Oral Contraceptives and Hormone Replacement Therapy Do Not Increase the Incidence of Arterial Thrombosis in a Nonhuman Primate Model

Dwight A. Bellinger, J. Koudy Williams, Michael R. Adams, Erika K. Honoré, Diane E. Bender

Abstract—Older oral contraceptive (OC) formulations containing high doses of potent synthetic estrogens and progestins are associated with increased risk of thrombosis. To examine the effects of current low-dose OC and hormone replacement therapy (HRT) regimens on arterial thrombosis, premenopausal and surgically postmenopausal cynomolgus monkeys were divided into four treatment groups. Premenopausal monkeys were given either no OCs or ethinyl estradiol and levonorgestrel as an OC at a dose equivalent to that currently given to women. Postmenopausal monkeys were given either no HRT or conjugated equine estrogens and medroxyprogesterone as an HRT at a dose equivalent to that currently given to women. The monkeys were fed an atherogenic diet containing these treatments for 27 to 30 months. At the end of this time, arterial thrombosis was evaluated with a standardized stenosis/injury procedure in the left carotid artery. Blood flow velocity was monitored for cyclic or permanent occlusive thrombosis. The current OC and HRT regimens did not increase the susceptibility of the artery wall to develop an occlusive thrombus following injury and stenosis. In fact, there was a reduction in the incidence of thrombosis in the OC animals compared with untreated controls. Increased amounts of atherosclerosis were associated with an increased incidence of occlusive arterial thrombosis. Several selected coagulation parameters [von Willebrand factor, protein C, lipoprotein(a), and platelet aggregation] did not appear to be associated with either the amount of atherosclerosis or incidence of arterial thrombosis. (Arterioscler Thromb Vasc Biol. 1998;18:92-99.)

Key Words: oral contraceptives ■ hormone replacement therapy ■ arterial thrombosis ■ atherosclerosis ■ cynomolgus monkeys

The effects of OCs and HRT on arterial thrombosis are unclear. Older OC formulations containing high doses of potent synthetic estrogens and progestins are associated with increased risk of thrombosis. This association is based on a number of reports of increased venous thrombosis, coronary heart disease, and thrombotic strokes.1-3 However, the current lower-dose OC formulations are considered to have less thrombotic risk compared with previous formulations.4-5 Furthermore, reports of increased coagulation parameters (plasma fibrinogen, prothrombin, and factors VII and VIII)6-7 associated with use of OCs have not correlated well with risk of thrombotic events. Present thought would support the notion that OC use is associated only with increased thrombotic risk in women who smoke8-9 and in selected populations prone to thrombosis.10 In contrast, HRT in postmenopausal women is associated with reduced risk of coronary heart disease.11 How HRT lowers coronary heart disease risk is unclear, but evidence exists that HRT has beneficial effects on plasma lipoprotein concentrations,12-14 inhibits progression of atherosclerosis,15 inhibits arterial wall accumulation of LDL,16 and reduces plasma Lp(a) concentrations.17,18 However, there is still concern about the effect of HRT on thrombotic events. This concern is sufficient to direct many physicians to stop HRT in women with a history of thrombotic disease. Much of this concern seems to be based on preconceived notions about exogenous estrogen/progestin administration in premenopausal women taking OCs. There is a paucity of information available about the effects of current OC and HRT formulations on risk of arterial thrombosis. Therefore, the purpose of this study was to examine the effects of current commonly used OC and HRT formulations on arterial thrombosis in a well-characterized animal model of arterial thrombosis in the setting of diet-induced atherosclerosis.

