Evidence is contradictory regarding the effects of natural or surgical menopause on "female protection" against coronary artery atherosclerosis. We evaluated atherosclerosis, plasma lipids, blood pressure, and carbohydrate tolerance in 21 ovariectomized and 23 intact female cynomolgus macaques fed a moderately atherogenic diet for 30 months. We also evaluated the influence of social dominance status, with particular emphasis on a possible relationship with ovarian endocrine function. Atherosclerosis was two to 10 times as extensive in coronary, carotid, and iliaco-femoral arteries of the ovariectomized females; this could be explained, in part, by 15% to 20% increases in total plasma and LDL cholesterol concentrations. Socially dominant intact females were protected against advanced atherosclerotic lesions (plaques) of the coronary arteries, while subordinate females and ovariectomized females were not. Increased susceptibility to advanced coronary artery atherosclerosis in subordinate intact females may have been related in some way to chronic ovarian dysfunction observed in seven of 12 of these individuals. As a group, subordinate intact females also had enlarged adrenal glands, suggestive of mechanisms that may influence atherogenesis independently. The results indicate that, in this species, ovariectomy and chronic ovarian dysfunction related to subordinate social status are associated with a more atherogenic plasma lipid pattern and abolish "female protection" against coronary artery atherosclerosis.

We have shown previously that female cynomolgus macaques share with premenopausal white women a relative protection against coronary artery atherosclerosis and are at lower risk of ischemic heart disease than are white men of similar age. However, evidence is contradictory regarding the effects of natural or surgical menopause on this relative "female protection." Some studies have found evidence for increased severity of coronary artery atherosclerosis and increased risk of ischemic heart disease in women with natural or surgical menopause, while others have found no relationship between menopausal status and severity of atherosclerosis or risk of ischemic heart disease. In the only previous study of this subject in nonhuman primates, ovariectomy had no effect on the extent of atherosclerosis in hysterectomized baboons. We have shown previously that female cynomolgus macaques share with premenopausal white women a relative protection against coronary artery atherosclerosis when compared to age-matched males. In those earlier studies, we also demonstrated a relationship between social dominance status and extent of coronary artery atherosclerosis. Socially dominant (i.e., aggressive) females had significantly less atherosclerosis than socially subordinate (i.e., submissive) females. An important finding from the latter study was an association among social subordination, chronic ovarian dysfunction, and increased extent of coronary artery atherosclerosis. Since chronic ovarian endocrine deficiency may approximate the endocrinologic deficiency associated with ovarian failure (i.e., menopause), these data offered preliminary evidence supporting the hypothesis that ovarian insufficiency or menopause is associated with increased coronary artery atherosclerosis.

In this report we describe a study designed to determine the effect of absolute ovarian deficiency (i.e., surgically-induced menopause) on diet-induced atherosclerosis of the aorta, coronary, iliaco-femoral, and carotid arteries; and on total plasma cholesterol (TPC) concentration, plasma high density lipoprotein (HDL) cholesterol (HDLC) concentration,
blood pressure, and carbohydrate tolerance in the cynomolgus macaque. We also sought to characterize further the influence of psychosocial factors on the extent of atherosclerosis in ovariectomized and intact females and sought to determine if there was a relationship between ovarian endocrine function and extent of atherosclerosis in intact females.

**Methods**

**Animals**

The animals studied were 52 adult female cynomolgus monkeys (*Macaca fascicularis*) imported from Malaysia (Primate Imports, Port Washington, New York). These females were chosen from a group of 300 females on the basis of similar mid-range TPC responses to a cholesterol-containing diet.

**Diet**

The animals were fed for 30 months a moderately atherogenic diet designed to approximate the nutritional composition of the diet of an average North American woman. The diet contained 44% of calories as fat and 0.4 mg cholesterol per calorie of diet (Table 1). The diet was prepared in our facility in 10 kg batches and fed in bulk to each social group to provide 150 calories/kg/animal/day.

