Effects of Estrogen and Progesterone on Plasma Lipoproteins and Experimental Atherosclerosis in the Baboon (*Papio* sp.)


We determined the effect of estrogen and progesterone on plasma cholesterol and lipoprotein cholesterol concentrations and on arterial lesions in 24 ovariectomized and hysterectomized baboons fed a high-cholesterol/high-saturated-fat diet. These baboons were divided into four groups: untreated control (C); estrogen, 100 μg/kg/week injected i.m. (E); progesterone, 3 mg/kg/day (P); and estrogen plus progesterone (E+P). The treatment regimen continued for 18 months. Cholesterol levels in plasma and lipoproteins were measured before hormone treatment and at 3, 10, and 18 months of treatment. Postheparin plasma lipoprotein lipase (LPL) activity was also measured during the treatment. After 18 months of hormone treatment, baboons were necropsied and arterial lesions were measured. Hormone treatment significantly influenced plasma cholesterol (P>C>[E+P]>E) and very low density lipoprotein plus low density lipoprotein (VLDL+LDL) cholesterol (P>C>[E+P]>E), with very little effect on high density lipoprotein (HDL) cholesterol concentration. The E+P group had a significantly higher HDL cholesterol concentration than did the P group. The (VLDL+LDL)/HDL cholesterol ratios in the E and E+P groups were significantly lower than those in the P and C groups. LPL activities were significantly lower in the E group compared with those in the E+P and P groups. Hormone treatment significantly influenced lesions in four (innominate, carotid, iliac, and abdominal aorta) of seven arteries. The P group had the most fatty streaks, and the E+P group had the least. Multiple regression analysis suggested that hormone treatment influenced arterial lesions, both through effects on VLDL+LDL cholesterol and (VLDL+LDL)/HDL cholesterol ratio and through an effect independent of lipoproteins. (Arteriosclerosis and Thrombosis 1991;11:23-31)

It is well known that premenopausal women have less atherosclerosis and a lower incidence of coronary heart disease than do men and that the incidence of coronary heart disease in women increases after natural or surgical menopause.1,2 Premenopausal women who have had bilateral ovariectomy demonstrate a higher incidence of myocardial infarction unless estrogen therapy is begun immediately after the ovariectomy.3,4 These associations have led to the concept that estrogen may protect against atherosclerosis and coronary heart disease. A number of studies in experimental animals have shown a hypolipidemic effect of estrogen.5-7 Our studies in cholesterol-fed rabbits suggested that the hypolipidemic effect of estrogen was mediated by a decrease in very low (VLDL) and intermediate (IDL) density lipoproteins.8 Estrogen treatment enhanced the uptake of cholesterol-rich VLDL by isolated, perfused rabbit livers9 due to an increase in the activity of the apolipoprotein (apo) B/E receptor.10-12 In human subjects with type III hyperlipoproteinemia, estrogen exerted a hypolipidemic effect and normalized lipoprotein levels by accelerating the removal of cholesterol-rich VLDL.13 Estrogen treatment in premenopausal women increased apo A-I but decreased hepatic lipase activity.14 Premenopausal women had lower levels of low density lipoproteins (LDL) and higher levels of high density lipoproteins (HDL) than did men at all ages beyond puberty.15 Thus, it appears that estrogen given alone produces an antiatherogenic lipoprotein profile. In contrast, oral contraceptives containing a low dose of estrogen combined with a medium or high dose of progestins produce the opposite effect,16,17 an observation suggesting that progestin-containing agents produce an atherogenic lipoprotein pattern. The effects of estrogen and...
Animals and Diet

Experimental Design

orally and cottonseed oil, the estrogen vehicle, the untreated control group received placebo (a fig bar in a fig bar and was injected with the estradiol vehicle weekly. The E+P group received a combination of β-estradiol 17-cypionate and progesterone at the same doses that were given to the separate E and P groups. In the first block, estrogen was administered at a dosage of 100 μg/kg the first week and was increased by 50 μg/kg/week until the baboons were stabilized at a dose of 200 μg/kg/week. The progesterone in the first block was started at 4 mg/kg/day and was raised to 6 mg/kg/day over a period of 6 weeks. After 7 months, the baboons in the first block were left untreated for 4.5 months to reduce the inflammation of their sex skin. Treatments were then resumed at reduced levels: estrogen at 100 μg/kg/week and progesterone at 3 mg/kg/day. Baboons were treated for 3 weeks and then taken off treatment for 1 week as described previously. This cycle of treatment allowed the sex skin of the baboons to become less inflamed during the off-treatment period. The changes in hormone doses of block 1 baboons were made to determine the optimal doses of estrogen and progesterone. The hormone treatment was given to these baboons for a total of 18 months. The final doses of estrogen and progesterone in the first block and the same treatment schedule were used for baboons in the second block. The second block of baboons was also treated with hormones for 18 months.

