Plaque Changes and Arterial Enlargement in Atherosclerotic Monkeys After Manipulation of Diet and Social Environment

Jay R. Kaplan, Stephen B. Manuck, Michael R. Adams, J. Koudy Williams, Thomas C. Register, and Thomas B. Clarkson

To study the effects of dietary and social manipulations on lesion progression in male monkeys with established atherosclerosis, 83 animals fed a diet containing 1 mg cholesterol per kcal for 14 months were either necropsied (baseline group, n=21) or assigned to one of three experimental conditions: 1) a diet containing a high amount of fat and cholesterol and a stressful social situation (HiFC-stress, n=18); 2) a diet lower in fat and cholesterol and a stressful social situation (LoFC-stress, n=21); or 3) the low-fat, low-cholesterol diet and a nonstressful social situation (LoFC-no stress, n=23).

After 28 months, all animals were necropsied. Coronary atherogenesis was arrested among monkeys in the LoFC-stress and LoFC-no stress conditions compared with that of animals in the baseline condition (plaque areas of 0.35 mm², 0.30 mm², and 0.38 mm², respectively). Lesions in animals fed the LoFC diet (both stress and no-stress groups) were significantly smaller than those in monkeys in the HiFC-stress condition (0.96 mm²). Furthermore, aortic cholesterol content was significantly decreased and luminal areas were relatively larger among monkeys in both LoFC conditions compared with animals in the baseline and HiFC-stress conditions (p<0.05 for all). The results demonstrate that a low-fat, low-cholesterol diet can halt plaque development, reduce arterial cholesterol content, and permit compensatory arterial enlargement, processes that were unaffected by social stress in this investigation. (Arteriosclerosis and Thrombosis 1993;13:254-263)

KEY WORDS: atherosclerosis • cholesterol • cynomolgus macaques • dietary fat • lesion regression • psychosocial stress

Epidemiological and experimental evidence indicates that excessive consumption of saturated fat and cholesterol elevates serum cholesterol concentrations, which in turn accelerate atherogenesis. Conversely, it has been suggested that plasma cholesterol lowering arrests lesion progression and may cause regression of established lesions. Because psychosocial factors (e.g., stress) also appear to enhance the risk of coronary heart disease events in human beings and atherogenesis in animals, it may be hypothesized that reduction of behavioral stress will similarly ameliorate an existing atherosclerotic condition.

Data from recent angiographic studies are consistent with the hypothesis that lesion progression can be arrested and perhaps reversed by reducing serum cholesterol concentrations. There is also preliminary evidence that psychosocial factors may influence regression. However, demonstrating that regression of atherosclerosis may be achieved in patients by any intervention is difficult for two reasons: 1) both lesions and arteries undergo remodeling during atherogenesis (e.g., medial thinning and especially the compensatory enlargement of arteries that is thought to occur in response to lesion development) and 2) atherosclerosis may develop diffusely or focally, with both types of development sometimes represented in adjoining arterial regions.

There are measurement limitations associated with present angiographic techniques. Quantitative angiography, as currently applied in the clinical setting, evaluates how much of the lesion protrudes into the arterial lumen ("stenosis"). Reduced stenosis seen on repeated angiograms is usually accepted as evidence of atherosclerosis regression. Yet angiographic measurements of lumen stenosis can confound arterial remodeling with alterations in lesion extent or morphological characteristics, neither of which can be visualized directly with biplane cineangiography. The presence of angiographically undetectable, diffuse atherosclerosis in the "normal" section of an artery adjacent to a focal lesion can further compromise interpretation by providing a false baseline against which luminal intrusion is assessed. Finally, quantitative angiography does not assess arterial function.
As a result of the foregoing problems, coronary arteriography provides a relatively narrow view of atherosclerosis regression that focuses on an indirect measure of lesion size. By contrast, studies of atherosclerosis regression in animal models can evaluate arterial function and size as well as directly measure lesion extent, severity, and composition. Radical reduction of serum lipid concentrations in nonhuman primates decreased both the size and cholesterol content of atherosclerotic lesions and significantly improved vasomotor function.28-35 Therefore, alleviation of existing atherosclerosis, whether by dietary or behavioral intervention, could occur in at least four ways: 1) by retarding or reversing lesion progression, 2) by altering lesion composition, 3) by remodeling the lesion-containing artery, or 4) by enhancing the artery’s functional integrity as measured by vasomotor activity.