**Group Composition**

After the animals had been fed the experimental diet for 6 months, 25 were ovariectomized and 27 were left with intact functional ovaries. The animals lived in social groups consisting of one male and four to six females. There were five groups of ovariectomized females and five groups of intact females. Males residing in groups with intact females were vasectomized to prevent pregnancies. Animals were anesthetized with ketamine hydrochloride (15 mg/kg body weight) administered intramuscularly prior to ovariectomy or vasectomy. Subsequent injections of ketamine were given as required to maintain surgical anesthesia. 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 committee. Each social group lived in a pen that measured 2.5 m × 3.3 m with outdoor exposure and a natural light/dark cycle.

**Psychosocial Observations**

The behavioral repertoire of macaques is well known; the majority of behaviors observed in a social context may be categorized as aggressive, submissive, or affiliative, and can be described by a relatively limited number of stereotyped motor patterns. Our methods for identifying and recording these behavioral motor patterns in cynomolgus monkeys have been described previously. Aggressive, submissive, and affiliative actions and responses (Table 2) were recorded by technicians using a focal sampling technique and an electronic data collection device (Electro General Corporation, Minnetonka, Minnesota). Each animal was observed two or three times per week. An average of 190 focal observations 15 minutes in length was made on each animal over a 90-week period.

**Determination of Social Status**

Aggressive interaction was defined as one individual directing aggressive behavior (Table 2) toward another individual; this individual responds with submissive behavior (Table 2). A typical aggressive interaction consisted of one individual directing a threat at another and eliciting a submissive gesture.

<table>
<thead>
<tr>
<th>Table 2. Behaviors of Female Cynomolgus Macaques</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Aggression Contact</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Noncontact</td>
</tr>
<tr>
<td></td>
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<td></td>
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<tr>
<td>Submission</td>
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</tr>
<tr>
<td>Affiliation</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>g/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactalbumin</td>
<td>4.6</td>
</tr>
<tr>
<td>Casein</td>
<td>10.2</td>
</tr>
<tr>
<td>Gelatin</td>
<td>1.6</td>
</tr>
<tr>
<td>Sucrose</td>
<td>20.9</td>
</tr>
<tr>
<td>Dextrin</td>
<td>20.9</td>
</tr>
<tr>
<td>Crisco</td>
<td>7.3</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>7.3</td>
</tr>
<tr>
<td>Lard</td>
<td>7.3</td>
</tr>
<tr>
<td>Hegsted salt mixture</td>
<td>3.6</td>
</tr>
<tr>
<td>Vitamin mix (void of vitamin D)</td>
<td>2.3</td>
</tr>
<tr>
<td>Vitamin D₃ in corn oil</td>
<td>0.6 (250 IU)</td>
</tr>
<tr>
<td>Alphacel</td>
<td>4.8</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.2</td>
</tr>
<tr>
<td>Bananas</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
</tr>
</tbody>
</table>
(lip smack or grimace) in response. More severe (contact) aggression occurred much less frequently. This typically consisted of one individual grabbing or biting another and eliciting a scream and a physical flight response.

As in previous experiments, social rankings were determined on the basis of outcomes of aggressive interactions and not on the rates of performance of aggressive behaviors or on their severity. The female that defeated all but the first-ranking monkey. The female that defeated all but the first-ranking monkey was designated as the second-ranking monkey, etc. In general, dominance relationships determined on the basis of outcomes of aggressive behaviors or on their severity. The female that defeated all but the first-ranking monkey was designated as the first-ranking monkey. The female that defeated all but the first-ranking monkey was designated as the second-ranking monkey, etc. In general, dominance relationships within groups were transitive, i.e., if Monkey 1 was dominant to Monkey 2 and Monkey 2 was dominant to Monkey 3, then Monkey 1 was dominant to Monkey 3 also.

The following procedure was used to determine a dominance score for each monkey. First, the number of animals over which a given monkey was dominant was recorded as a dominance score for that monkey in each 12-week observation period. These scores were next averaged for each monkey over all eight observation periods. Those monkeys having an average score greater than 2.0 (i.e., those monkeys that were, on average, first or second-ranking in their groups) were designated as dominant animals in the experiment, while those having an average score of 2.0 or less were labeled as subordinate.

