Atherosclerosis of Cynomolgus Monkeys Hyper- and Hyporesponsive to Dietary Cholesterol
Lack of Effect of Vasectomy

Thomas B. Clarkson, Nancy J. Alexander, and Timothy M. Morgan

A moderately atherogenic diet was fed to young adult cynomolgus macaque males that were observed to be either hypo- or hyperresponsive to dietary cholesterol and who were randomized into groups to be either vasectomized or sham-vasectomized. The extent of atherosclerosis was found to be considerably greater at all arterial sites studied for the monkeys that were hyperresponsive to dietary cholesterol. The differences in atherosclerosis development among the hyperresponder monkeys occurred primarily in the proximal portions of the coronary arteries, the proximal and distal portions of the common carotid arteries, and only in the most proximal portions of the femoral arteries. There were no significant effects of vasectomy or sham vasectomy on atherosclerosis extent in either the hyper- or the hyporesponding groups, although there was a suggestion of somewhat larger lesions in the left circumflex coronary artery of hyperresponder monkeys that were vasectomized and somewhat smaller atherosclerotic lesions in the left common carotid arteries of vasectomized monkeys. The data presented here do not support our first report of worsened atherosclerosis among cynomolgus monkeys fed diets high in cholesterol. The findings of the current study are consistent with recent epidemiological studies of vasectomized and nonvasectomized human males.

The repeated injection of animals with foreign antigens exacerbates atherosclerosis. There are similarities between the repeated injections of a foreign antigen and the body's reaction to sperm after vasectomy. Sperm continue to be produced at prevasectomy rates, soluble antigens from sperm enter the circulation, the sperm are seen as foreign proteins by the body, and antisperm antibodies develop. Because of the immune response to vasectomy, there has been concern over the past several years about possible long-term health effects of the procedure. Some of the concern arose from our report nearly a decade ago that vasectomized cynomolgus macaques fed diets high in cholesterol developed more extensive atherosclerosis than did sham-vasectomized monkeys fed the same diet. After that report, several case-control epidemiological studies were undertaken in an attempt to determine whether there was any evidence that coronary heart disease increased among vasectomized men; there was no evidence that vasectomy increased the incidence of coronary heart disease in the general population. The apparent difference in vasectomy effects on arteries between humans and monkeys has been unclear.

We and others have considered the possibility that our first observation of vasectomized animals was based on too small a sample and that an exacerbating effect of vasectomy might not be seen if much larger groups of monkeys were studied. Additionally, we were concerned with the extreme atherogenicity of the diet that was used (inducing total plasma cholesterol concentrations of 600 to 800 mg/dl).

More recently, we reported on the results of a study designed to examine the effect of a moderately athero-
concentrations were either slightly elevated (hyporesponders) or considerably elevated (hyperresponders). Consistent with our studies of squirrel monkeys, we found atherosclerosis to be more extensive among hyper- as compared to hyporesponder cynomolgus monkeys. Additionally, there were suggestions that vasectomy may have resulted in slightly reduced amounts of atherosclerosis at some arterial sites; however, we could not determine whether such small differences were due to somewhat lower total plasma cholesterol concentrations and low density lipoprotein cholesterol concentrations. In general, it is our conclusion that vasectomy had no significant effects on the extent of atherosclerosis among either hyper- or hyporesponding cynomolgus monkeys.

Methods

Animals

The animals used were 66 male cynomolgus monkeys imported as adults from Malaysia (average age 7.5 years, estimated by dentition). They were selected from a population of about 300 adult male Macaca fascicularis that had been fed a diet containing approximately 0.34 mg/kcal of cholesterol for 2 months. Blood samples for determination of total plasma cholesterol and high density lipoprotein (HDL) cholesterol concentrations were taken from all animals at weeks 6 and 8 during this pretest period. The 34 animals selected as being hyporesponsive to dietary cholesterol had an average plasma cholesterol concentration of 222 mg/dl. In contrast, the 32 animals selected as being hyperresponsive to dietary cholesterol had an average plasma cholesterol concentration of 422 mg/dl. After selection, the animals were brought to the Bowman Gray School of Medicine, the diet was continued, and plasma cholesterol concentration tests were repeated to confirm their hypo- and hyperresponsiveness.

