Carotid Artery Atherosclerosis in Cholesterol-fed Female Cynomolgus Monkeys

Effects of Oral Contraceptive Treatment, Social Factors, and Regional Adiposity

Carol A. Shively, Jay R. Kaplan, and Thomas B. Clarkson

Female cynomolgus monkeys, a previously established model of carotid and coronary artery atherosclerosis, were used to study the relationships between potential risk factors and carotid artery atherosclerosis. Over a 24-month treatment period, one-third of the monkeys (n=25) were given the oral contraceptive Ovral, one-third of the monkeys (n=26) were given the oral contraceptive Demulen, and the remaining monkeys constituted a control group (n=26). At necropsy, the atherosclerosis extent was measured in the left and right common carotid arteries and the left and right carotid bifurcations. Plasma lipid concentrations, regional adiposity, and social status were related to carotid artery atherosclerosis extent. The relationships between regional adiposity and social status and carotid artery atherosclerosis were accounted for, at least in part, by plasma lipid concentrations. Oral contraceptives had an adverse effect on plasma cholesterol concentrations and a protective effect against carotid artery atherosclerosis after adjusting for their effect on plasma lipids. The net result of these effects was little or no change in atherosclerosis extent in the carotid arteries due to oral contraceptive treatment. (Arteriosclerosis 10:358-366, May/June 1990)

Animal models offer the opportunity to study cerebral artery atherosclerosis more directly than is possible using human subjects. Cynomolgus macaques (Macaca fascicularis), which have been used successfully for studies of coronary artery atherosclerosis, are being used with increasing frequency to study various aspects of cerebral artery atherosclerosis. As yet, no studies have used this model to evaluate among females the relationship between typical cardiovascular risk factors and cerebral atherogenesis.

In the current study we considered several risk factors known to be associated with coronary heart disease or stroke in women to determine whether they were also risk factors for carotid artery atherosclerosis in this animal model. The variables evaluated included social factors, regional obesity, and plasma lipid concentrations. Additionally, oral contraceptive (OC) treatment was investigated as a potential risk factor because of equivocal evidence of an association with the incidence of atherothrombotic brain infarction in women.

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Table 1. Composition of Moderately Atherogenic Diet

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Grams/100 grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purina monkey chow-25</td>
<td>75.70</td>
</tr>
<tr>
<td>Dry egg yolk</td>
<td>8.20</td>
</tr>
<tr>
<td>Brewer’s yeast</td>
<td>4.00</td>
</tr>
<tr>
<td>Raw wheat germ</td>
<td>2.00</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.10</td>
</tr>
<tr>
<td>Lard</td>
<td>10.00</td>
</tr>
</tbody>
</table>

adjusted for body weight and caloric intake; the third group served as a control group. One treatment group received ethynodiol diacetate, 1.0 mg/day; ethinyl estradiol, 50 μg/day (Demulen, Searle, San Juan, Puerto Rico). The other treatment group received norgestrel, 0.5 mg/day; ethinyl estradiol, 50 μg/day (Ovral, Wyeth Laboratories, Philadelphia, PA) throughout the 24-month experimental period.

Four animals died during the experimental period of causes unrelated to the experiment (mainly gastrointestinal disorders). The left carotid bifurcation of one animal was not evaluated. Thus, findings presented here are based on 72 animals for the left carotid bifurcation and 73 animals for all other results reported.

Menstrual Cycle Evaluations

All females were trained to enter a restraint cage and present themselves for blood sample collection and vaginal swabbing. All animals were swabbed three times per week to document vaginal bleeding throughout the pre-experimental and experimental phases of the study. Blood samples were collected twice weekly for progesterone determinations during months 3 through 7 of the pre-experimental phase and months 1, 2, 4, 6, 11, 12, 17, and 18 of the experimental phase.