Importantly, ranking of individual monkeys was consistent over the course of the experiment (p < 0.01, Kendall's coefficient of concordance).

Assessment of Menstrual Cycliclty and Ovarian Endocrine Function

The monkeys were habituated to daily handling involving minimal restraint. They learned to run from their primary enclosures into transfer cages and from there through a tunnel, individually or as pairs, into a squeeze cage. The monkeys were also trained to present a leg for venipuncture, and at this time a swab was inserted into the vagina for daily determination of the presence or absence of menstrual bleeding. Blood samples were collected at 7 days following the onset of menstruation (follicular phase) and at 3-day intervals beginning 12 days following the onset of menstruation (luteal phase) for progesterone determinations. Additionally, blood samples for estradiol determinations were collected daily during the periovulatory period of two cycles of a subset of animals. All blood samples were collected between 0900 and 1200 hours.

Plasma was separated by centrifugation and frozen at −18°C until assayed. Progesterone and estradiol were extracted with diethyl ether and assayed without chromatography by using tritiated steroids in a radioimmunoassay (RIA) procedure. The progesterone RIA utilized an antiserum that has been characterized previously for use in the macaque.

An anovulatory cycle was defined as a cycle in which plasma progesterone concentration remained below 2.0 ng/ml with no evidence of a postovulatory increase. Peak luteal phase plasma progesterone concentrations between 2.0 and 4.0 were considered indicative of luteal phase (plasma progesterone) deficiency.

Plasma Lipid Concentrations

Monthly determinations were made of TPC and HDLC. These determinations were made in our Lipid Analytic Laboratory, which is in compliance with the Cooperative Lipid Standardization Program of the Department of Health and Human Services. For purposes of analyses, the mean of all monthly determinations of TPC, HDLC, and TPC:HDLC were calculated for each animal.

Blood Pressure

Systolic and diastolic blood pressure were recorded at 3-month intervals. Animals were anesthetized with ketamine hydrochloride (15 mg/kg) for recording of blood pressure with a Doppler ultrasound instrument (Arteriosonde 1010, Roche, Cranbury, New Jersey). For purposes of analyses, means of the seven observations for each animal were calculated. Previous work in our laboratory has shown that blood pressures recorded on ketamine-anesthetized macaques correlate highly with those recorded while the same animals were fully conscious.

Carbohydrate Tolerance

Carbohydrate tolerance was assessed three times during the experiment. The animals were fasted for 18 hours and anesthetized with ketamine HCl. Ketamine anesthesia does not influence plasma glucose and insulin responses to intravenous glucose administration in macaques. Blood samples for determination of glucose concentration were collected before glucose administration and at 5, 10, 20, 30, 40, and 60 minutes after intravenous administration of 50% glucose (0.75 g/kg body weight). Samples were collected in sodium fluoride, and glucose determinations were made with the Beckman Glucose AutoAnalyzer. For purposes of analyses, K values were calculated for each glucose tolerance test and the mean of the three K values was calculated for each animal.

Necropsy and the Measurement of Atherosclerosis

At the time of necropsy, animals were deeply anesthetized with pentobarbital and were then exsanguinated. The cardiovascular system was flushed with normal saline and perfused with 10% neutral buffered formalin under a pressure of 100
mm of mercury. After pressure fixation, five serial blocks were taken from each of the left circumflex, left anterior descending, and right coronary arteries. One section from each block was stained with Verhoff van Gieson stain. These sections were projected, and the area occupied by intima and intimal lesion was measured with a Zeiss Mop III Image Analyzer (digitizer). The extent of coronary artery atherosclerosis for each animal was expressed as the median intimal (plaque) area (in mm$^2$) of the 15 sections of coronary artery.