Unfortunately, most dietary studies of atherosclerosis regression in monkeys have employed intervention diets extremely low in fat and cholesterol. It is unlikely that such diets, resulting in total plasma cholesterol (TPC) concentrations below 160 mg/dl, will be consumed for any length of time by many human beings, which limits the extent to which these studies can be extrapolated. Moreover, psychosocial interventions have not been previously evaluated in experimental investigations of regression. Accordingly, the two objectives of the current study were to 1) assess the antiatherogenic effects of a diet moderately low in saturated fat and cholesterol and 2) determine whether any such effects would be modulated by the presence of social stress. We used a low-fat, low-cholesterol diet based on current recommendations of the American Heart Association, the National Cholesterol Education Program, and other organizations.6-7 In addition, we employed a psychosocial stressor (periodic reorganization of social group membership) that has been previously shown to potentiate atherogenesis in this species. It was expected that all animals fed the low-fat, low-cholesterol diet would show evidence of atherosclerosis regression (compared with untreated control animals), with the greatest regression in monkeys living in unstressed social groups. The postmortem evaluations included direct measurements of lesion size and cholesterol content as well as arterial remodeling.

Methods

Animals

The study used 100 male cynomolgus macaques (Macaca fascicularis) imported from Indonesia as adults (aged 5-12 years, as estimated from dentition). This species has a well-known susceptibility to diet-induced coronary artery atherosclerosis86 and has been used previously to model behavioral influences on atherosclerosis.14,15,37,38 Animals were housed in social groups of four or five monkeys each, in identical pens of 1.8 x 3.2 x 2.5 m with outdoor exposure and natural light. Analyses were based on 83 monkeys, as approximately 4% of the animals died each year from bacterial infections unrelated to the experimental manipulation. All procedures involving animals were conducted in compliance with state and Federal laws, standards of the Department of Health and Human Services, and guidelines established by the institutional Animal Care and Use Committee.

Experimental Design

An atherogenic diet containing 1 mg cholesterol per kcal (induction diet in Table 1) was fed to all animals for 14 months to induce atherosclerosis. During this time, the monkeys were housed in stable (unstressed) social groups. After the induction phase, animals were assigned to four experimental conditions that were balanced for plasma cholesterol responses to the experimental diet.

Condition 1: Baseline (n=21). These monkeys were immediately necropsied, and the presence and extent of atherosclerosis were confirmed by pathological examination.

Animals in the remaining three conditions were treated for 28 months (the treatment phase), after which all were necropsied.

Condition 2: High-fat, high-cholesterol diet/unstable social environment (HiFC-stress) (n=18). These monkeys consumed a diet consistent with the current upper quintile of cholesterol consumption in the United States (0.20 mg cholesterol per kcal and about 45% of calories from fat, predominantly saturated; see Table 1). Furthermore, animals lived in social groups made unstable by monthly reorganization.14,15,38 This condition was designed to model a situation in which human beings with some atherosclerosis consume a high-fat, high-cholesterol diet and live under conditions of significant social stress.

Condition 3: Low-fat, low-cholesterol diet/unstable social environment (LoFC-stress) (n=21). These monkeys ate a diet based on that currently recommended for

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Induction diet</th>
<th>HiFC</th>
<th>LoFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein, USP</td>
<td>8.00</td>
<td>8.00</td>
<td>8.00</td>
</tr>
<tr>
<td>Lactalbumin</td>
<td>8.00</td>
<td>8.00</td>
<td>8.00</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>36.00</td>
<td>36.00</td>
<td>35.00</td>
</tr>
<tr>
<td>Dextrin</td>
<td>6.00</td>
<td>6.00</td>
<td>6.00</td>
</tr>
<tr>
<td>Sucrose</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Applesauce</td>
<td>4.50</td>
<td>4.50</td>
<td>4.50</td>
</tr>
<tr>
<td>Lard</td>
<td>6.00</td>
<td>6.00</td>
<td>7.00</td>
</tr>
<tr>
<td>Butter</td>
<td>3.00</td>
<td>3.00</td>
<td>...</td>
</tr>
<tr>
<td>Safflower oil</td>
<td>4.00</td>
<td>4.00</td>
<td>1.20</td>
</tr>
<tr>
<td>Beef tallow</td>
<td>7.00</td>
<td>7.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Dried egg yolk</td>
<td>3.00</td>
<td>3.00</td>
<td>0.37</td>
</tr>
<tr>
<td>Crystalline cholesterol</td>
<td>0.37</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Complete vitamin mixture</td>
<td>2.50</td>
<td>2.50</td>
<td>2.50</td>
</tr>
<tr>
<td>Balanced mineral mixture</td>
<td>4.63</td>
<td>5.00</td>
<td>...</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>2.00</td>
<td>2.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Alphacel</td>
<td>...</td>
<td>...</td>
<td>15.43</td>
</tr>
</tbody>
</table>