Lipid and apoprotein levels in plasma and lipoproteins were measured periodically.27 Estrogen and progesterone levels were also measured by radioimmunoassay (Radioassay Systems Labs, Inc., Carson, Calif.). As reported earlier,26 baboons in the E and E+P groups had similar levels of estradiol in the plasma, which were 10-fold higher than in the C group (7.6±1.5 pg/ml in C versus 73.2±9.9 pg/ml in the E group). Similarly, progesterone-treated baboons had a twofold increase (16.8±2.5 ng/dl in the C group versus 36.4±1.2 ng/dl) in the P group, and 17-cypionate of (The Upjohn Co., Kalamazoo, Mich.) in cottonseed oil, which was injected i.m., and was fed a fig bar daily. The P group received progesterone (Sigma Chemical Co., St. Louis, Mo.) orally in a fig bar and was injected with the estradiol vehicle weekly.

Methods

 Twenty-four adult female baboons (Papio sp.), ranging from 4 to 9 years of age and with average plasma and lipoprotein cholesterol responses to a high-cholesterol/high-saturated-fat (HCHF) diet (7 weeks), were selected for these studies. Baboons used for these studies had approximately twofold increases in plasma LDL levels and did not have HDL₃ and lipoprotein(a) (Lp[a]) in their plasma. All the animals were ovaricetomized and hystercetomized after being maintained on the HCHF diet for 8 weeks. After surgery, baboons were allowed to recover for 4 weeks after which they were started on hormone treatments. Baboons were maintained on the HCHF diet during the recovery period and for the duration of hormone treatment. The composition of the diet was similar to that described earlier25 and is given in Table 1. The source of the saturated fat, which provided 40% of the total calories, was lard, and the cholesterol content of the diet was 1.7 mg/kcal.

Separation of Plasma Lipoproteins

Analysis of lipoproteins was performed at 3, 10, and 18 months after beginning the hormone treatment. Blood (10 ml) was collected in tubes containing EDTA (1 mg/ml), and plasma was obtained by low-speed centrifugation at 6°C. The plasma was treated with sodium azide (2 mg/dl), chloramphenicol (0.5 mg/dl), gentamicin sulfate (1 mg/dl), and phenylmethylsulfonyl fluoride (0.05 mmol/dl). The plasma (2 ml), adjusted to a density of 1.30 g/ml,
was layered with solutions of different densities, from 1.21 to 1.006 g/ml, in SW41 Ti rotor tubes as described earlier. The plasma was then ultracentrifuged in an SW41 Ti rotor with a Beckman ultracentrifuge Model L8-70 or L8-70M (from Beckman Instruments Inc., Palo Alto, Calif.) at 39,000 rpm (175,000g) for 24 hours at 6°C. After ultracentrifugation, the tube contents were fractionated by puncturing the tube at the bottom as described previously. On the basis of density, lipoprotein fractions were pooled to represent VLDL+IDL (d<1.019 g/ml), LDL (d=1.019–1.050 g/ml), and HDL (d=1.050–1.21 g/ml).

Cholesterol Quantification in Plasma and Lipoproteins

Cholesterol in plasma and lipoproteins was measured by enzymatic method assay kits (Sigma).

Collection of Postheparinized Plasma and Assay of Lipoprotein Lipase Activity

Fasted baboons were injected with heparin derived from porcine intestine (Elkins-Sinn, Inc., Cherry Hill, N.J.), 50 units/kg body wt, into the femoral vein. At 10 minutes after heparin injection, a 5.0-ml blood sample was taken, placed on ice, and centrifuged to recover the plasma.