The carotid arteries and the aorta were opened longitudinally and were immersion-fixed in 10% buffered formalin. These segments were then stained with Sudan IV and isopropanol. For the thoracic and abdominal segments of aorta (thoracic: 10 cm beginning just distal to the aortic arch; abdominal: 10 cm beginning just distal to the branch of the celiac artery), gross evaluation by three investigators provided estimates of the total surface area affected with plaques. Next, five representative plaque cross sections were taken from each of the two aortic segments; plaque thickness (in mm) was measured in these microscopic slides by using the digitizer. Plaque "volume" was computed (in mm$^3$) as the total surface area covered with plaque, multiplied by the mean intimal thickness of the plaque sections. At the carotid bifurcation, one standard cross section was taken for microscopic evaluation; in taking this section, bifurcation pads ("intimal cushions") were avoided. Lesion area and thickness were measured (in mm$^2$ and mm, respectively) with the digitizer. Five serial sections were taken from each common carotid and iliaco-femoral artery and median intimal area (in mm$^2$) was determined for each by using the digitizer.

All aspects of necropsy and measurement of atherosclerosis were done by individuals who were unaware of the social status and ovarian status of the animal.

### Adrenal Glands and Hearts

Adrenal glands were removed, trimmed, and weighed at necropsy. To correct for influence of body weight on adrenal weight, the ratio of total adrenal weight (right plus left) to body weight was calculated, and this figure was used as an indicator of adrenocortical activity. The hearts were also weighed at necropsy. The ratio of heart weight to body weight was used in all analyses.

### Results

#### Animals

In the 24 months following group establishment, four ovariectomized females and four intact females died of causes unrelated to the experiment. Data reported here are based on the remaining 21 ovariectomized females and 23 intact females.

#### Effects of Ovariectomy on Atherosclerosis and Other Variables

##### Atherosclerosis

The extent of atherosclerosis (intimal area) in each of the three major coronary arteries and the average intimal area of the three arteries are summarized in Table 3. Coronary artery atherosclerosis was two to three times more extensive in the ovariectomized females.

The extent of atherosclerosis at the other sites is summarized in Table 4. In the ovariectomized females, the extent of atherosclerosis was greater at both common carotid arteries, both carotid bifurcations, and both iliaco-femoral arteries.

#### Plasma Lipids, Blood Pressure, Carbohydrate Tolerance

Plasma lipid data are summarized in Table 5. TPC and LDL cholesterol (LDLC; calculated by subtracting HDLC from TPC) were higher in the ovariectomized females.

---

### Table 3. Extent of Atherosclerosis in Major Coronary Arteries (Median Values) of Female Cynomolgus Monkeys

<table>
<thead>
<tr>
<th>Coronary Artery</th>
<th>Ovariectomized (n = 21)</th>
<th>Intact (n = 23)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left anterior descending</td>
<td>0.072</td>
<td>0.016</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Left circumflex</td>
<td>0.183</td>
<td>0.067</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Right coronary artery</td>
<td>0.021</td>
<td>0.007</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Average of the three coronary arteries</td>
<td>0.091</td>
<td>0.036</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

Mann-Whitney U test was used to determine significance.

---

### Table 4. Extent of Atherosclerosis In Aorta, Carotid, and Iliaco-Femoral Arteries (Median Values) of Female Cynomolgus Monkeys

<table>
<thead>
<tr>
<th>Site</th>
<th>Ovariectomized (n = 21)</th>
<th>Intact (n = 23)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thoracic aorta (mm$^2$)</td>
<td>3.51</td>
<td>0.53</td>
<td>&lt;0.09</td>
</tr>
<tr>
<td>Abdominal aorta (mm$^2$)</td>
<td>2.12</td>
<td>1.14</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Right common carotid (mm$^2$)</td>
<td>0.083</td>
<td>0.008</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>Left common carotid (mm$^2$)</td>
<td>0.095</td>
<td>0.015</td>
<td>&lt;0.004</td>
</tr>
<tr>
<td>Right carotid bifurcation (mm$^2$)</td>
<td>1.001</td>
<td>0.531</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Left carotid bifurcation (mm$^2$)</td>
<td>0.756</td>
<td>0.316</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Right iliaco-femoral (mm$^2$)</td>
<td>0.046</td>
<td>0.017</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Left iliaco-femoral (mm$^2$)</td>
<td>0.061</td>
<td>0.014</td>
<td>&lt;0.004</td>
</tr>
</tbody>
</table>