Design and Allocation of Monkeys to Groups

The design of the experiment was a stratified randomized trial in which animals either hypo- or hyperresponsive to dietary cholesterol were randomized to be

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of monkeys</th>
<th>TPC</th>
<th>HDLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyporesponders</td>
<td>Vx (Group 1)</td>
<td>17</td>
<td>221 ± 11.6</td>
</tr>
<tr>
<td></td>
<td>Sham Vx (Group 2)</td>
<td>17</td>
<td>222 ± 11.4</td>
</tr>
<tr>
<td>Hyperresponders</td>
<td>Vx (Group 3)</td>
<td>16</td>
<td>423 ± 18.7</td>
</tr>
<tr>
<td></td>
<td>Sham Vx (Group 4)</td>
<td>16</td>
<td>426 ± 19.2</td>
</tr>
</tbody>
</table>

Data are the means (mg/dl) for the groups ± SE of the mean; the range of values is in parentheses.

Vx = vasectomy, Sham Vx = sham vasectomy, TPC = total plasma cholesterol concentration, HDLC = high density lipoprotein cholesterol concentration.

Table 2. Composition of Moderately Atherogenic Diet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>gm/100 gm</th>
<th>Protein (gm)</th>
<th>Fat (gm)</th>
<th>Carbo (gm)</th>
<th>Calories/100 gm of diet</th>
<th>Chol (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein USP</td>
<td>9.0</td>
<td>9.0</td>
<td>—</td>
<td>—</td>
<td>36.0</td>
<td>—</td>
</tr>
<tr>
<td>Lactalbumin</td>
<td>8.0</td>
<td>8.0</td>
<td>—</td>
<td>—</td>
<td>32.0</td>
<td>—</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>33.5</td>
<td>3.5</td>
<td>0.33</td>
<td>28.5</td>
<td>131.0</td>
<td>—</td>
</tr>
<tr>
<td>Dextrin</td>
<td>10.0</td>
<td>—</td>
<td>—</td>
<td>10.0</td>
<td>40.0</td>
<td>—</td>
</tr>
<tr>
<td>Sucrose</td>
<td>3.6</td>
<td>—</td>
<td>3.6</td>
<td>—</td>
<td>14.4</td>
<td>—</td>
</tr>
<tr>
<td>Applesauce</td>
<td>0.7</td>
<td>0.003</td>
<td>trace</td>
<td>0.168</td>
<td>0.68</td>
<td>—</td>
</tr>
<tr>
<td>Butter</td>
<td>23.50</td>
<td>0.10</td>
<td>19.00</td>
<td>0.10</td>
<td>171.90</td>
<td>70.5</td>
</tr>
<tr>
<td>Hefsted salt mix</td>
<td>3.80</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Complete vitamin mix</td>
<td>2.60</td>
<td>—</td>
<td>—</td>
<td>2.6</td>
<td>10.4</td>
<td>—</td>
</tr>
<tr>
<td>Alphacel</td>
<td>5.22</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.08</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>80.0</td>
</tr>
</tbody>
</table>

Total         | 100.00    | 20.60        | 19.33    | 44.97      | 436.38                  | 150.5    |

Equivalent to 0.35 mg cholesterol/kcal of diet.

Protein = 19% of calories, fat = 40% of calories, and carbohydrate = 41% of calories.
The double-ligation technique, and the other half were sham-vasectomized by subjecting them to the same surgical procedure but without ligation or resection of the vas deferens.

Table 3. Body Weight and Clinical Pathologic Observations

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Baseline</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>HO-VX</td>
<td>5.17 ± 0.22</td>
<td>5.37 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>HO-SHAM</td>
<td>4.97 ± 0.13</td>
<td>5.30 ± 0.18*</td>
</tr>
<tr>
<td></td>
<td>HP-VX</td>
<td>4.83 ± 0.12</td>
<td>5.22 ± 0.12†</td>
</tr>
<tr>
<td></td>
<td>HP-SHAM</td>
<td>4.81 ± 0.19</td>
<td>5.19 ± 0.19*</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dl)</td>
<td>HO-VX</td>
<td>15.53 ± 0.71</td>
<td>12.65 ± 0.73†</td>
</tr>
<tr>
<td></td>
<td>HO-SHAM</td>
<td>14.82 ± 0.87</td>
<td>12.58 ± 0.51†</td>
</tr>
<tr>
<td></td>
<td>HP-VX</td>
<td>15.25 ± 0.81</td>
<td>12.53 ± 0.87*</td>
</tr>
<tr>
<td></td>
<td>HP-SHAM</td>
<td>16.50 ± 0.96</td>
<td>13.40 ± 1.09*</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>HO-VX</td>
<td>0.96 ± 0.03</td>
<td>1.04 ± 0.04‡</td>
</tr>
<tr>
<td></td>
<td>HO-SHAM</td>
<td>0.98 ± 0.04</td>
<td>1.06 ± 0.03*</td>
</tr>
<tr>
<td></td>
<td>HP-VX</td>
<td>1.00 ± 0.04</td>
<td>1.13 ± 0.04*</td>
</tr>
<tr>
<td></td>
<td>HP-SHAM</td>
<td>1.04 ± 0.05</td>
<td>1.15 ± 0.04</td>
</tr>
</tbody>
</table>