Methods

Animals

Sixty-six adult (7 to 10 years of age) female cynomolgus monkeys (Macaca fascicularis) were imported from Indonesia (Institut Pertanian Bogor, Bogor, Indonesia) and fed monkey chow (High Protein Monkey Chow, Ralston Purina Co) during a 3-month quarantine period. The monkeys were then fed their experimental diets for 27 to 30 months. All diets were moderately atherogenic and contained 0.28 mg cholesterol/kcal, with 40% calories from fat.
The premenopausal monkeys received the atherogenic diet either with (n=15) or without (n=13) a triphasic OC (Triphasil, Wyeth-Ayerst). On days 1 to 6 of the month, monkeys received ethinyl estradiol at 0.0020 mg/kg body wt and levonorgestrel at 0.0035 mg/kg body wt. On days 7 to 11 of the month, monkeys received ethinyl estradiol at 0.0027 mg/kg body wt and levonorgestrel at 0.0050 mg/kg body wt. On days 12 to 21 of the month, monkeys received ethinyl estradiol at 0.0020 mg/kg body wt and levonorgestrel at 0.0083 mg/kg body wt. On days 22 to 28, monkeys received no hormone in their diets. These doses were calculated to approximate the dose of triphasic OCs given to women. Doses were adjusted for species differences in body weight and body metabolism.

Monkeys used for the postmenopausal hormone replacement part of the experiment were ovariocec tomized at the beginning of the treatment phase of the experiment and then received the atherogenic diet with (n=24) or without (n=14) HRT. The HRT consisted of CEE (Premarin, Wyeth-Ayerst) and MPA (Cycrin, Wyeth-Ayerst) added to the diet. The dose of both hormones was 0.04 mg CEE · kg body wt⁻¹ · d⁻¹ and 0.16 mg MPA · kg body wt⁻¹ · d⁻¹ added to the diet. These doses were chosen to approximate the daily doses of these hormones given to women (0.625 and 2.5 mg, respectively) and were calculated on the basis of body weight and metabolic rate differences between species.

Monkeys lived in social groups of 4 to 6 animals. All procedures were performed at the Comparative Medicine Clinical Research Center of the Bowman Gray School of Medicine in accordance with state and federal laws. Animal protocols were approved by the Bowman Gray Animal Care and Use Committee and conformed to guidelines set forth by the American Association for Accreditation of Laboratory Animal Care and by National Institutes of Health publication 86–23, Guide for the Care and Use of Laboratory Animals.

Blood Sample Analysis for Coagulation Parameters

All blood samples were taken from monkeys after an overnight fast. Monkeys were sedated with ketamine hydrochloride (10 to 15 mg/kg IM), and blood samples were taken from a femoral vein. Blood samples were collected periodically at 3, 18, and 26 months after the beginning of the atherogenic diet for the premenopausal groups and at 21 and 28 months after the beginning of the atherogenic diet for the premenopausal groups. Blood samples were drawn at various cycles of hormonal treatments, with the exception of the last OC samples, which were drawn at matched hormonal cycles. Blood samples were collected into sodium citrate from the femoral vein after sedation with ketamine hydrochloride (15 mg/kg). Blood analysis at the time of collection consisted of platelet aggregation studies with PRP with ADP (10⁻³ and 10⁻⁴ mol/L), manual platelet counts of PRP and whole blood, and thrombin clotting times with PPP. PPP was frozen for a later analysis of vWF antigen by ELISA (vWF antibody, Daco) and protein C activity by a chromogenic assay (Coamate, Chromogenix). vWF antigen and protein C activity are expressed as a percent of a standard pool. The standard pool was made from adult female cynomolgus monkeys unrelated to this study and not receiving any hormone therapy. Blood samples were also collected before and 12 months after the atherogenic diet for analysis of plasma concentrations of Lp(a). Plasma Lp(a) concentrations were determined with an ELISA for Lp(a) that was developed at Bowman Gray School of Medicine Lipoprotein Core Laboratory.

Arterial Thrombosis

The premenopausal monkeys received the atherogenic diet either with (n=15) or without (n=13) a triphasic OC (Triphasil, Wyeth-Ayerst). On days 1 to 6 of the month, monkeys received ethinyl estradiol at 0.0020 mg/kg body wt and levonorgestrel at 0.0035 mg/kg body wt. On days 7 to 11 of the month, monkeys received ethinyl estradiol at 0.0027 mg/kg body wt and levonorgestrel at 0.0050 mg/kg body wt. On days 12 to 21 of the month, monkeys received ethinyl estradiol at 0.0020 mg/kg body wt and levonorgestrel at 0.0083 mg/kg body wt. On days 22 to 28, monkeys received no hormone in their diets. These doses were calculated to approximate the dose of triphasic OCs given to women. Doses were adjusted for species differences in body weight and body metabolism.