All p values were significant, as measured by two-tailed Mann-Whitney U test.
Table 5. Plasma Lipids, Blood Pressure, and Carbohydrate Tolerance in Intact and Ovariectomized Females

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ovariectomized (n = 21)</th>
<th>Intact (n = 23)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total plasma cholesterol (TPC)</td>
<td>363 ± 16.8</td>
<td>312 ± 16.0</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>HDL cholesterol (HDLC)</td>
<td>38 ± 2.8</td>
<td>42 ± 2.8</td>
<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol (TPC-HDLC)</td>
<td>325 ± 17.2</td>
<td>268 ± 17.7</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>TPC:HDLC</td>
<td>12.4 ± 1.2</td>
<td>9.5 ± 1.0</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>100 ± 2.6</td>
<td>97 ± 2.9</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>57 ± 1.3</td>
<td>58 ± 2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Carbohydrate tolerance (K)</td>
<td>3.03 ± 0.13</td>
<td>3.44 ± 0.14</td>
<td>&lt;0.04</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Student's one-tailed t test was used to determine the significance for all variables except carbohydrate tolerance, the significance for which was determined by two-tailed test.

Behavior

Rates of aggressive interactions between females were similar among ovariectomized and intact females (7.3 vs 7.3 per hr, p > 0.2, Mann Whitney U test). However, aggressive interactions between males and ovariectomized females occurred with about twice the frequency as aggressive interactions between males and intact females (6.9 vs 3.4 per hr, p < 0.0003, Mann Whitney U test).

Psychosocial Influences on Ovarian Function and Coronary Artery Atherosclerosis

Social Status and Coronary Artery Atherosclerosis

Relationships between social dominance status and coronary artery atherosclerosis in ovariectomized and intact females are summarized in Figure 1. Plotting the data in this fashion makes apparent the differences among groups in the relative degree of lesion progression, as indicated by lesion size and morphologic characteristics. Coronary artery atherosclerosis had progressed beyond fatty streaks or small fatty plaques to larger lesions characterized more accurately as well-developed plaques in nine of 21 ovariectomized females and in only three of 23 intact females. This threefold difference in prevalence of the more advanced lesions was significant statistically (p < 0.03, chi-square test).

The three intact females that developed more advanced coronary artery atherosclerosis were subordinate individuals (Figure 1). Thus, the prevalence of the more advanced lesions among socially subordinate intact females (three of 12) was not different from that of the ovariectomized females (p > 0.2, chi-square test), while socially dominant females were totally protected against the more advanced lesions, with none of 11 animals affected (p < 0.02, chi-square test). Also apparent is that social dominance was not associated with protection against the more advanced lesions among the ovariectomized females (p < 0.01, chi-square test) where six of 10 dominant females were affected. These findings suggest that the presence of intact ovaries may be an important factor in the protective effect of social dominance observed here and in a previous experiment. We therefore sought to determine if ovarian dysfunction might explain the lack of protection among some subordinate females.

Ovarian Function, Plasma Lipids and Atherosclerosis

Ovarian dysfunction was common among the subordinate females. They had significantly higher rates of anovulation (26.5% vs 3.5%) (p < 0.001, chi-square test) and progesterone-deficient luteal phases (24.3% vs 8.9%) (p < 0.0001, chi-square test). Also apparent is that social dominance was not associated with protection against the more advanced lesions among the ovariectomized females (p < 0.01, chi-square test) where six of 10 dominant females were affected. These findings suggest that the presence of intact ovaries may be an important factor in the protective effect of social dominance observed here and in a previous experiment. We therefore sought to determine if ovarian dysfunction might explain the lack of protection among some subordinate females.