Postheparin plasma lipoprotein lipase activity was assayed by a glycerol-based triglyceride substrate as described by Nilsson-Ehle and Schotz. Activity was determined by incubating the enzyme preparation with 1.0 M and 0.15 M sodium chloride with 0.1 ml diluted substrate (containing 0.008 ml heat-inactivated baboon serum, 0.017 ml of the glycerol tri[9,10(n)3H]oleate stock (Amersham, Arlington Heights, Ill.), and 0.075 ml 0.2 M Tris with 3% bovine serum albumin [Miles, Scientific, Naperville, Ill.], pH 8.0). After 30 minutes at 37°C, released fatty acids were extracted and measured as described by Nilsson-Ehle and Schotz. Enzyme activity was expressed as micromoles of free fatty acid released per hour per milliliter of plasma. Under the assay conditions, virtually all (>95%) of the lipase activity was inhibited with 1.0 M sodium chloride.

Necropsy Procedure

After 18 months on hormone treatment, the baboons were immobilized with ketamine hydrochloride (10 mg/kg), anesthetized with pentothenal, and exsanguinated. The aorta and the coronary, iliac-femoral, brachial, and carotid arteries were opened longitudinally, fixed with the adventitia adherent to chip board in 10% buffered formalin, stained with Sudan IV, and packaged in plastic bags.

Grading of Atherosclerosis

Fatty streaks were defined as Sudan IV-stained intimal areas that were elevated slightly or not at all above the surrounding intimal surface. Fibrous plaques were defined as firm, distinctly elevated areas, regardless of whether their intimal area was stained for lipid. These definitions are similar to those used for grading human lesions. One of the coinvestigators (H.C.M.) estimated the percentage of intimal surface involved with each type of lesion in each of the following arterial segments: the aortic arch from the aortic valve to the ligamentum arteriosus; the thoracic aorta from the ligamentum arteriosus to the origin of the celiac artery; the abdominal aorta from the origin of the celiac artery to the iliac bifurcation; the entire innominate artery; the left and right carotid arteries from their origins to and including their trifurcations; the left and right brachial arteries from their origins to the branchings at the elbows; the left and right iliac-femoral arteries from their origins to the branchings of the femoral arteries at the knee; and the right, circumflex, and left anterior descending coronary arteries. Values for paired arteries were averaged for statistical analysis.

These evaluations were made independently for each segment and in random order without knowledge of the treatment group from which the specimen was derived. Independent regrading of all 24 specimens by the same grader indicated an acceptable intraobserver variability. The intraclass correlation coefficients between the two grades were 0.839 for the aortic arch, 0.866 for the innominate artery, 0.918 for the brachial arteries, 0.948 for the carotid arteries, 0.860 for the iliac-femoral arteries, 0.899 for the thoracic aorta, and 0.876 for the abdominal aorta.

All the lesions encountered were simple fatty streaks as delineated by Sudan IV staining, except for one small atypical plaque in the abdominal aorta of one animal that was not considered a result of the experimental treatment. The coronary arteries contained no lesions, and, therefore, no analyses are presented for them.

Statistical Analyses

The main effects of hormone treatment on cholesterol concentrations in plasma, VLDL+LDL, and HDL, (VLDL+LDL)/HDL cholesterol ratio, and postheparin lipolytic activity were analyzed separately by analysis of variance (ANOVA). ANOVA with repeated measures, with pretreatment and 3 months of treatment as the trial factors, was used to determine the effect of initial hormone treatment on plasma and lipoprotein cholesterol. Multiple comparisons of group means from equal sample sizes were done with Duncan's multiple-range test, and means from unequal sample sizes were compared with Tukey's honestly significant difference test. Serum lipid concentrations and lipoprotein lipase activity were log transformed before analysis to better meet the distributional assumption that variances are normally distributed among the treatment groups for the ANOVA. The assumption of normal distribution of variance among groups appeared not to be violated after examination of residuals. All results from transformed data are presented as the mean plus and minus 95% confidence intervals.
TABLE 2. Mean Plasma and Lipoprotein Cholesterol Concentrations by Diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>n</th>
<th>Total cholesterol (mg/dl)</th>
<th>LDL cholesterol (mg/dl)</th>
<th>HDL cholesterol (mg/dl)</th>
<th>(VLDL+LDL)/HDL cholesterol (ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chow</td>
<td>24</td>
<td>103</td>
<td>36</td>
<td>65</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(97-110)</td>
<td>(31-41)</td>
<td>(59-71)</td>
<td>(0.45-0.67)</td>
</tr>
<tr>
<td>HCHF</td>
<td>24</td>
<td>179</td>
<td>67</td>
<td>108</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(167-191)</td>
<td>(59-77)</td>
<td>(97-119)</td>
<td>(0.52-0.76)</td>
</tr>
<tr>
<td>Significance level</td>
<td>0.0009</td>
<td>0.0009</td>
<td>0.0009</td>
<td>0.098</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean, with 95% confidence intervals in parentheses. There were no significant differences between treatment groups. See text for explanation of abbreviations.