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Arterial Thrombosis

At the end of the dietary treatment phase of the experiment, each monkey in the experiment was anesthetized with ketamine hydrochloride (10 to 15 mg/kg body wt, IM) and butorphanol (0.025 mg/kg body wt, IM). Additional doses of each agent were given as needed to maintain anesthesia. Palpebral reflex and response to pain (toe pinch) were used as criteria to monitor the level of anesthesia. The ventral neck region was shaved, and an incision was made to expose the left common carotid.

The left carotid artery was dissected free of surrounding tissue over a 2-cm segment. A 5-mm Goldblatt clamp was placed proximally on the carotid artery, and a 20-MHz Doppler ultrasonic crystal was applied distally so that flow was not impaired. The Doppler crystal was energized by a range-gated pulsed Doppler unit, and the signal was range-gated to maximum clarity.

A schematic diagram of the steps in the injury/stenosis procedure is given in Fig 1. After a 30-minute period of stabilization following the surgical preparation, the carotid artery was stenosed by closing the Goldblatt clamp sufficiently to block reactive hyperemia. A 20-second total occlusion was used to induce reactive hyperemia and confirm the arterial blockade. A 30-minute period of observation for CFR or PCF followed stenosis. CFR was characterized by a gradual reduction in

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**Selected Abbreviations and Acronyms**

- CEE = conjugated equine estrogen
- CFR = cyclic flow reduction
- HRT = hormone replacement therapy
- Lp(a) = lipoprotein(a)
- MPA = medroxyprogesterone acetate
- OC = oral contraceptive
- PCF = permanent cessation of flow
- PPP = platelet-poor plasma
- PRP = platelet-rich plasma
- vWF = von Willebrand factor

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Plasma Hormone Concentrations

Plasma concentrations of estradiol and medroxyprogesterone were measured as described previously. Plasma concentrations of estradiol and medroxyprogesterone were measured by ELISA methods in Dr Mark Wilson's laboratory at Emory University.

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**Arterial Thrombosis Protocol**

1. Surgical preparation of left carotid artery
2. Stabilization period
3. 30-min. stenosis with Goldblatt clamp; observe for CFR or PCF (reactive hyperemia blockade)
   - CFR or PCF=stop experiment (artery thrombosed)
   - no CFR or PCF
4. Reopen clamp
5. Stabilization
6. Injury of artery with forceps
7. 10-min. of observation
8. CFR or PCF=stop experiment (artery thrombosed)
9. Repeat injury and stenosis protocol
10. CFR or PCF=stop (not thrombosed)

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**Figure 1.** Schematic diagram of injury/stenosis procedure.
flow to zero followed by a sudden spontaneous return of flow to the baseline level. PCF was characterized by a gradual reduction in flow velocity, but, unlike CFR, there is no spontaneous return of blood flow. If neither CFR nor PCF occurred, the Goldblatt clamp was released after another 20-second occlusion to confirm that reactive hyperemia had been blocked throughout the period of observation.

After the stenosis was removed by opening the Goldblatt clamp and blood flow velocity stabilized, the carotid artery was injured in the area where the clamp was applied. Injury was induced by three occlusions of the artery with spring-loaded forceps (Castroviejo Needle Holder, J. Sklar, Inc.). After 10 minutes of observation, the Goldblatt clamp was closed to block reactive hyperemia. If CFR or PCF occurred before or after the clamp was closed, flow velocity was monitored for 20 minutes and the experiment was stopped for that artery. If, within 30 minutes, neither CFR or PCF was seen, the clamp was reopened, and after stabilization of flow velocity, the pinch injury was repeated. If flow velocity remained unchanged, the Goldblatt clamp was tightened a third time for a final 30-minute period of observation. The results were expressed as the frequency of carotid arteries per treatment group that developed PCF, CFR, or both. At the end of the experiment, the artery segment was ligated, removed, and fixed with 4% paraformaldehyde.