*Composition is in grams per 100 grams of diet.
†HiFC, high fat, high cholesterol; contains 0.19 mg cholesterol per kcal of diet.
§LoFC, low fat, low cholesterol; contains 0.05 mg cholesterol per kcal of diet.
human beings (0.05 mg cholesterol per kcal and about 30% of calories from fat and 10% of calories from unsaturated fatty acids; Table 1). Like the animals in condition 2, these animals also lived in social groups perturbed by monthly reorganization. This condition was designed to model a situation in which human beings with atherosclerosis undergo cholesterol lowering but live under conditions of significant social stress.

**Condition 4: Low-fat, low-cholesterol diet/stable social environment (LoFC-no stress) (n=23).** These monkeys consumed the low-fat, low-cholesterol diet and lived in social groups of unchanging membership. This condition was designed to model a situation in which human beings with atherosclerosis undergo cholesterol lowering and live in a stable social setting.

**Psychosocial Manipulation**

In cynomolgus monkeys, the instability induced by reorganization of membership across social groups is often accompanied by an acute increase in the intensity of agonistic behavior, as animals attempt to reestablish their social relationships. In the current study, monkeys assigned to unstable conditions 2 and 3 were subjected to monthly reorganization of group membership during the treatment phase such that each animal was housed with either three or four new monkeys as a result of each manipulation.

**Clinical Observations**

After a 15-hour fast, animals were anesthetized with ketamine hydrochloride (0.10 mg/kg body wt i.v.) for all blood sampling and physical measurements.

**Plasma cholesterol concentrations.** Blood samples for determining TPC and high density lipoprotein cholesterol (HDLc) concentrations were taken three times during the induction phase of the experiment and at 6-month intervals thereafter. Samples were evaluated using previously described procedures in a laboratory that fully complies with the Centers for Disease Control–National Heart, Lung, and Blood Institute Lipid Standardization Program.

**Blood pressure.** Mean blood pressure in millimeters of mercury was recorded with the use of a Doppler ultrasound apparatus (Arteriosonde 1010, Roche). Measurements were taken once during the induction phase and twice during the treatment phase. Previous work has shown that indirect measurements of blood pressure recorded under ketamine hydrochloride anesthesia correlate well with blood pressure measurements obtained from the same animals while conscious.

**Body weight and ponderosity.** Ponderosity was measured as the body weight of the animal divided by the distance between the suprasternal notch and the pubic symphysis in grams per centimeter. Body length was measured once during the induction phase and twice during the treatment phase; body weight was measured monthly.

**Behavioral Observations**

Thirty-minute observations were made of each social group twice per week to evaluate behavior. Hence, each animal was observed approximately 110 times during the induction phase and 225 times during the treatment phase. The outcomes of all aggressive, submissive, and affiliative interactions occurring within a social group were recorded on an electronic data recording device (Tandy TRS model 100) using an ad libitum sampling technique. All observations were made between 9 AM and 4 PM, with times of day balanced across groups. After collection, the data were transmitted to a VAX computer for calculation of five behavioral indices encompassing a broad range of social interaction: 1) the rate of fighting (fight initiations per monkey per hour); 2) the rate of intense fighting (initiations of intense fights per monkey per hour); and 3) the percentage of time spent a) in passive body contact, b) in grooming, or c) sitting alone at a distance.

**Postmortem Evaluations**

**Coronary artery and aortic atherosclerosis.** At necropsy, all animals were anesthetized with ketamine hydrochloride (30 mg/kg body wt i.v.) and then injected with sodium pentobarbital (60 mg/kg body wt i.v.). While deeply anesthetized, the animals were exsanguinated and the cardiovascular system flushed with 0.1 M phosphate buffer. The heart was removed, and the coronary arteries were perfused with 10% neutral buffered formalin under a pressure of 100 mm Hg for 1 hour. As in previous experiments, 15 tissue blocks (each 3 mm long) were cut perpendicularly to the long axis of the three major coronary arteries after pressure fixation. Five of these were serial blocks from the left circumflex (LCX), five from the left anterior descending (LAD), and five from the right coronary (RCA) artery. One section was cut from each block and stained with Verhoeff-van Gieson's stain and projected onto a grid; the area occupied by intimal lesion (i.e., the area between the internal elastic lamina and the arterial lumen) was measured (in square millimeters) using a computer-assisted image analyzer (MOP III, Zeiss). Previous work has demonstrated a high interobserver reliability (r=0.96) for intimal area measurements obtained by this technique in our laboratory.