The percentages of intimal surface area involved with fatty streaks were also analyzed by ANOVA. An examination of residuals of the variance of lesion data indicated that variances appeared to be normally distributed among all treatment groups, with the exception of the abdominal aorta. The percentages of intimal surface area involved with fatty streaks in the abdominal aorta were transformed using square roots to better meet the normal distribution assumptions for the ANOVA. Associations of lesions with steroid hormone treatment, VLDL + LDL cholesterol, and (VLDL + LDL)/HDL cholesterol ratio as predictor variables were analyzed by multiple linear regression. We have reported effects at p<0.1 to achieve a better balance between Type I and Type II statistical errors.

Results

Effect of Challenge Diet on Plasma and Lipoprotein Cholesterol Concentrations Before Hormone Treatment

Plasma and lipoprotein cholesterol values for baboons on the chow and challenge diets before treatment are given in Table 2. Plasma cholesterol concentration was increased by 75% after feeding the HCHF diet. There were similar increases in LDL and HDL cholesterol. The (VLDL + LDL)/HDL cholesterol ratio was not influenced by the challenge diet. There were no significant differences among the treatment groups in plasma and lipoprotein cholesterol values before hormone treatment.

Effect of Initial Hormone Treatment on Plasma and Lipoprotein Cholesterol Concentrations

The effects of 3 months of each hormone treatment on plasma and lipoprotein cholesterol concentrations are described in Table 3. There was a significant treatment by time (pretreatment and at 3 months’ treatment) interaction effect on plasma cholesterol, VLDL + LDL cholesterol, and HDL cholesterol concentrations and on the (VLDL + LDL)/HDL cholesterol ratio. Total plasma cholesterol decreased significantly in baboons receiving estrogen and estrogen plus progesterone therapy, whereas total plasma cholesterol did not change in baboons receiving progesterone and placebo control. LDL cholesterol decreased in both the E (not significant) and E + P (significant) groups but increased in C (significant) and P (not significant) groups. HDL cholesterol declined significantly in the E, E + P, and P groups. (VLDL + LDL)/HDL cholesterol ratios significantly increased in the C and P groups but not in the E and E + P groups.

Effect of Long-term Hormone Replacement Therapy on Plasma and Lipoprotein Cholesterol Concentrations

The effects of 18 months of hormone replacement therapy on mean plasma and lipoprotein cholesterol concentrations are reported in Table 4. Plasma cholesterol concentrations in the E group were significantly lower than in the P group. The E group had

TABLE 3. Mean Plasma Cholesterol and Lipoprotein Cholesterol Levels After Initial Sex Hormone Replacement Therapy

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Total cholesterol (mg/dl)</th>
<th>VLDL+LDL cholesterol (mg/dl)</th>
<th>HDL cholesterol (mg/dl)</th>
<th>(VLDL+LDL)/HDL cholesterol (ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>160</td>
<td>68</td>
<td>93</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(143-180)</td>
<td>(60-101)</td>
<td>(68-109)</td>
<td>(0.3-1.33)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.45-1.06)</td>
<td></td>
<td></td>
<td>(0.67-1.24)</td>
</tr>
<tr>
<td>Estrogen</td>
<td>6</td>
<td>187</td>
<td>68</td>
<td>117</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(152-231)</td>
<td>(55-85)</td>
<td>(58-109)</td>
<td>(0.42-0.81)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.21-0.73)</td>
<td></td>
<td></td>
<td>(0.61-0.90)</td>
</tr>
<tr>
<td>Estrogen +</td>
<td>6</td>
<td>188</td>
<td>70</td>
<td>101</td>
<td>0.60</td>
</tr>
<tr>
<td>progesterone</td>
<td></td>
<td>(166-211)</td>
<td>(56-87)</td>
<td>(85-120)</td>
<td>(0.46-0.77)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.30-0.93)</td>
<td></td>
<td></td>
<td>(0.4-0.73)</td>
</tr>
<tr>
<td>Progesterone</td>
<td>6</td>
<td>181</td>
<td>73</td>
<td>87</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(155-211)</td>
<td>(56-96)</td>
<td>(68-116)</td>
<td>(0.45-1.06)</td>
</tr>
</tbody>
</table>