Tissue Evaluation of Injury and Atherosclerosis

Transverse sections of the injury/stenosis site were examined with light microscopy for the amount of arterial injury and presence of thrombus. The cross sections were divided into eight equal segments and analyzed for the presence or absence of smooth muscle damage, medial hemorrhage, disruption of the internal elastic lamina, and mural thrombus formation. Smooth muscle damage was defined as pale-staining cytoplasm and contracted and pyknotic nuclei. The mural thrombus was defined as occlusive arterial thrombosis. Smooth muscle damage or medial hemorrhage.

Arterial cross sections stained with Verhoeff–van Gieson’s stain were projected onto a digitizer and analyzed morphometrically (Imagepro, Media Cybernetics). Morphometric measurements included luminal area, intimal area, and medial area. The amount of injury was expressed as the percentage of segments with smooth muscle damage or medial hemorrhage.

Arterial cross sections stained with Verhofe-van Giesen’s stain were projected onto a digitizer and analyzed morphometrically (Imagepro, Media Cybernetics). Morphometric measurements included luminal area, intimal area, and medial area. The amount of atherosclerosis was expressed in two ways: the intimal area as a percentage of the area within the internal elastic lamina (percentage of luminal narrowing) or the intimal area as a percentage of the medial area. All sections were examined and measured blinded, without the observer knowing the treatment group.

Immunohistochemistry

Representative sections were evaluated from all four groups for the presence of vWF. Sections were taken from the injury/stenosis site from 16 premenopausal monkeys (8 receiving OCs and 8 not receiving OCs) and 23 postmenopausal monkeys (12 receiving HRT and 11 not receiving HRT). These included sections from 18 monkeys that had occlusive arterial thrombosis and 21 that had no alterations in flow velocity. Sections were deparaffinized in xylene, rehydrated in graded alcohol, and immunostained. Primary antibodies were localized with appropriate biotinylated secondary antibody and tertiary avidin-biotin complex stain (Vector Laboratories). Control slides were stained without a primary antibody. Sections were counterstained with Mayer’s hematoxylin and examined by light microscopy.

TABLE 1. Incidence of PCF and CFR After Injury and Stenosis

<table>
<thead>
<tr>
<th>Group</th>
<th>Total No.</th>
<th>No. With PCF</th>
<th>No. With CFR</th>
<th>No. With No PCF or CFR</th>
<th>Incidence of Arterial Thrombosis (PCF and CFR), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>OC</td>
<td>15</td>
<td>2</td>
<td>1*</td>
<td>12</td>
<td>20 (3 of 15)†</td>
</tr>
<tr>
<td>No OC</td>
<td>13</td>
<td>4</td>
<td>3</td>
<td>6</td>
<td>54 (7 of 13)</td>
</tr>
<tr>
<td>HRT</td>
<td>24</td>
<td>7</td>
<td>6</td>
<td>11</td>
<td>54 (13 of 24)</td>
</tr>
<tr>
<td>No HRT</td>
<td>15</td>
<td>2</td>
<td>4</td>
<td>9</td>
<td>40 (6 of 15)</td>
</tr>
</tbody>
</table>

*This one was equivocal.
†14.2% (2 of 14) if the equivocal one is excluded.

Statistical Analysis

All measured variables were reported as mean±SEM. Comparisons between means for all groups were made by an ANOVA and Student’s t test with a confidence level of 95%. The Wilcoxon signed-rank test was used to compare paired data. Nonparametric analyses were made with the Kruskal-Wallis and the Mann-Whitney U tests with a confidence level of 95%. The χ² test for a contingency table and Fisher’s exact test were used to compare frequency data and the Spearman’s rank correlation to compare the linear relationships with a 95% confidence level. Statistical analyses were performed with a computer program (StatMost, Dataxiom Software, Inc).