The abdominal aorta was also fixed in 10% neutral buffered formalin. After fixation the aorta was opened longitudinally along the posterior surface, and five crow's-foot sections were cut perpendicularly to the long axis. These cross sections of aortic intima were then paraffin fixed, stained with Verhoeff-van Gieson's stain, and projected onto a flat grid. The area occupied by intimal lesion was measured using a computer-assisted image analyzer.

**Arterial remodeling (artery size).** Arterial enlargement is known to occur during progression of atherosclerosis in monkeys and human beings as well as during regression in monkeys; the process of enlargement is considered to be an aspect of arterial remodeling. Verhoeff-van Gieson's-stained sections prepared for determination of coronary artery atherosclerosis were evaluated for two indices of arterial remodeling: 1) artery size (the area circumscribed by the internal elastic lamina) and 2) lumen size (artery size minus the area occupied by the lesion).

**Aortic free and ester cholesterol.** One-centimeter-long sections of abdominal aorta taken from a point adjacent to the mesenteric arteries were minced and homogenized in 4 ml chloroform/methanol (2:1, vol/vol) followed by an overnight extraction at 25°C. The organic phase was removed, and the tissue was washed twice for 1 hour each at 25°C with chloroform/methanol (2:1, vol/vol). The pooled organic phase was extracted twice...
with 4 ml 0.9% NaCl followed by one wash with distilled water to remove hydrophilic components. The organic phase was dried under nitrogen and resolubilized in chloroform, and a portion of the extract was saponified and assayed for total cholesterol content using o-phthalcyanide.35 Free and esterified cholesterol were separated by thin-layer chromatography before quantification. Data were corrected for losses based on recoveries of 3H-cholesterol added before tissue homogenization. Data are expressed as milligrams of cholesterol per gram wet weight of tissue and as milligrams of cholesterol per gram dry weight of tissue. Dry weight is delipidated dry weight of the tissue obtained after lipid extraction.

Statistical Analyses
Serum lipid concentrations (TPC and HDLC) and blood pressure, weight, and ponderosity measurements were subjected to separate repeated-measures analyses of variance (ANOVA). Data collected during the induction phase were analyzed according to the later assignment of animals to the baseline or the three treatment conditions using one grouping factor (condition: baseline, HiFC-stress, LoFC-stress, or LoFC-no stress) and a variable number of repeated measures, depending on the frequency with which each variable was sampled in this portion of the study. Similarly, data collected during the treatment phase of the experiment were analyzed by ANOVA using one grouping factor reflecting the three treatments (condition: HiFC-stress, LoFC-stress, or LoFC-no stress) and a variable number of repeated measures. All post hoc comparisons involving antemortem measures were performed using Scheffé’s procedure.43 Each of the five behavioral indices was represented by the mean of data recorded in 110 observations for each animal in the induction phase and 225 observations in the treatment phase. The individual values that related to the initiation of fighting or intense fighting had a nonparametric distribution. These data were analyzed using a Kruskall-Wallis one-way ANOVA44; the grouping factor had four levels (baseline, LoFC-no stress, LoFC-stress, and HiFC-stress) for data collected during the induction period and three levels (LoFC-no stress, LoFC-stress, and HiFC-stress) for data collected during