*p<0.05 between pretreatment and posttreatment (3 months) values. 95% confidence intervals in parentheses. See text for explanation of abbreviations.
Table 4. Effect of Long-term Hormone Therapy on Plasma Cholesterol and Lipoprotein Cholesterol Concentrations

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Plasma cholesterol (mg/dl)</th>
<th>VLDL+LDL cholesterol (mg/dl)</th>
<th>HDL cholesterol (mg/dl)</th>
<th>(VLDL+LDL)/HDL cholesterol (ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>161 (133–195)</td>
<td>77 (58–101)</td>
<td>80 (62–104)</td>
<td>0.95 (0.54–1.40)</td>
</tr>
<tr>
<td>Estrogen</td>
<td>6</td>
<td>144* (129–160)</td>
<td>55† (47–66)</td>
<td>87 (76–99)</td>
<td>0.63± (0.52–0.77)</td>
</tr>
<tr>
<td>Estrogen + progesterone</td>
<td>6</td>
<td>159 (143–176)</td>
<td>60 (46–77)</td>
<td>98 (88–108)</td>
<td>0.61 (0.47–0.81)</td>
</tr>
<tr>
<td>Progesterone</td>
<td>6</td>
<td>171 (160–184)</td>
<td>88§ (68–114)</td>
<td>76</td>
<td>1.16§ (0.71–1.89)</td>
</tr>
</tbody>
</table>

95% confidence intervals in parentheses. See text for explanation of abbreviations.

*Significantly different (p<0.05) from progesterone group.
†Significantly different (p<0.05) from control group.
‡Significantly different (p<0.05) from estrogen and estrogen+progesterone groups.
§Significantly different (p<0.05) from estrogen and estrogen+progesterone groups.
||Significantly different (p<0.06) from estrogen+progesterone group.

significantly lower VLDL+LDL cholesterol than did the P and C groups. The E+P group also had significantly lower VLDL+LDL cholesterol than the P group. The E+P group had a higher HDL cholesterol concentration than the P group. The (VLDL+LDL)/HDL cholesterol ratio was also influenced by the hormone treatment. The E+P group had a significantly lower (VLDL+LDL)/HDL cholesterol ratio than did the P and C groups. The E group also had a (VLDL+LDL)/HDL cholesterol ratio significantly different from P and C groups.

The changes in VLDL+LDL cholesterol and the (VLDL+LDL)/HDL cholesterol ratio during the experiment are summarized in Figures 1 and 2, respectively. During the treatment period (3–21 months), VLDL+LDL cholesterol and (VLDL+LDL)/HDL cholesterol ratio were analyzed for treatment effect with time as a trial factor by repeated-measures ANOVA. There was no significant time-by-treatment interaction on VLDL+LDL cholesterol concentrations. In contrast to VLDL+LDL cholesterol, there was a significant time-by-treatment interaction on (VLDL+LDL)/HDL cholesterol ratios. The P and C groups increased their (VLDL+LDL)/HDL cholesterol ratios during hormone treatment, but the E and E+P groups did not.

Effect of Hormone Replacement Therapy on Postheparin Plasma Lipoprotein Lipase Activity

The effect of hormone replacement therapy on postheparin plasma lipoprotein lipase activity in different treatment groups is reported in Table 5. Baboons treated with estrogen had significantly lower plasma lipoprotein lipase activity than that in the E+P and P groups. Hepatic triglyceride lipase activity measured at pH 8.8 and in the presence 1.0 M

![Figure 1](http://atvb.ahajournals.org/)

**Figure 1.** Line plot showing mean values for VLDL+LDL-C (mg/dl) as a function of time (months) in ovariectomized and hysterectomized baboons in control (○), estrogen (■), estrogen plus progesterone (△), and progesterone (▲) groups. Each group had six baboons. Mean values denoted by arrows A and B are for the chow and high-cholesterol/high-fat diet, respectively, before ovariectomy and hysterectomy. Baboons were ovariectomized and hysterectomized and treated with hormones after the lipoprotein measurement denoted by arrow B. All baboons were maintained on the high-cholesterol/high-fat diet throughout the hormone treatment period. VLDL+LDL-C, very low plus low density lipoprotein cholesterol.