Figure 1. Extent of coronary artery atherosclerosis (mean lesion area) in dominant and subordinate, ovariectomized and intact females. Each data point represents one animal. Lesions less than 0.10 mm² are primarily fatty streaks or small fatty plaques. Lesions greater than 0.20 mm² are more accurately characterized as well developed plaques.
test). Overall, evidence of ovarian endocrine dysfunction was found in 50.8% of all menstrual cycles of subordinate females as compared to 12.4% of all cycles of dominant females \((p < 0.001, \chi^2\text{-test})\).

Preexisting ovarian dysfunction may have resulted in behavioral alterations that resulted in subordinate social status, or, conversely, social subordination may have resulted in impaired ovarian function. To clarify this relationship, we examined behavioral data from 12 females that were anovulatory or amenorrheic for 3 months or longer. Six females had experienced a decrease in social status (i.e., social subordination) from the preceding 3-month period, while two had experienced an increase. In the 3-month period following the prolonged anovulatory period and during which ovulation resumed, seven of the 12 females experienced an increase in status while only one experienced a decrease. Thus, social subordination was more likely to be associated with prolonged anovulation or amenorrhea, while a return to normal ovarian function was more likely to be associated with increased social dominance status \((p = 0.04, \text{Fisher's exact test})\).

Luteal phase plasma progesterone concentration was used as an index of ovarian endocrine function. Each data point in Figure 2 represents mean peak plasma progesterone concentrations for all menstrual cycles of each female. Females had an average of 19 cycles during the experimental period. Subordinate females had lower mean peak plasma progesterone concentrations \((4.3 \text{ ng/ml})\) than did dominant females \((7.2 \text{ ng/ml})\) \((p < 0.003, \text{Student's t test})\). As can be seen in Figure 2, there is great individual variability in plasma progesterone concentrations, and therefore in the frequency or degree of ovarian endocrine dysfunction. The seven females with plasma progesterone concentrations below the mean of 4.3 ng/ml were the most frequently affected, having an average of 70.0% endocrinologically abnormal cycles. To assess the influence of severe ovarian dysfunction on plasma lipids and coronary artery atherosclerosis (intimal area), we compared these seven intact females with high frequency \((70.0\%)\) of endocrinologically abnormal cycles to the 16 remaining intact females with low frequency of ovarian endocrine dysfunction \((16.8\%)\) and to the 21 ovariectomized females (Table 6). The seven females with chronic ovarian dysfunction could not be distinguished from the ovariectomized females in any variable; but they had significantly lower HDLC, significantly higher TPC:HDLC, and a significantly greater extent of coronary artery atherosclerosis than did the 16 intact females with relatively normal ovarian endocrine function (Table 6).

To further characterize ovarian endocrine function, we examined plasma estradiol concentrations in cycles of a subset of females in which blood samples had been collected daily during the periovulatory period \((n = 32\) cycles from 16 monkeys). Figure 3 illustrates periovulatory plasma estradiol concentrations plotted by days, with Day 0 defined as the day of the estradiol peak. Estradiol was lower on the day of the estradiol peak \((t = 2.33, p < 0.03)\) and one day before \((t = 2.3, p < 0.04)\) in those cycles with deficiencies in peak plasma progesterone \((n = 10)\) as compared to normal cycles \((n = 16)\). Plasma estradiol in anovulatory cycles \((n = 6)\) was significantly lower than in normal cycles or progesterone-deficient cycles.

### Table 6. Plasma Lipids and Coronary Artery Atherosclerosis in Ovariectomized Females, Females with Ovarian Dysfunction, and Intact Females with Normal Ovarian Function