**Table 2. Clinical Measurements During Induction** and Intervention Phases

<table>
<thead>
<tr>
<th>Groups</th>
<th>TPC (mg/dl)</th>
<th>HDLC (mg/dl)</th>
<th>Body weight (g)</th>
<th>Ponderosity index (g/cm)</th>
<th>Mean BP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Induction</strong></td>
<td></td>
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<tr>
<td>Baseline (n=21)</td>
<td>642±126</td>
<td>23±5</td>
<td>5.5±1.0</td>
<td>178±21</td>
<td>76.2±9.4</td>
</tr>
<tr>
<td>HiFC-stress (n=18)</td>
<td>652±94</td>
<td>21±5</td>
<td>5.6±1.0</td>
<td>176±19</td>
<td>67.1±12.1</td>
</tr>
<tr>
<td>LoFC-stress (n=21)</td>
<td>656±114</td>
<td>21±7</td>
<td>5.7±1.0</td>
<td>180±21</td>
<td>66.8±11.0</td>
</tr>
<tr>
<td>LoFC-no stress (n=23)</td>
<td>690±168</td>
<td>23±4</td>
<td>5.6±1.0</td>
<td>179±18</td>
<td>69.8±12.3</td>
</tr>
<tr>
<td><strong>Intervention</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HiFC-stress (n=18)</td>
<td>355±80†</td>
<td>40±17†</td>
<td>5.8±0.6</td>
<td>191±19</td>
<td>67.5±13.1</td>
</tr>
<tr>
<td>LoFC-stress (n=21)</td>
<td>176±30†</td>
<td>66±13†</td>
<td>6.0±0.8</td>
<td>197±22</td>
<td>67.1±7.0</td>
</tr>
<tr>
<td>LoFC-no stress (n=23)</td>
<td>182±34†</td>
<td>65±18†</td>
<td>6.2±1.2</td>
<td>201±31</td>
<td>68.6±9.4</td>
</tr>
</tbody>
</table>

TPC, total plasma cholesterol; HDLC, high density lipoprotein cholesterol; BP, blood pressure; HiFC, high-fat, high-cholesterol diet; LoFC, low-fat, low-cholesterol diet. Values are mean±SD.

*No significant effects of condition on any variable were observed.
†Condition: F2,9=76.3, p<0.01.
‡Condition: F2,9=15.2, p<0.01.

**Table 3. Behavioral Indices Recorded During Induction** and Intervention Phases

<table>
<thead>
<tr>
<th>Groups</th>
<th>Median fights initiated†</th>
<th>Median intense fights initiated‡</th>
<th>Time spent (%)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Passive BC</td>
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<tr>
<td><strong>Induction</strong></td>
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<td></td>
</tr>
<tr>
<td>Baseline (n=21)</td>
<td>1.52</td>
<td>0.92</td>
<td>16.1±12.0</td>
</tr>
<tr>
<td>HiFC-stress (n=18)</td>
<td>1.92</td>
<td>1.29</td>
<td>21.3±13.8</td>
</tr>
<tr>
<td>LoFC-stress (n=21)</td>
<td>3.09</td>
<td>1.45</td>
<td>16.1±8.5</td>
</tr>
<tr>
<td>LoFC-no stress (n=23)</td>
<td>1.80</td>
<td>1.12</td>
<td>15.4±7.4</td>
</tr>
<tr>
<td><strong>Intervention</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HiFC-stress (n=18)</td>
<td>3.44</td>
<td>1.36‡</td>
<td>11.5±7.0†</td>
</tr>
<tr>
<td>LoFC-stress (n=21)</td>
<td>3.23</td>
<td>1.50‡</td>
<td>10.4±4.8§</td>
</tr>
<tr>
<td>LoFC-no stress (n=23)</td>
<td>2.66</td>
<td>1.05§</td>
<td>5.2±4.8§</td>
</tr>
</tbody>
</table>

BC, body contact; HiFC, high-fat, high-cholesterol diet; LoFC, low-fat, low-cholesterol diet. Values are median or mean±SD.

*No significant differences between conditions for any behavioral index were observed.
†Per monkey per hour.
‡Significant differences among groups (Kruskall-Wallis test, p<0.04); stress conditions significantly greater than no-stress condition.
§Significant differences among groups (F2,9=7.83, p<0.01); stress conditions significantly greater than no-stress condition.
FIGURE 1. Panel A: Section of left circumflex coronary artery from a monkey in the baseline group. The intimal lesions consist of foam cells, extracellular lipid, smooth muscle cells, and collagen. Panel B: Section of left circumflex coronary artery from a monkey in the LoFC-no stress group. The intimal lesions contain fewer foam cells and more collagen than those from the baseline group. Panel C: Coronary artery section from an animal in the LoFC-stress group. Lesions in this group had somewhat more foam cells but were otherwise like those in the LoFC-no stress group. Panel D: Coronary artery lesion typical of progressing atherosclerosis in the cynomolgus monkey. Lesions contained abundant foam cells, extracellular lipid, and collagen. Verhoeff–van Gieson's stain, ×42. LoFC, low fat, low cholesterol.

the treatment phase of the experiment. Values related to the percentage of time spent in various activities were parametrically distributed and analyzed for both phases of the experiment using an ANOVA with a single grouping factor containing either four (induction phase) or three (treatment phase) levels.