![Figure 2](http://atvb.ahajournals.org/)

**Figure 2.** Line plot showing mean values for (VLDL+LDL)/HDL-C ratio in ovariectomized and hysterectomized baboons in control (○), estrogen (■), estrogen plus progesterone (△), and progesterone (▲) groups. Each group had six baboons. Details of the hormone treatments are described in Figure 1. (VLDL+LDL)/HDL-C, ratio of very low plus low density lipoprotein to high density lipoprotein cholesterol.
TABLE 5. Effect of Steroid Treatment on Postbeparinlzed Plasma Lipoprotein Lipase Activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>LPL activity (μmol FFA/hr/ml plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>21.1 (14.6–30.5)</td>
</tr>
<tr>
<td>Estrogen</td>
<td>6</td>
<td>14.6 (11.6–18.5)</td>
</tr>
<tr>
<td>Estrogen+progesterone</td>
<td>6</td>
<td>30.9* (19.5–49.1)</td>
</tr>
<tr>
<td>Progesterone</td>
<td>5</td>
<td>31.3* (23.7–40.7)</td>
</tr>
</tbody>
</table>

LPL, lipoprotein lipase; FFA, free fatty acids. 95% confidence intervals in parentheses. *Significantly different from estrogen-treated group at p<0.010.

Effect of Hormone Replacement Therapy on Arterial Lesions

The percent of intimal surface area involved with lesions in major arteries is reported in Table 6. Hormone replacement therapy significantly influenced the extent of these lesions in the innominate, carotid, and iliac arteries and the abdominal aorta. The effects of hormone treatment on fatty streaks in the brachial artery (p=0.094) and aortic arch (p=0.134) approached statistical significance. The highest percentage of surface involved by lesions was observed in baboons treated with progesterone alone. In contrast, the least extensive fatty streaks occurred in baboons treated with a combination of estrogen and progesterone. In the innominate artery, the surface area involved with fatty streaks was significantly more in the P group than in the other treatment groups. The extent of lesions in the carotid artery in the E and E+P groups was significantly less than that in the C or P group. In the iliac artery, the extent of lesions was significantly higher in the P group compared with the C and E+P groups. Baboons treated with estrogen and progesterone had significantly less atherosclerosis in the abdominal aorta than did the P group. There were no measurable lesions in the coronary arteries of baboons in any treatment group.

TABLE 6. Mean Arterial Lesions After 18 Months of Hormone Treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent of surface area with fatty streaks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arteries</td>
</tr>
<tr>
<td></td>
<td>Aortic arch</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
</tr>
<tr>
<td>Estrogen</td>
<td>6</td>
</tr>
<tr>
<td>Estrogen+progesterone</td>
<td>6</td>
</tr>
<tr>
<td>Progesterone</td>
<td>5</td>
</tr>
<tr>
<td>Effect of treatment</td>
<td>p=0.134</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *Significantly different from control, estrogen, and estrogen+progesterone groups at p<0.05. †Significantly different from estrogen and estrogen+progesterone groups at p<0.05. ‡Significantly different from control and estrogen+progesterone group at p<0.05. §Significantly different from estrogen and estrogen+progesterone groups at p<0.05.

Regression Analysis of Lesions

Plasma VLDL+LDL cholesterol was positively correlated with lesions in the innominate (r=0.679, p=0.0001), carotid (r=0.559, p=0.006), iliac (r=0.554, p=0.005), and brachial (r=0.506, p=0.014) arteries and in the abdominal aorta (r=0.658, p=0.001). Similarly, the plasma (VLDL+LDL)/HDL cholesterol ratio was positively associated with lesions in the innominate (r=0.691, p=0.009), carotid (r=0.465, p=0.025), iliac (r=0.583, p=0.003), and brachial (r=0.398, p=0.06) arteries and in the abdominal aorta (r=0.702, p=0.0009). To determine whether the observed effects of hormone replacement therapy on lesions in the innominate, brachial, carotid, and iliac arteries and in the abdominal aorta (Table 6) resulted from the observed treatment effects on VLDL+LDL cholesterol and the (VLDL+LDL)/HDL cholesterol ratio, we analyzed the extent of lesions by multiple linear regression analysis. The (VLDL+LDL)/HDL cholesterol ratio, when added with hormone treatment, significantly increased the prediction of lesions for the abdominal aorta (p<0.05) and for the innominate (p<0.025) and iliac (p<0.025) arteries but not for the carotid and brachial arteries. Hormone treatment, when added with (VLDL+LDL)/HDL cholesterol ratio, significantly increased the prediction of lesions in the carotid (p<0.05), brachial (p<0.1), innominate (p<0.05), and iliac (p<0.1) arteries and the abdominal aorta (p<0.1). When adjusted for hormone therapy, the (VLDL+LDL)/HDL cholesterol ratio still was associated with lesions in the innominate (partial correlation coefficient=0.538, p=0.008) and iliac (partial correlation coefficient=0.524, p=0.009) arteries and abdominal aorta (partial correlation coefficient=0.478, p=0.018) but not in the carotid (partial correlation coefficient=0.185) and brachial (partial correlation coefficient=0.199) arteries. Similar results were obtained with VLDL+LDL cholesterol as the predictor in place of the (VLDL+LDL)/HDL cholesterol ratio.