The records of vaginal bleeding and the results of progesterone determinations indicated that the control group females maintained menstrual cycles throughout the experimental phase. During this time, 206 cycles from 24 animals were sampled for plasma progesterone concentrations. In 180 of these cycles (87.4%), plasma progesterone concentrations rose rapidly to levels greater than 2 ng/ml, indicating that ovulation had occurred. Among the 23 monkeys treated with Ovral, 214 plasma progesterone samples were evaluated during the experimental phase, and in one instance, the progesterone concentration was greater than 2 ng/ml. Among the 26 monkeys treated with Demulen, plasma progesterone concentrations were evaluated 243 times during the experimental phase and were never found to be elevated.

Evaluation of Drug Administration Regimen

Plasma concentrations of levonorgestrel (the active isomer of norgestrel) and norethindrone (the major metabolite of ethynodiol diacetate) were assessed by radioimmunoassay by using established procedures. Blood samples were collected for the evaluation of levonorgestrel and norethindrone 3 to 5 hours after feeding during months 2, 6, 12, 18, and 20 of the experimental period to determine serum levels resulting from treatment. The concentrations of levonorgestrel and norethindrone sampled throughout the experimental period indicated that the animals were receiving the contraceptives in the amounts intended. Identical sampling during pre-experimental month 5 resulted in nondetectable levels of these progestins. The within-assay variation was less than 12%. All samples were run in two assays.

Evaluation of Adiposity

Body weight and body length (the distance between the pubis symphysis and the suprasternal notch) were measured every 6 months during the experimental period. The ponderosity index (POND) was calculated as the ratio of body weight (g) to body length (cm). Skinfold thickness was measured at the same time immediately inferior to the inferior angle of the scapula (subscapular skinfold) and over the triceps muscle midway along the posterior brachium (triceps skinfold) by using spring-loaded calipers. A ratio of subscapular/triceps skinfold thickness was calculated (SS:TRI). This ratio is identical to one used previously to identify a regional adiposity effect on coronary artery atherosclerosis in female cynomolgus monkeys and represents the ratio of central to peripheral fat deposition. The mean values of each adiposity measure were calculated from all values collected during the experimental period. The SS:TRI skinfold ratio distribution was divided at the mean to produce two regional adiposity groups: a high central fat group (n=36) and a low central fat group (n=37).
Evaluation of Lipids and Lipoproteins

Blood samples were collected for the determination of TPC and HDLc concentrations bimonthly during the pre-experimental and experimental periods. TPC and HDLc concentrations were determined by using the Lipid Research Clinics methodology.26,27 Mean values were calculated for the pre-experimental period and for the experimental period for use in analysis.

Evaluation of Blood Pressure

Evaluations of systolic (SBP) and diastolic (DBP) blood pressures with the Dinamap Research Monitor (Model 1245) were conducted at 2-month intervals pre-experimentally and at 3-month intervals during the experimental period. The mean values were calculated for the pre-experimental period and for the experimental period for SBP and DBP for use in analysis.

Determination of Social Status

As in previous experiments, social status 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.28 In each group, the female that defeated all others, as evidenced by her ability to consistently elicit submissive responses, 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. Dominance scores were determined once pre-experimentally and then bimonthly throughout the experimental period. Preliminary analysis revealed the scores to be stable throughout the experiment. Hence, a mean value for the entire experimental period was computed for use in analysis.

As in prior experiments, the social status hierarchy was divided into two groups: dominants and subordinates. Because of animal loss, two social groups contained three animals. Ten social groups contained four animals, and six social groups contained five animals. The dominant group included all females who were, on average, first or second ranking in social groups of five and four, and first ranking in social groups of three females. Because of the variation in group size, there were fewer females considered dominant (n=32) than subordinate (n=41).

Necropsy Procedure and Measurement of Atherosclerosis

At the time of necropsy, the animals were given a bolus intravenous injection of sodium pentobarbital. Then the cardiovascular system was flushed with normal saline and perfused with 10% neutral buffered formalin at a pressure of 100 mm Hg. The arteries were dissected free, flushed briefly with normal saline, opened longitudinally, and fixed flat on cardboard in 10% neutral buffered formalin.