*Mean during experimental period significantly (p<0.05) different from the baseline mean value. †Mean during experimental period significantly (p<0.001) different from the baseline mean value. ‡Hyperresponders significantly (p<0.05) different from the hyporesponders.


either vasectomized or sham-vasectomized. The animals were allocated to the two groups so they would be as equal as possible within each stratum in terms of total plasma cholesterol concentrations, HDL cholesterol concentrations, and age. The design of the experiment and the plasma lipid concentrations of the four groups of animals are shown in Table 1.

Diet

A moderately atherogenic diet designed to mimic the composition of diets often consumed by North American humans who are at high risk for coronary heart disease was used in this study (Table 2). The diet contained 40% of calories from fat and 0.35 mg cholesterol per calorie. The diet was mixed with 24 ml of H2O per 100 grams dry weight constituents, and 100 grams was fed to each animal twice a day. Both hypo- and hyperresponsive animals consumed all of the diet. Body weight gains were the same (see Table 3).

To ensure that plasma cholesterol concentrations remained within desired ranges, it was necessary to make two reductions in the amount of dietary cholesterol. At month 4 of the 18-month study, the amount of dietary cholesterol was reduced to 0.25 mg/kcal. At month 11, a further reduction was made to 0.18 mg/kcal, and this amount was fed for the remainder of the experiment.

Surgical Procedures

After 6 months of consumption of the cholesterol-containing diet, half of the animals in each dietary cholesterol response group were vasectomized by the double-ligation technique, and the other half were sham-vasectomized by subjecting them to the same surgical procedure but without ligation or resection of the vas deferens.

Health Profile Observations

During the course of the pre-experimental and experimental periods, body weights were determined at 2-month intervals. At 6-month intervals, total serum protein concentrations, blood urea nitrogen concentrations, serum creatinine concentrations, urinary protein concentrations, hematocrits, hemoglobin concentrations, and enumeration of erythrocytes/leukocytes were measured in the Clinical Pathology Laboratory of the Department of Comparative Medicine using standard quality-controlled procedures.

Blood Pressure

Systolic and diastolic blood pressure measures were recorded on monkeys sedated with ketamine hydrochloride by using a Doppler ultrasound apparatus (Arteriosonde 1010, Roche, Cranberry, NJ). Blood pressures were determined at 10 different intervals during the course of the study.

Plasma Lipid Concentrations

Blood samples for determination of total plasma cholesterol and HDL cholesterol concentrations were taken at 2-month intervals during the course of the study. Total plasma cholesterol determinations (in mg/dl) were done by using the AutoAnalyzer II procedure.24 HDL cholesterol concentrations (in mg/dl) were assessed by the heparin manganese precipitation procedure, as described in the Lipid Research Clinics manual.25 All plasma lipid evaluations were evaluated in our Lipid Analytic Laboratory, which is in complete compliance with the Cooperative Lipid Standardization Program of the U.S. Department of Health and Human Services. Animals were fasted 24 hours before sampling, and during the sampling procedures they were restrained with ketamine hydrochloride (15 mg/kg).