<table>
<thead>
<tr>
<th>Monkey group</th>
<th>TPC (mg/dl)</th>
<th>HDLC (mg/dl)</th>
<th>TPC:HDLC</th>
<th>Intimal area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovariectomized ((n = 21))</td>
<td>363 ± 16.8</td>
<td>37 ± 2.8</td>
<td>9.6</td>
<td>0.195 ± 0.043</td>
</tr>
<tr>
<td>Ovarian dysfunction ((n = 7))</td>
<td>342 ± 32.8</td>
<td>32 ± 2.0</td>
<td>10.7</td>
<td>0.225 ± 0.105</td>
</tr>
<tr>
<td>Normal intact ((n = 16))</td>
<td>296 ± 18.5</td>
<td>46 ± 3.4</td>
<td>6.4</td>
<td>0.030 ± 0.005</td>
</tr>
<tr>
<td>(p = 0.003) ((2 \text{ vs } 3))</td>
<td>NS</td>
<td>&lt;0.02</td>
<td>&lt;0.03</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Student’s two-tailed \(t\) test was used to determine significance. Intimal area data required logarithmic transformation for analysis.

TPC = total plasma cholesterol; HDLC = high density lipoprotein cholesterol.
Adrenal Weight, Heart Weight

Subordinate intact females had larger adrenal glands (203 vs 168 mg/kg body weight) \( (p < 0.05, \) Mann-Whitney U test) and larger hearts (8.63 vs 7.25 g/kg body weight) \( (p < 0.05, \) Student’s t test) than did dominant intact females. Among ovariectomized females, adrenal weight in dominant females did not differ from that of subordinate females (206 vs 217 mg/kg, \( p > 0.1 \) Mann-Whitney U test), but both had larger adrenal glands than dominant intact females \( (p = 0.05, \) Mann-Whitney U test). Also, heart weights were not different between dominant and subordinate ovariectomized females (7.26 vs 6.54 g/kg), but both had smaller hearts than subordinate intact females \( (p < 0.05, \) Student’s t test).

Correlations Between Variables and Coronary Artery Atherosclerosis

Variables that correlated with mean coronary artery intimal area among intact females were: HDLC \( (\rho = -0.44, p < 0.05) \), LDLC \( (\rho = 0.60, p < 0.01) \), TPC:HDLC \( (\rho = 0.61, p < 0.01) \), mean peak plasma progesterone concentration \( (\rho = -0.40, p < 0.05) \), and heart weight:body weight \( (\rho = 0.45, p < 0.05) \).

Variables that correlated with mean coronary artery intimal area among ovariectomized females were: HDLC \( (\rho = -0.82, p < 0.01) \) and TPC:HDLC \( (\rho = 0.70, p < 0.01) \).

Also of interest were the relatively strong correlations between mean peak plasma progesterone concentrations and HDLC in subordinate intact females \( (\rho = 0.84, p < 0.001) \) and all intact females \( (\rho = 0.60, p < 0.01) \).

Discussion

The evidence has been contradictory regarding the effect of estrogen deficiency due to ovariectomy or natural menopause on coronary artery atherosclerosis in women. Effects on traditional risk factors have appeared to be minimal. Blood pressure and carbohydrate tolerance have appeared to be unchanged, while TPC and LDLC were significantly elevated in postmenopausal women.

The results described here show that ovariectomy of adult female cynomolgus macaques results in a significantly greater extent of atherosclerosis in the coronary arteries and also in the femoral and carotid arteries when compared to females with intact ovaries. These differences in the extent of atherosclerosis can be explained in part by increased TPC, TPC:HDLC, and LDLC. As in human females, there was no effect on blood pressure and only a slight effect on carbohydrate tolerance.

We also found in this experiment and in a previous experiment that socially dominant intact females were protected against advanced coronary artery atherosclerosis. In the current experiment, socially dominant ovariectomized females were not similarly protected. This finding suggests that the protective effect of social dominance is mediated by the presence of intact ovaries. However, intact ovaries seem to be protective only if they are functioning normally, as suggested by the finding that chronic ovarian dysfunction associated with social subordination was in turn associated with a more atherogenic plasma lipid pattern and greatly increased extent of coronary artery atherosclerosis.