For each animal, intimal area, artery size, and lumen area were expressed as the mean (in square millimeters) of five sections from each of the coronary arteries; thus, there were three values (LAD, LCX, and RCA) for each measure. The mean extent of atherosclerosis (in square millimeters) of five sections of aorta was taken as the index of aortic atherosclerosis for each animal. All arterial data were logarithmically transformed to ensure homogeneity of variance and then evaluated using a repeated-measures ANOVA with one between- and one within-subjects factor (condition, LoFC-stress × artery, LAD, LCX, and RCA). All post hoc evaluations of postmortem measurements were done with Dunnet's procedure, using the baseline condition as a control against which the three treatment conditions were compared. All tests of significance were two-tailed at p < 0.05.

Results

Clinical Observations

Induction phase. First, data were analyzed to establish that animals assigned to the baseline or the three treatment conditions did not differ in serum lipid, blood pressure, weight, or ponderosity measurements during the induction phase (Table 2). Condition had no effect on any of these parameters.

Treatment phase. During the treatment phase, condition had a significant effect on TPC and HDLC but not
on blood pressure or body size (Table 2). Post hoc evaluations demonstrated that TPC was significantly lowered and HDLC raised among all monkeys consuming the LoFC diet (both stress and no-stress groups) compared with animals in the HiFC-stress condition.

**Behavioral Observations**

**Induction phase.** The five behavioral indices were analyzed to determine whether differences existed during the induction phase among animals later assigned to the baseline or the three treatment conditions (Table 3). There were no significant differences in any of these behaviors.

**Treatment phase.** Analysis of the behavioral data collected during the treatment phase demonstrated that monkeys in stressed conditions (HiFC-stress and LoFC-stress) engaged in a significantly higher rate of intense fighting and in significantly more passive affiliation than did their counterparts in the LoFC-no stress condition (Table 3). No other differences were observed.

**Postmortem Findings**

**Appearance of the arteries.** Figure 1 contains photomicrographs of typical histological sections of LCXs from animals from the four groups. Figure 1A is typical of atherosclerotic lesions seen most commonly in the baseline group. There was marked intimal thickening composed of intracellular and extracellular lipid, smooth muscle cells, and macrophages and increased amounts of collagen. In Figure 1B, we have illustrated a typical lesion from the LoFC-no stress group. The lesions in that group consist of thickened intimas, few foam cells, and abundant smooth muscle cells and collagen. In Figure 1C, we have illustrated a typical lesion from an animal in the LoFC-stress group. The lesions consisted of a thickened intima, a few foam cells, and abundant amounts of collagen and smooth muscle cells. Finally, Figure 1D shows an example of a coronary artery lesion like those seen among the animals that continued the HiFC-stress regimen. Compared with the baseline animals, the intimal thickenings were greater, and the lesions continued to be composed primarily of foam cells with some smooth muscle cell proliferation and abundant extracellular lipid and collagen.

**Coronary artery atherosclerosis.** There were significant effects of condition and artery on lesion area; the interaction between the factors was not significant. The statistical tests are summarized in Table 4, whereas Figure 2 depicts atherosclerosis extent in each condition (averaged data from each of the three arteries). Post hoc comparisons indicated that animals in the HiFC-stress condition had significantly larger lesions than did baseline monkeys; however, baseline animals did not differ significantly in lesion size from animals in either of the LoFC conditions (stress or no stress). Also, lesions in the LAD and LCX were significantly larger than those in the RCA.

There were no significant correlations between serum lipid levels, blood pressure, or body size measurements of collagen. In Figure 1B, we have illustrated a typical lesion from the LoFC-no stress group. The lesions in that group consist of thickened intimas, few foam cells, and abundant smooth muscle cells and collagen. In Figure 1C, we have illustrated a typical lesion from an animal in the LoFC-stress group. The lesions consisted of a thickened intima, a few foam cells, and abundant amounts of collagen and smooth muscle cells. Finally, Figure 1D shows an example of a coronary artery lesion like those seen among the animals that continued the HiFC-stress regimen. Compared with the baseline animals, the intimal thickenings were greater, and the lesions continued to be composed primarily of foam cells with some smooth muscle cell proliferation and abundant extracellular lipid and collagen.