Antisperm Antibody Measurements

Both sperm agglutination and sperm immobilization antibody assays26,27,28 were used to assess the immune response to vasectomy.14,15,16 Results from sperm agglutination antibody (SpAgg) assays are reported in integers representing the lowest titer of serum from a test animal that would agglutinate a given volume of normal sperm. Values ranged from 0 to 8 and represent serum dilutions of 1: 10, 20, 40, 80, 160, 320, 640, or 1280 with 0 being <1:10. Animals with high SpAgg concentrations would
have a high dilution factor and thus a high integer value. Numbers for the complement dependent sperm immobi-
ization (Splmm) antibody assays are the result of the following equation:

\[
\text{Splmm} = 1 - \frac{\% \text{ motile sperm in test serum}}{\% \text{ motile sperm in control serum}}
\]

The range of possible values is 0 to 1, with 0 representing low antibody concentration in the experimental serum and 1 representing high antibody titers in test animals.

**Measurements of Atherosclerosis**

**Thoracic and Abdominal Aorta**

The thoracic and abdominal aorta were dissected from each animal. The arteries were cleaned of excess adventitia, were opened longitudinally, were mounted flat on cardboard, and were immersion-fixed in 10% neutral buffered formalin for 1 week. After fixation, the arteries were stained with Sudan IV in isopropyl alcohol. To evaluate the percent intimal involvement, the arteries were photographed and the photographs were then digitized to calculate the percentage of total surface area affected with plaque and/or fatty streaks.

To evaluate lesion extent, five standard blocks (whole width of aorta) were cut from each artery by using a celluloid template for block selection. The blocks were 3 mm long and were cut perpendicular to the long axis of the artery. After embedding in paraffin, a 5 \(\mu\)m section was cut from each block and was stained with Verhoeff van Gieson stain.

The stained artery cross-sections were then projected with a projection microscope onto a digitizer tablet. Using a hand-held stylus and a computer-assisted digitizer, we measured the cross-sectional intimal area of each artery section.

**Coronary Arteries**

At necropsy, the heart was removed and the coronary arteries were perfused with 10% neutral buffered formalin under a pressure of 100 mm Hg. After pressure fixation, 17 tissue blocks (each 3 mm in length) were cut perpendicularly to the long axis of the coronary arteries. Six of these were serial blocks from the left circumflex, six were from the left anterior descending artery, and five were from the right coronary artery (Figure 1). Two sections were cut from each block and were stained with either hematoxylin and eosin or Verhoeff van Gieson. The stained artery cross-sections were then projected, and the area occupied by intima and/or intimal lesion was measured. The extent of coronary artery atherosclerosis for each animal was expressed as the cross-sectional intimal area (in mm\(^2\)) of each of the sections of each of the coronary arteries. Coronary artery sections were also projected and digitized by a second investigator; a comparison of cross-sectional intimal areas based on the two sets of measurements yielded a reliability coefficient of 0.96.

<table>
<thead>
<tr>
<th>Table 4. Hematologic Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
</tr>
<tr>
<td>Leukocyte counts</td>
</tr>
<tr>
<td>(x10(^3) /mm(^3))</td>
</tr>
<tr>
<td>Total monocyte</td>
</tr>
<tr>
<td>(x10(^3) /mm(^3))</td>
</tr>
<tr>
<td>HP-VX</td>
</tr>
<tr>
<td>HP-SHAM</td>
</tr>
</tbody>
</table>

*Mean during experimental period significantly (p<0.05) different from the baseline mean value. †Vasectomized animals significantly (p<0.05) different from the sham-vasectomized animals. ‡Vasectomized animals significantly (p<0.001) different from the sham-vasectomized animals.

<table>
<thead>
<tr>
<th>Table 5. Blood Pressure Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
</tr>
<tr>
<td>(mm Hg)</td>
</tr>
<tr>
<td>HP-VX</td>
</tr>
<tr>
<td>HP-SHAM</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
</tr>
<tr>
<td>(mm Hg)</td>
</tr>
<tr>
<td>HP-VX</td>
</tr>
<tr>
<td>HP-SHAM</td>
</tr>
</tbody>
</table>

*Mean during experimental period significantly (p<0.05) different from the baseline mean value.


**Iliacofemoral and Carotid Arteries**

The iliacofemoral and common carotid arteries, including the carotid bifurcations, were removed at necropsy, were opened longitudinally on the anterior surface, and were placed in 10% neutral buffered formalin for immersion fixation. For both the iliacofemoral and common carotid arteries, five standardized blocks were taken, and the prepared sections were stained with hematoxylin and eosin and with Verhoeff van Gieson stains. Cross-sectional intimal areas were determined for each of the arteries as described for coronary arteries. The size of the carotid bifurcation prevented taking more than a single standardized block. The carotid bifurcation was processed as described for the common carotid arteries, and the extent of atherosclerosis was determined as the cross-sectional intimal area.

