominal aorta and other arterial segments, we reported earlier that the extent of induced lesions (fatty streaks and raised lesions) was similar in the two studies. Thus, it appears that in this nonhuman primate species, the coronary arteries and aorta do not behave identically with respect to progression of atherosclerotic lesions. With hypercholesterolemia alone, a plateau of lesion progression is apparently reached in
the aorta and other arterial segments but not in the coronary arteries in this primate species.

With respect to lesion regression, our study also indicates that diet-induced atherosclerotic lesions in the coronary arteries behave differently from those in the aorta and in most other arterial segments. We reported that after the 1.9-year regression period, both the thoracic and abdominal aorta and 5 of 8 arterial segments showed regression of fatty streaks. We also found that cholesterol content decreased in all arterial segments except the abdominal aorta after the 1.9-year regression period. In contrast, in the coronary arteries after the 1.9-year regression, not even a suggestion of a decrease in the extent of lesions was observed by morphometric measures, viz, mean intimal thickness, maximum intimal thickness, and percent luminal reduction (Table 1). Thus, during both progression and regression, atherosclerotic lesions in the coronary arteries behaved differently from those in aortic and other arterial segments.

The light microscopic studies consistently showed less intimal lipid, not only in group R5 animals in which the morphometric measures were lower, but also in group R4 animals in which the morphometric measures clearly showed no evidence of a decrease (Fig 4 and Table 1). These findings suggest that there were compositional changes in the lesions in terms of the amount and distribution of lipid before lesion size was affected by lowering plasma cholesterol levels. These changes included a lesser amount of intimal lipid, a shift from intracellular to extracellular lipid, and a shift of some lipid from the intima to the media.

In all groups, there were "nonresponders" to the atherogenic diet, with few experimentally induced coronary lesions as judged by microscopic studies. With this consideration, the data concerning lesion size and distribution of intimal lipid seem to indicate that those animals that developed the most lesions in the coronary arteries during the lesion-induction period almost certainly had smaller lesions and contained less intimal lipid than those in group P. Our previous 2-year study had also indicated that regression of lesions in the coronary arteries occurred only in those animals that developed the most severe lesions, even though lesions underwent regression to a much greater extent in the aorta, carotid arteries, and other peripheral arterial segments. Clarkson et al. in a study of atherosclerosis regression in rhesus monkeys, concluded that increased luminal size during regression
TABLE 4. Mean Intimal Thickness, Percentage of Intima Containing Lipid, Density of Lipid, Intimal Lipid Grade, and Amount of intracellular and Extracellular Lipid Accumulation In 1-μm-Thick Sections of Coronary Artery Lesions In the Progression and the Two Regression Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>P (15)</th>
<th>R4 (13)</th>
<th>R5 (13)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean intimal thickness (range)</td>
<td>2.5 (0.3-5)</td>
<td>2.5 (0.3-4)</td>
<td>1.9 (0.25-12)</td>
</tr>
<tr>
<td>Percent of intimal thickness with lipid (range)</td>
<td>69 (0-100)</td>
<td>41 (0-80)</td>
<td>28 (0-85)</td>
</tr>
<tr>
<td>Density of lipid in areas with lipid (range)</td>
<td>2.7 (0-4)</td>
<td>1.5 (0-3)</td>
<td>1.8 (0-4)</td>
</tr>
<tr>
<td>Mean intimal lipid grade (range)</td>
<td>73 (0-200)</td>
<td>36 (0-168)</td>
<td>39 (0-408)</td>
</tr>
<tr>
<td>Amount of lipid accumulated, intracellular:extracellular</td>
<td>1:0.9</td>
<td>1:3.6</td>
<td>1:2.2</td>
</tr>
<tr>
<td>Intracellular lipid, MFC:SMCL</td>
<td>1:0.2</td>
<td>1:1.5</td>
<td>1:2.9</td>
</tr>
</tbody>
</table>

P Indicates progression group, R4 and R5, regression groups; MFC, macrophage foam cells; and SMCL, smooth muscle cells with lipid droplets. Figures in parentheses in column headings are number of animals in each group.

*One-micron-thick cross sections were prepared from only 13 of the 14 animals in this group.