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Table 5. Pearson Correlations (r) Between Artery Size/Lumen Area and Intimal Area Within Conditions*

<table>
<thead>
<tr>
<th>Artery size/intimal area</th>
<th>Baseline</th>
<th>HiFC-stress</th>
<th>LoFC-stress</th>
<th>LoFC-no stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>21</td>
<td>18</td>
<td>21</td>
<td>23</td>
</tr>
<tr>
<td>lumen area/intimal area</td>
<td>0.96</td>
<td>0.87</td>
<td>0.85</td>
<td>0.84</td>
</tr>
</tbody>
</table>

HiFC, high-fat, high-cholesterol diet; LoFC, low-fat, low-cholesterol diet.

*Correlations represent the mean of correlations across three arteries within each animal at p<0.01 except as noted.

\( t_p < 0.10 \), \( t_p < 0.05 \).

during the induction phase and extent of coronary artery atherosclerosis. TPC and HDLC (measured during the treatment phase) were significantly correlated with the extent of coronary artery atherosclerosis, but only among animals in the HiFC-stress condition (TPC: \( r=0.43 \); HDLC: \( r=-0.49 \); \( p<0.05 \) for both).

**Coronary artery size.** Condition and artery (LAD, LCX, or RCA) had a significant effect on artery size; however, the artery \times condition interaction was not significant (Table 4). Figure 3 shows artery size in each condition, with averaged findings from all three arteries. Post hoc evaluations indicated that monkeys in each of the three treatment conditions (HiFC-stress, LoFC-stress, and LoFC-no stress) had significantly larger arteries than did animals in the baseline condition. Further, the LAD and LCX were larger than the RCA in all monkeys.

Lumen area was also significantly affected by condition (Figure 3) and by artery; additionally, there was a significant artery \times condition interaction (Table 4). Post hoc analysis of these findings indicated that lumens were significantly larger among monkeys in the three treatment conditions than among baseline animals. Further, lumens were significantly larger in the LAD and LCX than in the RCA. Evaluation of the condition \times artery interaction indicated that lumen size differed more significantly among conditions within the RCA than within the LAD or LCX; in all three arteries, however, baseline animals had significantly smaller lumens than did animals in the three treatment conditions.

Finally, the relation between artery size to lumen size ratio and intimal area was examined among animals in each condition (Table 5). Both lumen and artery size were associated significantly with intimal area within each condition.

**Atherosclerosis in the abdominal aorta.** Figure 4 illustrates that animals in the four conditions differed significantly in lesion extent (statistical tests shown in Table 5), with post hoc comparisons demonstrating significantly larger lesions among animals in the HiFC-stress condition than among baseline monkeys. The baseline animals did not differ from those in the LoFC conditions (stress or no stress). Finally, there were no significant associations between serum lipid concentrations, mean blood pressure, or body size (as measured during the induction or treatment phases) and the extent of atherosclerosis in the abdominal aorta.

**Aortic free and esterified cholesterol.** Aortic cholesterol content was determined for a subset of the animals (n=36) randomly selected from each treatment group (Figures 5 and 6). When calculated on a per unit wet weight of tissue basis, esterified cholesterol levels were significantly affected by condition, whereas free cholesterol was only marginally affected (Table 4). Post hoc evaluation indicated that significantly less esterified cholesterol was present in the aortas of monkeys fed the LoFC diet (stress and no stress) than in the aortas of animals in the baseline or HiFC-stress conditions, which could not be distinguished from each other by these values. Similarly, when calculated on a per unit dry weight of tissue basis, esterified cholesterol level differed significantly among conditions, whereas free cholesterol varied marginally among conditions (Table 4). Post hoc tests demonstrated that significantly less esterified cholesterol per unit dry weight of tissue was...
present in the aortas of monkeys that had consumed the LoFC diet (stress and no stress) than in the aortas of animals in either the baseline or HiFC-stress conditions.

Discussion

Atherosclerosis regression is a complex process encompassing changes in both morphological and functional characteristics. Lesion, artery, and lumen size (aspects of remodeling) and lesion composition were measured directly in the current investigation. Atherosclerosis was first induced in all animals by feeding a HiFC diet for 14 months. We found that progression of atherosclerosis in the coronary arteries and abdominal aorta was entirely arrested in monkeys that then consumed a low-fat diet based on current recommendations for human beings. In contrast, lesions became considerably enlarged in monkeys initially fed an induction diet that thereafter remained relatively hypercholesterolemic and were subjected to social stress during the treatment phase. It has previously been reported in nonhuman primates that preexisting lesions actually may decrease after consumption of “regression diets,” but this has only been shown when such diets contained less saturated fat and cholesterol than is generally recommended by current guidelines. Thus, our results may represent more accurately the expected outcome in human beings under usual dietary recommendations.