Discussion

Summary of Results

The present study demonstrates that hormone treatment in pharmacological doses influences the...
development of arterial lesions in ovariectomized and hysterectomized baboons. The least extensive lesions were observed in the group treated with a combination of estrogen and progesterone. Progesterone treatment alone, on the other hand, resulted in more extensive arterial lesions. During hormone treatment, progesterone-treated baboons also had greater plasma cholesterol (P>C>[E+P]>E), VLDL+LDL cholesterol (P>C>[E+P]>E), and (VLDL+LDL)/HDL cholesterol ratio (P>C>E>[E+P]), but lower HDL cholesterol (P<C<E<[E+P]) than the other treatment groups. Regression analysis suggested that hormone therapy influenced arterial lesions partly by affecting VLDL+LDL cholesterol and the (VLDL+LDL)/HDL cholesterol ratio, but hormone therapy also affected lesions independently of plasma lipoprotein levels.

**Effect of Estrogen on Lipoprotein Levels and Composition**

The effect of estrogen treatment in this study was mainly on VLDL+LDL cholesterol and (VLDL+LDL)/HDL cholesterol ratio. Estrogen treatment only slightly increased HDL cholesterol concentrations. The minimal effect of estrogen treatment on HDL cholesterol concentration may be due to the naturally occurring high concentration of HDL in the baboon. These results are consistent with those observed in humans and experimental animals. Walmant and Varenhorst\(^\text{34}\) reported that patients with prostatic carcinoma, who were treated daily with ethinyl estradiol orally and with polyestradiol phosphate i.m. once a month for 8 weeks, had lower plasma cholesterol concentrations. The HDL cholesterol increased by 53%, and the LDL cholesterol decreased by 25%. Krauss et al\(^\text{35}\) found a significantly higher level of HDL (predominantly HDL\(_2\)) in postmenopausal estrogen users compared with nonusers. Cauley et al\(^\text{38}\) also reported that menopausal women using estrogens had significantly higher total HDL and HDL\(_2\) cholesterol concentrations than did controls, without any difference in HDL\(_3\). Schaefer et al\(^\text{14}\) reported that estrogen-treated premenopausal women had increased plasma HDL\(_2\) cholesterol levels with no change in LDL levels. We found that HDL increased while LDL decreased during estrogen treatment of a normolipidemic postmenopausal woman.\(^\text{37}\)

A number of studies conducted in several animal species have shown a hypolipidemic effect of estrogen. Moskowitz et al\(^\text{38}\) reported lower serum cholesterol levels and less-severe coronary atherosclerosis in estrogen-treated rats fed an atherogenic diet. Similarly, Prichard et al\(^\text{3}\) have shown a lipid-lowering and antiatherogenic effect of estrogen in cholesterol-fed, male, White Carneau pigeons. Chao et al\(^\text{36}\) and Davis and Roheim\(^\text{7}\) have reported that administration of large amounts of ethinyl estradiol to male rats produced a profound hypolipidemia that involved all major lipoprotein classes. Our studies in rabbits showed that the hypolipidemic effects of estrogen were mediated by a decrease in VLDL and IDL alone.\(^\text{8}\)