**Statistical Analyses**

Comparisons of variables with multiple measurements for each monkey were performed by univariate repeated measures analysis of variance by using BMDP3V, which does not exclude cases with incomplete repeated measures. Repeated measures of body weight, clinicopathologic, hematologic, and blood pressure variables were measured across time, and repeated measures of intimal areas were done on multiple sections. There were only a few missing repeated observations: 2% in body weight,
Results

Health Profile Observations

In Table 3 we have summarized the results of the most important clinicopathologic observations taken both before the initiation of the experiment (baseline) and during the course of the experiment (experimental). Generally, the animals remained in good health, and there were no major differences in the health profile data between hypo- and hyperresponders. There was a modest gain in body weight during the course of the study, an average of half a kilogram.

Because we recognized that there was some possibility that the vasectomized animals might develop glomerulonephritis as a result of their circulating immune complexes (sperm antigen/antisperm antibody), multiple measurements were made of the blood urea nitrogen and plasma creatinine concentrations. The blood urea nitrogen concentrations of all animals in all four groups decreased during the course of the experiment. The decreases were clinically unimportant, statistically significant, and have often been noted in the improved health of feral animals. There were small increases in plasma creatinine concentrations during the experimental period in all groups. Although some of the increases were statistically significant, the magnitude of the increase was of no clinical significance, and there was no indication of a relation between hyper- or hyporesponsiveness to dietary cholesterol or vasectomy.

There were no effects of hyper- or hyporesponsiveness to dietary cholesterol on leukocyte counts. We have no explanation for the small differences among the groups during the baseline period. During the experimental period, the vasectomized animals had higher total leukocyte counts than did the sham-vasectomized animals, and...
there was no difference in the extent of this elevation between the hyper- and hyporesponding vasectomized animals (Table 4). Small sperm granulomas developed in the majority of the vasectomized animals, and it was our conclusion that the elevated leukocyte counts related to local low-grade infections associated with fistulotus tracts that developed from the sperm granulomas.

Monocyte adherence to endothelium is an important part of atherogenesis. We have reported that there are decreased numbers of monocytes adhering to the endothelial surfaces of the major arteries of vasectomized cynomolgus monkeys fed a moderately atherogenic diet. For that reason it was of interest to us to determine whether there were differences between vasectomized and sham-vasectomized hyper- and hyporesponder animals in the extent of total monocyte count decrease. As noted in Table 4, there were large decreases in total monocyte counts during the experimental period. There was also a significant effect of vasectomy on the magnitude of the decrease in monocyte counts. In keeping with our earlier observation that vasectomy was associated with reduced numbers of monocytes adhering to arterial surfaces, it was of interest to note that the vasectomized animals had significantly higher total monocyte counts. Whether the lower total monocyte counts of the sham-vasectomized animals and the higher counts of the vasectomized animals related to differences in arterial wall monocyte adherence is uncertain.

During the course of this experiment, we also measured total serum protein concentrations, urinary protein concentrations, hematocrits, hemoglobin concentrations, and enumerated erythrocytes. There were no differences that related to hyper- or hyporesponsiveness or to vasectomy, and we did not present those data in Table 4.

In Table 5 we have summarized our observations on blood pressure. There were no effects of hyper- or hyporesponsiveness to dietary cholesterol on either systolic or diastolic blood pressure. Similarly, there were no effects of vasectomy on blood pressure, although the hyporesponder sham-vasectomized animals had statistically significantly lower systolic blood pressures; the magnitude of that change is of no clinical significance.

**Antisperm Antibodies**

Both sperm agglutinating and sperm immobilizing antibody levels were measured in this study. The results indicate that there was a pronounced immune response to vasectomy. The degree to which hyper- and hyporesponding monkeys developed antisperm antibodies after vasectomized animals and the high density lipoprotein cholesterol concentration (TPC/HDLC) plotted as means for the groups during the 7-month pre-experimental period and the 18-month experimental period. The amount of dietary cholesterol fed during the times in which the total plasma cholesterol concentrations were measured are indicated. Hyporesponder vasectomized group (C), hyporesponder sham-vasectomized groups (M), hyperresponder vasectomized group (A), and hyperresponder sham-vasectomized group (B).