Significant compensatory arterial enlargement also occurred in the current experiment, indicated in part by a positive association between lesion size and both lumen and artery size within each condition. More importantly, animals in all three treatment conditions had larger arteries and lumens than did baseline animals. This arterial enlargement was probably not a function of maturation, as the animals were 5-12 years old when the study began. Notably, arterial enlargement more than compensated for lesion size: the arterial lumens of monkeys in the HiFC-stress condition were three times larger than in baseline monkeys. Further, once initiated, arterial enlargement continued even without lesion progression, as evidenced by the substantially enlarged lumens of monkeys in both LoFC conditions (stress and no stress) compared with those of baseline animals; this disparity in lumen size existed despite the presence of similarly sized lesions.

There may be limits to compensatory arterial enlargement. Among monkeys in the HiFC-stress condition, for example, lesions were much larger but lumens were only marginally larger than among animals in the LoFC conditions (stress and no stress). As a result, the lumen area to intimal area ratios among monkeys consuming the LoFC diet (stress or no stress) were three times larger than in animals in the HiFC-stress condition (Figure 3).

Currently, angiographic studies of atherosclerosis in human beings provide assessment of lumen stenosis but not lesion size. Since lumen stenosis is judged from artery and lumen size, an inaccurate representation of atherosclerosis may result if arterial remodeling (particularly enlargement) has occurred in response to lesion development. Notably, angiographic and morphological evidence from recent studies in human beings is consistent with the presence of compensatory arterial enlargement in the heart, particularly where atherosclerosis is of mild to moderate severity.22,23 Arterial enlargement in response to lesion development has also been reported in previous studies of nonhuman primates.30,38,46 Passive arterial distention or active growth of the media may mediate such enlargement, which probably would be limited once lesions became calcified or fibrotic.23 The foregoing data and our observations suggest that compensatory enlargement may accompany atherosclerotic regression and perhaps contribute to the reduced stenosis sometimes reported in angiographic studies of regression in human beings. Such reductions in stenosis are usually attributed, in the absence of direct measurements, strictly to alterations in lesion size.

Among other results, aortic lesion composition was significantly affected by the dietary manipulation. Hence, there was less esterified cholesterol (and marginally less free cholesterol) in the lesions of monkeys consuming the LoFC diet (stress and no stress) than in those of animals in the baseline or HiFC-stress conditions. The distribution of foam cells and cholesterol clefts in Figure 1 indicates that the dietary manipulation similarly affected lesion composition in all of the coronary arteries that were studied. Other investigations of regression in nonhuman primates have reported similar patterns of lesion composition after cholesterol lowering.32,47-49 Therefore, it might be speculated that analogous changes would take place in the arteries of human beings experiencing significant reductions in serum lipid levels.

Psychosocial stress had no effect on lesion, artery, or lumen size or on lesion composition. Elsewhere, however, we have shown in the same animals that the same psychosocial manipulation, but not diet or lesion extent, influenced vasomotor function (as indexed by in vivo coronary artery responses to infusion of acetylcholine, nitroglycerin, and saline).50 Specifically, the LoFC-no stress monkeys demonstrated relative arterial dilation after exposure to acetylcholine compared with the relative (and similar degree of) constriction observed among the stressed animals consuming either diet. This pattern of functional differences occurred despite equivalent lumen size and lesion extent for monkeys in the two regression-diet conditions and despite the markedly greater lesion and artery size among monkeys in the HiFC-stress condition. The observation that psychosocial stress influenced vasomotor responses suggests that, during dietary interventions, psychosocial factors may have a more immediate effect on arterial function than on lesion structure. Notably, it has been suggested that ameliorated endothelial function is a more relevant interventional goal than is reduced coronary artery lesion size.20

From these data, we conclude that a LoFC diet arrests the development of preexisting atherosclerosis and reduces the cholesterol content of lesions in this animal model. Arterial enlargement also occurred in all treatment conditions. Finally, while manipulation of a psychosocial stressor known to potentiate atherogenesis in this species did not influence lesion size or composition, such stress does appear to modulate coronary vasomotor activity.

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