To study the extent and severity of carotid artery atherosclerosis, five standard blocks (each 3 mm in length and representing proximal, middle, and distal portions of the artery) were cut perpendicularly to the long axis of the left common (LC) and the right common (RC) carotid arteries. One standard cross section was taken from each of the left (LCB) and right (RCB) carotid bifurcations, avoiding the bifurcation pads (intimal cushions). The tissue blocks were dehydrated through increasing concentrations of ethanol and were embedded in paraffin. Five 5 μm sections were cut from each block and were stained with Verhoeff van Gieson stain. The area occupied by the intima and intimal lesion was measured (mm²) using a Zeiss MOP III Image Analyzer. While the intimal area (IA) was determined from one section of the LCB and RCB, the values of all sections taken from the LC and the RC were averaged for use in analysis. The data were collected in a similar fashion from the coronary arteries and are presented elsewhere.32

Statistical Analysis

Logarithmic transformation of plasma lipid concentrations and lesion areas was used to reduce skewness and equalize group variances. Preliminary analyses using one-way analysis of variance (ANOVA, 1 x 3 Experimental Groups) and analysis of covariance (ANCOVA) adjusted for baseline factors used in the matched randomization were used to examine the potential influence of OC administration on the plasma lipid and adiposity measures reported here. The ANOVA and the ANCOVA elicited the same results; thus, only the results of the ANOVA are presented here. OC administration had no effect on whole-body POND (p=0.72) or body fat distribution as measured by the SS:TRI ratio (p=0.65). The independent effects of OC administration and regional adiposity on carotid artery atherosclerosis were examined by two-way ANOVA. A 2 x 3 ANOVA (Regional Adiposity x Experimental Group) was used to examine the effects of OC administration and regional adiposity on IA. Simultaneous analysis of social status, regional adiposity, and experimental group (three-way ANOVA) was not statistically feasible due to small cell sizes. Thus, supplemental ANOVAs (Status x Experimental Group) were used to examine the relationship between social status and carotid artery atherosclerosis. Pearson correlations were used to measure the degree of association between carotid atherosclerosis extent (IA), whole-body POND, BP, and plasma lipid concentrations. Finally, multiple-regression analyses were used to simultaneously assess the relative strength of the associations between significant risk variables and carotid artery atherosclerosis extent, with grouping variables added to account for the effects of OC treatment.29 Social status and regional adiposity were entered as continuous variables in these analyses. All bar graphs and the table depict the untransformed mean values ± SEM.

Results

Characteristics of Carotid Artery Lesions

Atherosclerosis extent (mean IA) in the common carotid arteries (mean value of LC and RC) and the carotid bifurcations (mean value of LCB and RCB) was significantly correlated (Table 2). Atherosclerosis extent was also significantly associated between the LC and the RC and between the LCB and the RCB (Table 2). Lesions of the common carotid and carotid bifurcation were similar in histologic characteristics, although atheroscle-
Table 2. Correlations between Clinical Pathological Variables and Carotid Artery Intimal Area

<table>
<thead>
<tr>
<th></th>
<th>SBP</th>
<th>DBP</th>
<th>POND</th>
<th>TPC</th>
<th>HDLC</th>
<th>LC</th>
<th>RC</th>
<th>LCB</th>
<th>RCB</th>
<th>Mean car</th>
<th>Mean car bif</th>
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<tbody>
<tr>
<td>SBP</td>
<td></td>
<td>0.86*</td>
<td>0.39*</td>
<td>0.16</td>
<td>0.11</td>
<td>0.07</td>
<td>0.12</td>
<td>0.14</td>
<td>0.17</td>
<td>0.10</td>
<td>0.14</td>
</tr>
<tr>
<td>DBP</td>
<td>0.19</td>
<td></td>
<td>0.05</td>
<td>0.04</td>
<td>0.01</td>
<td>0.01</td>
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<td>0.09</td>
<td>0.00</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>POND</td>
<td>0.14</td>
<td>0.14</td>
<td></td>
<td>0.19</td>
<td>0.20</td>
<td>0.06</td>
<td>0.14</td>
<td>0.19</td>
<td>0.04</td>
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<tr>
<td>TPC</td>
<td>-</td>
<td>0.60*</td>
<td></td>
<td>0.77*</td>
<td>0.78*</td>
<td>0.76*</td>
<td>0.69*</td>
<td>0.78*</td>
<td>0.79*</td>
<td></td>
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<tr>
<td>HDLC</td>
<td>-</td>
<td>0.56*</td>
<td></td>
<td>0.50*</td>
<td>0.56*</td>
<td>0.46*</td>
<td>0.53*</td>
<td>0.59*</td>
<td></td>
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</tr>
<tr>
<td>LC</td>
<td>-</td>
<td>0.95*</td>
<td></td>
<td>0.73*</td>
<td>0.69*</td>
<td>0.98*</td>
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<tr>
<td>RC</td>
<td>-</td>
<td>0.72*</td>
<td></td>
<td>0.74*</td>
<td>0.99*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.94*</td>
<td></td>
</tr>
<tr>
<td>LCB</td>
<td>-</td>
<td>0.64*</td>
<td></td>
<td>0.74*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.77*</td>
<td></td>
</tr>
<tr>
<td>RCB</td>
<td>-</td>
<td>0.73*</td>
<td></td>
<td>0.77*</td>
<td></td>
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<td></td>
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</tbody>
</table>