**Table 6.Thoracic and Abdominal Aorta Atherosclerosis of Hypo- and Hyperresponding Vasectomized and Sham-Vasectomized Cynomolgus Monkeys**

<table>
<thead>
<tr>
<th>Group</th>
<th>Thoracic</th>
<th>Abdominal</th>
<th>Thoracic</th>
<th>Abdominal</th>
</tr>
</thead>
<tbody>
<tr>
<td>HO-VX</td>
<td>26 ± 6</td>
<td>36 ± 7</td>
<td>0.4212 ± 0.0805</td>
<td>0.2996 ± 0.1014</td>
</tr>
<tr>
<td>HO-SHAM</td>
<td>33 ± 6</td>
<td>34 ± 8</td>
<td>0.5347 ± 0.1458</td>
<td>0.3617 ± 0.0717</td>
</tr>
<tr>
<td>HP-VX</td>
<td>45 ± 6</td>
<td>53 ± 6</td>
<td>0.7281 ± 0.1278</td>
<td>0.4305 ± 0.0601</td>
</tr>
<tr>
<td>HP-SHAM</td>
<td>41 ± 7</td>
<td>54 ± 6</td>
<td>0.9243 ± 0.2427</td>
<td>0.8429 ± 0.1462</td>
</tr>
</tbody>
</table>

*Percent of intimal surface affected with lesions. The data are the averages for the groups ± SE. †Lesion extent expressed as the average intimal area (mm²) for the groups ± SE.

Figure 6. The extent of coronary artery atherosclerosis observed in the left anterior descending (LAD), left circumflex (LCX), and right coronary (RCA) arteries. The data are the average intimal areas (IA) in mm² for each of the blocks studied. Hyporesponder vasectomized group (■), hyporesponder sham-vasectomized group (○), hyperresponder vasectomized group (▲), and hyperresponder sham-vasectomized group (●).

Serum Lipid Concentrations

In Figure 3 we have summarized the total plasma cholesterol concentrations of the four groups of animals during the 7-month pre-experimental period and the 18-month experimental period. As we have indicated, the animals were originally selected to be either hyper- or hyporesponsive to dietary cholesterol, and during the pre-experimental period before they were allocated to groups to be either vasectomized or sham-vasectomized, the average total plasma cholesterol concentrations were 424 and 222 mg/dl for the hyper- and hyporesponder groups, respectively. To evaluate the atherogenic consequences of hyperresponsiveness to dietary cholesterol and the effect of plasma cholesterol concentrations on vasectomized monkeys, it was our intent to maintain the hypo- and hyperresponder animals at about 200 or 400 mg/dl, respectively. To achieve this, it was necessary to reduce the amount of dietary cholesterol on two occasions. During the first 2 months, the total plasma cholesterol concentration increased in both hyper- and hyporesponders, and at the fourth month, the amount of dietary cholesterol was reduced from 0.35 to 0.25 mg/kcal. With the reduction in dietary cholesterol, the animals more nearly approximated the desired concentrations of 200 vs. 400 mg/dl but not to the extent that was desired. For that reason, at month 11 the amount of dietary cholesterol was reduced further to 0.18 mg/kcal, and the hyporesponding animals plateaued at nearly 200 mg/dl, while the hyperresponder groups plateaued at about 350 mg/dl.

The allocation of monkeys to be either vasectomized or sham-vasectomized among the hyper- and hyporesponder groups resulted in nearly identical total plasma cholesterol concentrations during the pre-experimental period in those subgroups. Unexpectedly, among both the hyper- and hyporesponder animals, vasectomy resulted in a smaller, but statistically significant, increase in total plasma cholesterol concentrations; the magnitude of that effect was more apparent among the hyporesponders than the hyperresponders. We have not seen in our previous studies any evidence for lower plasma cholesterol concentrations among vasectomized animals consuming atherogenic diets. In this study the differences were not large enough to have affected atherosclerosis.

The hyper- and hyporesponder effects on total plasma cholesterol concentration were consistent throughout the experiment, and the rank order of the animals within the groups was remarkably consistent (i.e., r = 0.81 between months 6 and 18). It is of interest that the magnitude of the differences in plasma cholesterol concentrations remained about the same through both the reductions in dietary cholesterol.