Results

Arterial Thrombosis

The incidence of arterial thrombosis after injury and stenosis is given in Table 1 and Fig 2. In the group receiving OCs, 2 had PCF and 1 had equivocal changes in flow that were difficult to interpret as CFR. There was no CFR or PCF in the other 12 monkeys in this group. The incidence of arterial thrombosis was 14.2% (2 of 14) if the one with equivocal flow reductions is excluded or 20% (3 of 15) if it is scored as CFR. In the premenopausal group not receiving OCs, 4 monkeys had PCF (one of these was preceded by CFR) and 3 had CFR. No CFR or PCF was observed in the other 6 monkeys in this group. Thus, the incidence of thrombosis was 53.9% (7 of 13) for this group. In the group of postmenopausal monkeys receiving HRT, 7 had PCF (one of which was preceded by CFR), 6 had CFR, and 11 had no CFR or PCF. The incidence of arterial thrombosis was 54% (13/24). In the group of postmenopausal monkeys not receiving HRT, 2 had PCF, 4 had CFR, and 9 had no CFR or PCF, for an arterial thrombosis incidence of 40% (6 of 15). The group of monkeys receiving OCs had a lower incidence of arterial thrombosis than the group of monkeys receiving HRT (P=0.03).

Figure 2. Incidence of arterial thrombosis after injury and stenosis.

One monkey in OC group had equivocal cyclic flow reductions. If this one is excluded, the difference in incidence of thrombosis is significantly lower in the group of monkeys receiving OCs than in the premenopausal monkeys not receiving OCs (P=0.03).
thrombosis than the premenopausal group not receiving OCs. This rate was significant when 14.2% was compared with 53.9% ($P=.033$, Fisher’s exact test). If the equivocal one is included, the value is $P=.059$. There was no significant difference in the incidence of arterial thrombosis in the treated and untreated postmenopausal groups ($P=.18$, Fisher’s exact test). When all four groups were compared using 14.2% for the OC group, the difference among groups was nearly significant ($P=.084$, $\chi^2$ test for a contingency table). If 20% was used, there was no significant difference among the groups ($P=.163$, $\chi^2$ test for a contingency table).

**Evaluation of Tissue Injury and Atherosclerosis**

The amount of injury to the vessel wall as expressed by the percentage of segments that had medial injury was similar among groups ($P=.97$, ANOVA). The amount of damage to the media was 85.0±29% for the premenopausal group receiving OCs and 81.3±32% for the group not receiving OCs. The postmenopausal group receiving HRT had 85.5±26% medial damage, and the group not receiving HRT had 85.3±22%.

The amount of atherosclerosis in the carotid arteries of the premenopausal and postmenopausal groups is given in Fig 3 as the percentage of luminal narrowing. The difference among groups was nearly significant ($P=.053$, Kruskal-Wallis test). The group of monkeys receiving OCs had less atherosclerosis than the control group and the postmenopausal groups. Pairwise comparisons revealed a nearly significant difference between the OC group and the premenopausal group not receiving OCs ($P=.069$, Mann-Whitney $U$ test). There was no significant difference between the treated and untreated postmenopausal groups ($P=.677$, Mann-Whitney $U$ test). Similar results were seen when atherosclerosis was expressed as a percentage of medial area.

To evaluate the relationship of thrombosis to the amount of atherosclerosis, each group was divided into those that formed occlusive thrombi and those that did not (Fig 4). The difference among these groups was nearly significant ($P=.055$, Kruskal-Wallis test). Within the HRT group, those that formed occlusive thrombi had a significantly greater amount of atherosclerosis than those in this group that did not ($P=.016$, Mann-Whitney $U$ test), and the difference in atherosclerosis was nearly statistically significant within the OC group ($P=.079$, Mann-Whitney $U$ test). The OC group had both the lowest incidence of thrombosis and the least amount of atherosclerosis.