*Critical value r=0.23, p=0.05, one-tail test.

SBP=systolic blood pressure, DBP=diastolic blood pressure, POND=ponderosity index, TPC=total plasma cholesterol, HDLC=high density lipoprotein cholesterol, LC=left common carotid artery, RC=right common carotid artery, LCB=left carotid bifurcation, RCB=right carotid bifurcation, Car=carotid artery, Bif=carotid bifurcation.

Figure 2. Photomicrograph of a Von Korff van Gieson-stained lesion of the left common carotid artery showing an amorphous matrix, cholesterol clefts, mineralization, necrosis, a broken internal elastic lamina, thin media, fibromuscular cap, and a few foam cells.

Atherosclerosis extent was generally greater at the bifurcation. The lesions typically contained foam cells, cholesterol crystal clefts, smooth muscle cells, and connective tissue. Necrosis, mineralization, and fibromuscular caps occurred occasionally. The internal elastic lamina was frequently broken at sites of more complicated lesions (Figure 2).

**Oral Contraceptive Treatment**

Atherosclerosis in the common carotid arteries was first considered as the mean IA of the left and right sides together, and then each side was evaluated separately. Two-way ANOVA (Experimental Group × Regional Adiposity) revealed that OC administration had no effect on atherosclerosis extent in the common carotid arteries considered together (p=0.96) or separately (RC: p=0.94, Figure 3A; LC: p=0.86, Figure 3B). Carotid bifurcation atherosclerosis was similarly considered as the mean of the two sides together, and then each side was evaluated separately. As in the common carotid arteries, OC administration had no effect on atherosclerosis extent in the carotid bifurcations whether considered together (p=0.86) or separately (RCB: p=0.57, Figure 4A; LCB: p=0.68, Figure 4B). Further, OC treatment did not significantly affect TPC (p=0.14); however, HDLC concentrations were significantly decreased (p=0.003, Table 3).

**Regional Adiposity**

In contrast to the lack of effect associated with OC administration, females with high central fat ratios...
Figure 3. Common carotid artery atherosclerosis by oral contraceptive group and regional adiposity. Open bars represent females with a low subscapular/triceps skinfold thickness (SS:TRI) ratio; hatched bars represent females with a high SS:TRI ratio. A. Right common carotid artery. B. Left common carotid artery.

Figure 4. Carotid bifurcation atherosclerosis by oral contraceptive group and regional adiposity. Open bars represent females with a low subscapular/triceps skinfold thickness (SS:TRI) ratio, and hatched bars represent females with a high SS:TRI ratio. A. Right carotid bifurcation. B. Left carotid bifurcation.

appeared to have more extensive atherosclerosis in the common carotid artery \( p=0.055 \). When the two arteries were considered separately, the effect was significant in the LC \( p=0.05 \), Figure 3B and of borderline significance in the RC \( p=0.06 \), Figure 3A). These effects were somewhat stronger at the carotid bifurcation, where females with high central fat also had significantly more atherosclerosis than did those with low central fat \( p=0.02 \). When each side was considered separately, the effect was again significant on the left side \( p=0.01 \), Figure 4B and only suggestive on the right \( p=0.10 \), Figure 4A). Regional adiposity and OC administration did not interact to significantly influence atherosclerosis at any of these sites (group by regional adiposity interaction: all \( p \) values > 0.20).