In Figure 4 we have summarized our observations on the HDL cholesterol concentrations during both the pre-
experimental and the postsurgical periods. The monkeys that were hyporesponsive to dietary cholesterol main-
tained significantly higher HDL cholesterol concentrations
throughout the experiment. Such a finding was expected,
since there is a general inverse relationship between total
plasma cholesterol concentrations and the concentrations
of HDL cholesterol in cynomolgus monkeys. There were
no effects of vasectomy in either the hyper- or hypore-
sponding groups. Interestingly, the rank order of HDL
cholesterol concentrations was not consistent (i.e., $r=0.35$
between months 6 and 18).

The effect of hyper- and hyporesponsiveness to dietary
cholesterol on the ratio of total plasma cholesterol to
HDL cholesterol concentrations are presented in Figure
5. As expected, there were major differences between
hyper- and hyporesponder animals in the ratio of total
plasma cholesterol to HDL cholesterol concentrations.
The ratio seen among the hyporesponding animals was
generally about 4 and did not change markedly with
reductions in dietary cholesterol. In contrast, in the
hyperresponding animals, the ratio increased to about
12 and decreased with each of the decreased dietary
cholesterol regimens, reaching a final ratio of about 7.
There were no significant effects of vasectomy among
either the hyper- or hyporesponder animals.

Atherosclerosis Development

Thoracic and Abdominal Aorta

The evaluation of thoracic and abdominal aorta ather-
sclerosis was done in two ways. First, we estimated the
percent of the intimal surface affected with lesions, and
then we determined the lesion extent expressed as aver-
age intimal area of those lesions (Table 6) In both the
thoracic and abdominal aorta there were significant differ-
ences between the hyper- and hyporesponder animals in
both percent involvement and lesion extent. There were
no effects of vasectomy. In the thoracic aorta, the hyper-
responder animals developed markedly more atheroscle-
rosis than did their hyporesponding counterparts. Consis-
tent with our recent earlier study,23 we found smaller
lesions among the vasectomized animals of both the
hyper- and hyporesponder groups. The thoracic aortic
differences did not reach statistical significance ($p=0.34$);
however, the differences in the abdominal aorta were
significant ($p=0.005$).

Coronary Arteries

For the three main coronary arteries, the descriptor
used for the amount of atherosclerosis was the mean
cross-sectional intimal area recorded for each of the
blocks as illustrated in Figure 1. In Figure 6 we have
summarized all of those observations for the left anterior
descending, left circumflex, and right coronary arteries. In
all three of the coronary arteries there was a significant
effect of hyperresponsiveness on the development of
coronary artery atherosclerosis (left anterior descending,
$p=0.019$; left circumflex coronary artery, $p<0.001$; and
right coronary artery, $p=0.006$). There were no significant
effects of vasectomy or sham vasectomy among the
hyper- and hyporesponding groups, although there was a
The extent of atherosclerosis observed in the left and right common carotid arteries. The data are the average intimal areas (IA) in mm² for each of the blocks studied. Hyporesponder vasectomized group (○), hyporesponder sham-vasectomized group (■), hyperresponder vasectomized group (△), and hyperresponder sham-vasectomized group (▲).

It was of particular interest to us to note that the more extensive coronary artery atherosclerosis seen among hyperresponsive animals occurred primarily in the most proximal one or two blocks. With the exception of the left circumflex coronary artery, the difference in the extent of atherosclerosis between hyper- and hyporesponding animals was minimal in the more distal blocks.

Left and Right Common Carotid Arteries

Diet-induced common carotid artery atherosclerosis in the cynomolgus monkey tends to occur primarily in the most proximal portions and in the more distal portions just before the carotid bifurcation. In Figure 8 we have illustrated our observations on the extent of atherosclerosis in the left and right common carotid arteries. In both the left and right common carotid arteries there was a highly significant effect of hyperresponsiveness for both arteries (p<0.001). The worsened atherosclerosis seen among the hyperresponder animals occurred primarily in the proximal and distal portions, with only minimal differences in the extent of atherosclerosis in the mid-portion of the common carotid arteries.