We had hoped to be able to reach conclusions regarding associations between the variables studied and the extent of atherosclerosis. Unfortunately, the relatively small number of animals studied and, in particular, the small number (12 of 44) affected with advanced atherosclerosis precluded the use of multivariate statistical approaches. Of the variables studied, plasma lipid and lipoprotein variables correlated most strongly with the extent of coronary artery atherosclerosis. However, these correlations were not sufficiently strong to rule out the possibility that other variables influence the atherosclerotic process. The finding of elevated adrenal weight in subordinate individuals leads us to speculate on possible atherogenic mechanisms that are independent of plasma lipids.

Numerous studies have identified a relationship between social subordination and increased adrenocortical activity or increased adrenal size in rodents and macaques. Adrenal enlargement, although perhaps a crude indicator, suggests chronic pituitary-adrenocortical or sympathethic-adrenomedullary stimulation in the subordinate individual. Both of these pathways have been proposed as independent atherogenic stimuli, although direct evidence for such an effect is lacking. Chronic adrenocortical stimulation also has been associated with changes in plasma lipids in a direction that would be considered more atherogenic.

Increased adrenocortical activity and altered hypothalamicpituitary function associated with social...
subordination50-51 have been shown to result in ovar-
ian dysfunction. This suggests that social subordina-
tion also may influence the extent of atherosclerosis
indirectly through neuroendocrine-based effects on
ovarian function. It is important to note that there are
alternative interpretations of this two-step relation-
ship. Neuroendocrine concomitants of social subor-
dination may influence atherogenesis independently
of the effects on ovarian function. Also, social status
and ovarian function may be influenced by an unre-
lated and unidentified variable or set of variables.

Ovariectomized females engaged in aggressive
interactions with males twice as frequently as did
intact females. Also, dominant and subordinate ova-
riectomized females did not differ in regard to adrenal
weight; both had large adrenal glands similar in
weight to those of subordinate intact females and
significantly larger than those of dominant intact fe-
male. These findings lead us to speculate that in-
creased aggression toward females by the males
within groups containing ovariectomized females re-
sulted in a more stressful social environment which,
in turn, may have contributed to the increased extent
of coronary artery atherosclerosis among ovariecto-
mized females.

Plasma progesterone concentration was used in
this study as an index of ovarian function and was
found to have a strong positive relationship with
HDLc and an inverse relationship with extent of
coronary artery atherosclerosis. Based on the exten-
sive literature on the influence of exogenous estro-
gens and progestogens,52 we conclude that this
probably does not represent a direct relationship be-
tween endogenous progesterone and HDLC, but in
fact may represent a relationship between relative
estrogen deficiency and low HDLC. The finding in a
relatively small (n = 32) subset of cycles of low
periovulatory plasma estradiol concentrations in
those cycles with abnormally low luteal phase pla-
ma progesterone concentrations supports the exis-
tence of a positive relationship between estrogen
deficiency and decreased HDLC.

Deficiencies in reproductive steroids associated
with absent or chronically impaired ovarian function
may also have an influence on atherogenesis inde-
pendent of plasma lipids. Evidence exists for the
presence of estrogen53-56 and progesterone54 recep-
tors in arterial endothelial and smooth muscle cells
in several mammalian species. Other studies have
shown that estrogen treatment in vivo or in vitro is
associated with reductions in arterial smooth muscle
cell proliferation,58 decreased collagen and elastin
production,60-63 and increased degradation of colla-
gen and elastin in arterial tissue.64 Thus, evidence
indicates that vascular estrogen receptors are phys-
ologically functional, and, therefore, deficiencies in
endogenous reproductive steroids may influence
atherogenesis directly at the level of the artery wall.

Taken together, the results of this study indicate
that, in this species, the ovaries play an important
role in the regulation of plasma lipids and lipoopro-
tins and in the atherosclerotic process. Normal
ovarian function is associated with a relatively non-
atherogenic plasma lipid pattern and protection
against advanced coronary artery atherosclerosis.
Ovariectomy or chronically impaired ovarian function
is associated with a more atherogenic plasma lipid
pattern and loss of “female protection” against coro-
nary artery atherosclerosis. “Female protection” is
most probably mediated by the reproductive steroids,
most probably by the estrogens.

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