**Social Status**

Two-way ANOVA (Social Status \( \times \) Experimental Group) revealed significantly less atherosclerosis in dominant females in the carotid bifurcations \( p=0.04 \). When each side was considered separately, this effect was significant in the RCB \( p=0.04 \), Figure 5A) but only suggestive in the LCB \( p=0.16 \), Figure 5B). There was no difference between dominants and subordinates in the common carotid arteries considered together \( p=0.26 \) or separately \( LC, p=0.24 \); RC, \( p=0.24 \). As in the previous analysis, OC administration had no effect on atherosclerosis (all \( p \) values > 0.50). Moreover, social status did not interact significantly with OC treatment to influence atherosclerosis at any of the four sites (all \( p \) values > 0.20).

**Covariation of Risk Variables**

Plasma lipid concentrations were highly correlated with atherosclerosis extent at the four arterial sites. The correlations with HDLC were significant and negative, and the correlations with TPC were significant and positive (Table 2). Mean plasma lipid concentrations for the three experimental groups are presented in Table 3.

To determine whether the effect of regional adiposity on carotid artery atherosclerosis was mediated by plasma lipid concentrations, multiple-regression analyses were used, with grouping variables added to account for OC treatment. The results are presented in Table 4. Initially, regional adiposity (the SS:TRI skinfold ratio) was entered as an independent variable, and mean common carotid atherosclerosis was entered as the dependent variable. The results confirmed a significant relationship between the two variables. When TPC was added to the equation, the relationship between the SS:TRI skinfold ratio and mean common carotid atherosclerosis disappeared, and
Table 3. Plasma Cholesterol Concentrations In Three Groups during Experimental Period

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ovral</th>
<th>Demulen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total plasma cholesterol</td>
<td>386 (28)</td>
<td>425 (36)</td>
<td>465 (31)</td>
</tr>
<tr>
<td>High density lipoprotein cholesterol*</td>
<td>54 (4.8)</td>
<td>32 (3.2)</td>
<td>43 (5.2)</td>
</tr>
</tbody>
</table>

Values are the means (SEM) and are given in mg/dl.

*P=0.003.

When the negative effects of OC on experimental period TPC were adjusted for in the multiple-regression equations, the relationships between atherosclerosis extent in both the common carotid arteries and the carotid bifurcations and treatment with Demulen became significant. These were inverse relationships, indicating that when the effects of TPC were accounted for, the residual association with Demulen treatment was protective against the development of atherosclerosis in these arteries. When the effects of HDLC were added to the equation, the relationship between atherosclerosis in these arteries and treatment with Ovral became significant. This, too, was in inverse relationship, indicating that the residual association with Ovral treatment was protective against the development of atherosclerosis. The inverse relationship with Demulen treatment, after the effects of HDLC were accounted for, increased in magnitude but did not reach significance (common carotid arteries: p=0.08; carotid bifurcations: p=0.07). These results suggest that OC treatment has a protective effect against carotid atherosclerosis, which only becomes apparent after the adverse effects on plasma cholesterol concentrations are taken into account. Thus, the net result of these two effects is little or no change in carotid artery atherosclerosis extent.

Social status also was used as an independent variable, with the grouping variables accounting for OC treatment to predict mean carotid bifurcation atherosclerosis using multiple-regression analysis (Table 4). (As the ANOVA indicated no effect of social status on common carotid atherosclerosis, this relationship was not considered using regression analyses.) This analysis confirmed that social status was a significant predictor of atherosclerosis. When TPC was added to the equation, the strength of the association between social status and carotid bifurcation atherosclerosis declined but remained significant, and TPC accounted for a large proportion of the variance in carotid bifurcation atherosclerosis. When HDLC was substituted for TPC, social status was no longer a significant predictor of carotid bifurcation atherosclerosis.