**Effect of Progesterone on Lipoprotein Levels and Composition**

Progestins with strong androgenic or antiestrogenic effects have been shown to decrease HDL\(_2\) selectively in premenopausal women, and this decrease was associated with increased hepatic lipase activity.\(^\text{39,40}\) Plasma HDL\(_2\) concentration was inversely correlated with postheparin plasma hepatic lipase activity in postmenopausal women.\(^\text{41}\) This observation suggested that the effects of progesterone may be mediated by hepatic lipase, as suggested for anabolic steroids. Progestins,\(^\text{42}\) and cynomolgus monkeys,\(^\text{46-48}\) oral contraceptives containing mainly progestins increased plasma triglycerides and decreased plasma HDL, especially HDL\(_2\). In cynomolgus monkeys treated with contraceptives containing both estrogen and progesterone, the HDL subfraction was decreased, with an increase in HDL\(_{3b}\) (as determined by gradient gels).

The main purpose of these studies was to investigate the effects of estrogen and progesterone, alone and in combination, on lipoprotein levels and composition and on arterial lesions in baboons. Our studies suggest that progesterone, when given alone to ovariectomized and hysterectomized baboons, increases VLDL+LDL cholesterol and the (VLDL+LDL)/HDL cholesterol ratio. In addition, most of the HDL in progesterone-treated baboons was in HDL\(_3\) range.\(^\text{26}\) The combined treatment with progesterone plus estrogen produced changes opposite to those observed in baboons treated with progesterone alone. Estrogen treatment decreased plasma postheparin lipoprotein lipase activity, but when estrogen was combined with progesterone, lipoprotein lipase activity was increased as it was with progesterone only. Thus, progesterone also counterbalanced the effect of estrogen in reducing lipoprotein lipase activity. These results suggested that estrogen and progesterone modulated lipoprotein levels and composition independently of each other in baboons. In some cases, estrogen and progesterone counterbalanced each other's effects on lipoprotein levels and metabolism, for example, apo B production\(^\text{49}\) and apo A-I catabolism.\(^\text{26}\) Estrogens and estrogen plus progesterone produced a lipoprotein pattern that was antiatherogenic, while progesterone alone resulted in a lipoprotein pattern that was atherogenic.

**Effect of Estrogen and Progesterone on Atherosclerotic Lesions**

Although studies in experimental animals have shown an antiatherogenic effect of exogenous estrogens,\(^\text{5,8,18-21}\) there are no reports on the effects of progesterone on atherosclerosis. Our study demonstrates that progesterone therapy increased atherosclerosis compared with controls, estrogen treatment, and estrogen plus progesterone treatment. Our ex-
experiment provides some evidence that the observed effects of hormone therapy on experimental atherosclerosis may be independent of lipoproteins. In the brachial and carotid arteries, hormone treatment predicted lesions independently of either VLDL+LDL cholesterol or the (VLDL+LDL)/HDL cholesterol ratio. In the abdominal aorta and innominate artery, the association of (VLDL+LDL)/HDL cholesterol ratio and VLDL+LDL cholesterol with lesions was reduced after adjusting for hormone therapy. However, because of the small numbers of baboons in each group, the relative contributions of each hormone therapy and lipoproteins to atherosclerosis could not be determined. The presence of an independent effect of hormone therapy on atherosclerosis as suggested in our study is consistent with similar studies in other animal models. Estrogen treatment retarded arterial lesion development in rabbits without an effect on plasma and lipoprotein cholesterol concentrations. Likewise, intravaginal ring treatment of female cynomolgus monkeys was associated with plaques that were larger than those in monkeys treated with oral contraceptives (containing both estrogen and progesterone) despite a similar reduction in plasma HDL cholesterol in both groups. Thus, it appears that estrogens have an antiatherogenic effect that is independent of reductions in plasma and lipoprotein cholesterol concentrations. This antiatherogenic effect of estrogens may involve a direct effect on the arterial wall, as suggested by some investigators. The primary effect of estrogen deficiency and estrogen replacement on coronary atherosclerosis in other models has been described. In our studies, lesion development was not noticed in coronary arteries in any treatment group. The beneficial effect of estrogen-replacement therapy was detected mainly on the innominate, brachial, iliac, and carotid arteries and the abdominal aorta. Although progesterone produces an atherogenic lipoprotein profile, it is not known whether progesterone influences atherosclerosis independently of lipoprotein levels. In our studies, progesterone treatment resulted in extensive arterial lesions. However, baboons given progesterone in combination with estrogen had much more extensive lesions than did the progesterone-treated group. 

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