In the left common carotid artery, the vasectomized animals had significantly less atherosclerosis (p=0.037). The effect was not seen in the right common carotid artery (p=0.209). The finding of small atherosclerotic lesions in

Table 7. Carotid Bifurcation Atherosclerosis of Hypo- and Hyperresponsive Vasectomized and Sham-Vasectomized Cynomolgus Monkeys

<table>
<thead>
<tr>
<th>Group</th>
<th>Left</th>
<th>Right</th>
</tr>
</thead>
<tbody>
<tr>
<td>HO-VX</td>
<td>0.458</td>
<td>0.308</td>
</tr>
<tr>
<td>HO-SHAM</td>
<td>0.707</td>
<td>0.550</td>
</tr>
<tr>
<td>HP-VX</td>
<td>0.723</td>
<td>0.952</td>
</tr>
<tr>
<td>HP-SHAM</td>
<td>0.700</td>
<td>0.956</td>
</tr>
</tbody>
</table>

Data are mm² and are the group averages.
the left common carotid artery is consistent with our recent observations of vasectomized cynomolgus monkeys fed moderately atherogenic diets. The differences are relatively small, are of no clinical significance, and may have occurred by chance, although there is evidence for diminished atherosclerosis in the more distal blocks of the right common carotid artery. Certainly there is no evidence for worsened atherosclerosis due to vasectomy among either the hyper- or hyporesponding animals.

**Carotid Bilirrucation**

In Table 7 we have summarized our observations on carotid bifurcation atherosclerosis among the hyper- and hyporesponsiveness to dietary cholesterol. The carotid bifurcation of cynomolgus monkeys is an unusual arterial site in that considerable atherosclerosis develops among feral animals consuming natural diets. Further, the association between plaque progression and the plasma lipid concentrations of cynomolgus monkeys is inconsistent. In this experiment there was a highly significant effect of hyperresponsiveness in the right carotid bifurcation (p<0.001), but there was no such effect in the left carotid bifurcation. The lack of an effect of hyperresponsiveness at the left carotid bifurcation probably relates to the rather large lesions found among sham-vasectomized hyporesponding animals. At the right carotid bifurcation, the lesions seen among the vasectomized animals tended to be smaller, but the effect was not statistically significant (p=0.090). Among the hyporesponding animals, the findings of smaller carotid bifurcation plaques is again consistent with our recent report.

**Left and Right Femoral Arteries**

Our observations on the extent of atherosclerosis in the left and right femoral arteries are summarized in Figure 9. There was a significant effect of hyperresponsiveness at both of these arterial sites (left femoral artery, p=0.015; right femoral artery, p=0.013). Such significant effects of hyper- and hyporesponsiveness relate almost entirely to the worsened atherosclerosis found in the most proximal block of those arteries. In subsequent blocks, the effect of hyperresponsiveness was minimal. There was no effect of vasectomy (left femoral artery, p=0.36; right femoral artery, p=0.14). There were large differences in susceptibility to diet-induced atherosclerosis between the proximal and distal portions of the femoral artery, and this finding suggests that these arterial sites may be useful for research on regional differences.

**Discussion**

The extent of atherosclerosis was found to be considerably greater at all arterial sites studied for the monkeys that were hyperresponsive to dietary cholesterol. The differences in atherosclerosis development among the hyperresponder monkeys occurred primarily in the proximal portions of the coronary arteries, the proximal and distal portions of the common carotid arteries, and only in the most proximal portion of the femoral arteries. Among the hyperresponder animals, there was no evidence that vasectomy increased atherosclerosis extent, although there was a suggestion of larger lesions in the left circumflex coronary artery but not in the left anterior descending and right coronary arteries. There was no effect of vasectomy on coronary artery atherosclerosis of the hyporesponder animals.

The data presented here do not support our first report of worsened atherosclerosis among vasectomized cynomolgus monkeys fed diets high in cholesterol. This study differs from the first reported study in several ways. First, much larger groups of animals were used. Second, the atherogenic diet fed to the animals was more typical of that consumed by North American human beings in fat and cholesterol content. Third, current morphometric methods for assessing lesion extent permit the collection of more extensive and precise observations of the arteries being studied. Finally, the large morphometric data bases lend themselves to more sophisticated statistical analyses. The finding in the current study that vasectomy has no effect on atherosclerosis in either hyper- or hyporesponding monkeys is consistent with recent epidemiological studies of human beings.

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Atherosclerosis of cynomolgus monkeys hyper- and hyporesponsive to dietary cholesterol.

Lack of effect of vasectomy.

T B Clarkson, N J Alexander and T M Morgan

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