When the effects of HDLC were accounted for in the equation with social status, the relationship between carotid bifurcation atherosclerosis and treatment with Ovral became significant. Thus, after the adverse effect of Ovral treatment on HDLC concentrations was taken into account, a protective effect of Ovral against diet-induced carotid bifurcation atherogenesis became apparent.

**Blood Pressure and Ponderosity**

Correlations between SBP, DBP, and atherosclerosis extent at the four carotid sites were nonsignificant. Fur-
Furthermore, after the deleterious effects of OCs on plasma cholesterol concentrations were taken into account, a protective effect of OCs against carotid atherosclerosis emerged. The net effect of adverse changes in plasma cholesterol concentrations, coupled with this apparent protective effect, resulted in no significant difference in carotid atherosclerosis extent. This finding is similar to that found in the coronary arteries, although the protective effect may be of greater strength in those arteries. Currently, it is hypothesized that this protection is due to the influence of estrogen; however, further work will be necessary to understand the mechanism of action.

Psychosocial stress has been found to have a potent influence on coronary artery atherosclerosis in cynomolgus macaques and coronary heart disease among human beings. Eaker et al. found that the Type A behavior pattern was related to the incidence of cerebrovascular disease in women in the Framingham Study. Among female cynomolgus monkeys, protection against coronary artery atherosclerosis is closely associated with dominant social status. In the current study, dominant female monkeys had significantly less carotid bifurcation atherosclerosis than did their subordinate counterparts. We have observed previously a number of physiological differences between dominant and subordinate females of this species, which could have mediated the differences between them in atherosclerosis extent reported here. Subordinate female cynomolgus monkeys have lower HDLC and social status was a significant predictor of carotid bifurcation atherosclerosis alone and when TPC

### Table 4. Relationships between Regional Adiposity (SS:TRI) and Carotid Atherosclerosis, and Social Status and Carotid Atherosclerosis without and with Adjustment for Plasma Lipid Concentrations

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Carotid bifurcation atherosclerosis*</th>
<th>Carotid bifurcation atherosclerosis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS:TRI</td>
<td>Ovral</td>
<td>Demulen</td>
</tr>
<tr>
<td>0.03</td>
<td>0.56</td>
<td>0.82</td>
</tr>
<tr>
<td>SS:TRI</td>
<td>Ovral</td>
<td>Demulen</td>
</tr>
<tr>
<td>0.28</td>
<td>0.01</td>
<td>0.32</td>
</tr>
<tr>
<td>Ovral</td>
<td>Demulen</td>
<td>SS:TRI</td>
</tr>
<tr>
<td>0.82</td>
<td>0.01</td>
<td>0.28</td>
</tr>
<tr>
<td>HDLC</td>
<td>TPC</td>
<td>SS:TRI</td>
</tr>
<tr>
<td>0.00</td>
<td>0.01</td>
<td>0.28</td>
</tr>
<tr>
<td>Demulen</td>
<td>SS:TRI</td>
<td>HDLC</td>
</tr>
<tr>
<td>0.08</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Ovral</td>
<td>Demulen</td>
<td>SS:TRI</td>
</tr>
<tr>
<td>0.02</td>
<td>0.07</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*Each column describes a series of three multiple-regression analyses, the first of which does not adjust for oral contraceptive effects on plasma lipid concentrations. The subsequent two analyses in each column adjust for either TPC or HDLC. Independent variables were chosen because of their significant association with the dependent variables or because they were major grouping variables in the experiment (oral contraceptive variables). The significance of the relationship between each independent variable and the dependent variable, within each of the multiple regression analyses, is represented by the p value.

SS:TRI = ratio of subscapular/triceps skinfold thickness, TPC = total plasma cholesterol concentration, HDLC = high density lipoprotein cholesterol concentration.
was entered into the equation. When HDLc was entered, the relationship between carotid bifurcation atherosclerosis and social status declined below the level of significance. This finding is consistent with the hypothesis that social status exerts its influence as a risk factor, at least in part, by lowering HDLc.