Intimal lipid grade was calculated as follows: intima thickness × % of intima thickness with accumulated lipid × density of accumulated lipid.

was partly due to increased artery size as well as to regression of intimal lesions. Thus, our results agree with those of Clarkson and colleagues\(^ {13,14}\) that diet-induced coronary atherosclerotic lesions regress towards the normal intima.

Although the morphometric measures in the coronary arteries of group R5 animals were not statistically significantly different from those in group P, the mean values in group R5 were 2% to 49% lower than in group P. Possible reasons for the lack of statistical significance are (1) the small number of animals studied and the wide variability and skewed distribution of lesions among animals (Fig 1), (2) "nonresponders" to the atherogenic diet in all groups, (3) measurement errors inherent in the morphometric methodology, and (4) the limited time allowed for regression to occur. However, the consistency and magnitude of decreases in the mean extent of lesions and the distribution of lesions among animals (Fig 3, lower portion) indicate that lesion regression had occurred to some extent. During the regression period the basal diet was low in cholesterol but high in saturated fatty acids; serum cholesterol levels returned to basal levels within 12 to 18 weeks of reinitiating the basal diet. Whether a decrease in the fat content of the diet and/or a change in the composition of dietary fat would have affected lesion regression in the coronary arteries more favorably cannot be answered from this study. Furthermore, whether extension of the regression period would have increased the extent of lesion regression in the coronary arteries also remains unanswered. In this context it is interesting to note that in the Cholesterol-Lowering Atherosclerosis Study (CLAS), regression of coronary artery lesions was documented angiographically in 16.2% of subjects at 2 years and in 17.9% at 4 years in the treatment group compared with 3.6% at 2 years and 6.4% at 4 years in the placebo group.\(^ {1}\)

Ideally, regression of atherosclerotic lesions should return the arterial wall to its normal or prediseased state. That the coronary arteries did not revert to "normal" in this study (Table 1) agrees with our previous studies\(^ {4,15}\) and those of other investigators.\(^ {3,7,8,10,13,14}\) It is probably unrealistic to expect that reversal of coronary atherosclerosis will ever be "complete," even under the most favorable conditions, including a longer period of lowered serum cholesterol levels. It has been a common experience that with regression of diet-induced atherosclerotic lesions, some deleterious residual changes remain, even after a long-term reduction of serum cholesterol levels. Angiographic studies in human patients with symptomatic coronary artery disease have also shown that with drastic treatment measures involving dietary, drug, surgical, or lifestyle changes, coronary artery lesions regressed only slightly, many

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**Fig 5.** Bar graph showing percentage of animals with varied intimal thickness in the proximal left anterior descending coronary artery in the progression group (P) and the two regression groups (R4 and R5).

**Fig 6.** Bar graph showing percentage of animals with varied intimal lipid grades calculated as described in the footnote to Table 4 in the proximal left anterior descending coronary artery in the progression group (P) and the two regression groups (R4 and R5).
lesions did not regress, and in many cases, lesions even continued to progress.  

In summary, this long-term study shows that diet-induced atherosclerotic lesions in the coronary arteries are capable of regression. After 5.4 years of lesion induction, in three coronary artery segments, mean intimal thickness, maximum intimal thickness, and percent stenosis decreased after a regression period of 3.7 years but not after 1.9 years. We conclude that regression of coronary atherosclerosis requires a much longer time than does regression of lesions in other arterial segments. These findings support the results of clinical trials in human subjects.  

Acknowledgments  
The study was supported by program project grant HL-08974 from the National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Md. We thank Martha Jiminez, Audrey Smith, Elizabeth Donnell, and Jimmie Leslie for expert technical help and Bernard Jackson for the care and handling of the animals. We especially thank Dr Peter Gerone, Director, and Dr Robert Wolfe, Head, Veterinary Services, Delta Regional Primate Research Center (DRPRC), for providing the facilities to house the animals and personnel (Astor Bridges and Gus West) at the DRPRC for care and handling of the animals. We thank Drs Miguel A. Guzman and Donald E. Mercante for help in statistical analyses. We are grateful to Dr Henry C. McGill, Southwest Foundation for Biomedical Research, San Antonio, Tex, for reviewing the manuscript.

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doi: 10.1161/01.ATV.14.12.2007

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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