Elevated heart rate response has been associated previously with exacerbated coronary artery and RCB atherosclerosis in female cynomolgus monkeys. It is possible that subordinate females in this experiment were likewise characterized by elevated heart rate response to challenge and similarly were at greater risk of developing exacerbated atherosclerosis. Whatever the mechanism(s), the apparent protective nature of high social status extends to the carotid bifurcation in these animals.

There are conflicting data relating whole-body obesity (measured as height/weight ratios) and atherothrombotic brain infarction. Only recently have subcategories of obesity, based on the anatomic location of fat deposition, been investigated as stroke risk factors. Body fat deposited on the trunk (central fat deposition), versus the periphery, has been found to be associated with stroke, as well as myocardial infarction, and hypertension, and plasma lipid concentrations. In women. Central fat deposition has also been found to be associated with exacerbated coronary artery atherosclerosis, as well as cardiovascular risk factors, in female cynomolgus monkeys. In the current study, the height/weight ratio (POND) was not associated with carotid artery atherosclerosis. However, the central deposition of fat on the trunk versus the periphery (SS:TRI) was moderately associated with carotid artery atherosclerosis. When plasma lipid variables were added to the equation, the association of regional adiposity and carotid atherosclerosis disappeared. This finding is compatible with the hypothesis that regional adiposity exerts its influence as a risk factor for carotid atherosclerosis by worsening plasma lipid profiles. However, the causal relationships implied in this hypothesis have yet to be shown.

Plasma lipid concentrations were the variables most strongly associated with exacerbated carotid artery atherosclerosis in this study. These variables were influenced by the response to dietary cholesterol and fat and to contraceptive steroid treatment. However, as previously described, plasma lipid concentrations are also associated with social status in cynomolgus monkeys and are affected by stress in human beings. Finally, plasma lipid concentrations are associated with regional adiposity in cynomolgus monkeys and in human beings. The multiple-regression analyses of lipids, regional adiposity, and atherosclerosis and of social status, lipids, and atherosclerosis suggest that the effects of social status and regional adiposity are not entirely independent. Rather, the association of each of these variables with carotid atherosclerosis may be mediated, at least in part, by plasma lipid concentrations.

While the relationship between coronary heart disease and plasma cholesterol concentrations is well established, there are conflicting data in human beings concerning the relationship between plasma cholesterol concentrations and carotid artery atherosclerosis or its clinical sequelae. In the current study of middle-aged female monkeys, HDLc and TPC were the most significant correlates of carotid artery atherosclerosis. There may be several reasons why this relationship was clear in monkeys but not in human beings. Measurement error may be a significant factor, as atherosclerosis extent was directly quantified in the current study, which is not possible, prospectively, in studies of human beings. Further, in several studies of human beings, geriatric populations were examined, and lipid determinations at that time may not be representative of lifetime lipid concentrations, which actually contribute to the relatively long process of atherogenesis. Also, geriatric populations may be biased because individuals at risk for atherosclerosis have probably died of coronary heart disease before the time of the study. Finally, there may be species differences in the contribution of risk factors, although prior research with this species on atherogenesis in the coronary arteries has provided indirect evidence to suggest that this is not the case.

Finally, we note the absence of an association between carotid artery atherosclerosis extent and BP. This lack of association may be related to low variability among these females in SBP or DBP and to the fact that these values were similar to those reported as normal for this species. Restricted variability in BP among normotensive women would tend to obscure a relationship between carotid artery atherosclerosis and BP.

In summary, the results of this study suggest that OCs do not increase the risk of cerebrovascular clinical events by exacerbating atherosclerosis of the common carotid artery or carotid bifurcation. Regional adiposity is associated with carotid artery atherosclerosis, and this effect appears to be mediated by plasma lipid concentrations. Social status is associated with carotid bifurcation atherosclerosis, and this effect also may be mediated, in part, by plasma lipid concentrations. Finally, plasma lipid concentrations independently and through their association with social stress factors and central fat deposition are closely associated with exacerbated carotid artery atherosclerosis in female cynomolgus monkeys.

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