**Combining all groups, the amount of atherosclerosis (luminal narrowing) was greater in those vessels that formed occlusive thrombi (37.6%) than in those that did not (16.2%) ($P=.011$, Mann-Whitney $U$ test). In addition, the incidence of thrombosis, 60.7% (17 of 28) for those animals with the most atherosclerosis (upper half), was significantly greater than the incidence of 29% (8 of 28) for those with the least atherosclerosis (lower half) ($P=.01$, Fisher’s exact test). With all groups combined, the occurrence of thrombosis (graded as 0=no flow alterations, 1=CFR, and 2=PCF) was significantly correlated to the amount of atherosclerosis ($r=.338$, $P=.01$, Spearman’s rank correlation).

**Immunohistochemistry**

A section of an injured stenosed vessel immunostained for vWF is shown in Fig 5. In normal vessels, vWF is confined to the endothelium and subendothelium. In the stenosed and injured vessels, the luminal surfaces that were denuded of endothelium often lacked staining for vWF. Staining for vWF was usually associated with areas that contained platelets. The damaged media accumulated vWF only in areas of medial hemorrhage that were platelet rich. Thrombi had a mixed staining pattern for vWF, with areas rich in red blood cells lacking vWF staining and areas rich in platelets staining heavily. Staining for vWF was not associated...
with uninjured plaque. There were no distinctive differences in the staining pattern among the groups.

Coagulation Parameters

The plasma concentrations of vWF antigen for the premenopausal and postmenopausal groups are given in Table 2 for 26 to 28 months after the atherogenic diet was begun. At this time point, the vWF antigen was significantly different among groups (P<.002, Kruskal-Wallis test). The OC group had lower vWF concentrations than the premenopausal group not receiving OCs (P=.018, Mann-Whitney U test). Although the HRT group had lower vWF concentrations than the untreated postmenopausal group, this difference was not significant (P=.21, Mann-Whitney U test). When all groups were combined, plasma vWF concentrations were not correlated with the amount of atherosclerosis (r = -.06, P = .67, Spearman’s rank correlation). High concentrations of plasma vWF were not associated with arterial thrombosis: the plasma vWF concentrations were similar for those that formed occlusive thrombi and those that did not (103.6 versus 102.2%, P = .62, Mann-Whitney U test).

There were no differences in plasma Lp(a) concentrations among the groups (P = .67, Kruskal-Wallis test) and no changes with 1 year of atherogenic diet feeding (P = .30, t test; P = .47, Mann-Whitney U test) (Table 2). There were no significant correlations between Lp(a) concentration and atherosclerosis (r = .168, P = .216, Spearman’s rank correlation), nor was there a difference in Lp(a) levels between monkeys that formed occlusive thrombi and those that did not (36.3 versus 36.0 mg/dL, P = .97, Mann-Whitney U test).

Platelet aggregation to ADP was observed in all monkeys. There was a significant difference among the groups at the last time point in the mean slope of the aggregation curve (P < .01, Kruskal-Wallis test) (Table 2). No differences in the mean slope of the aggregation curves were observed between the premenopausal monkeys receiving OCs and the premenopausal group not receiving OCs (P = 1.00, Mann-Whitney U test). No differences were observed between the treated and untreated postmenopausal groups (P = .22, Mann-Whitney U test). There was a significant difference in the slope of the aggregation curve between the premenopausal and postmenopausal animals (3.8 versus 6.2, P = .00, Mann-Whitney U test). There was no significant difference in platelet counts among groups at the final time point (P = .187, Kruskal-Wallis test) (Table 2) and no significant differences in slope or platelet count between those that formed occlusive thrombi and those that did not (P = .62 and P = .25, respectively, Mann-Whitney U test).

There were no differences among the groups for protein C plasma concentrations (P = .33, Kruskal-Wallis test) (Table 2). There was no significant correlation of protein C to atherosclerosis (P = .22, Spearman’s rank correlation) and no difference in protein C in animals that formed occlusive thrombi versus those that did not (111.7 versus 112.8% standard pool, P = .55, Mann-Whitney U test). The clotting times were within the normal range for all monkeys.

Plasma Hormone Concentrations

Plasma concentrations of estradiol in the OC and HRT groups of animals were 356±23 and 320±30 pmol/L, respectively. Plasma medroxyprogesterone concentrations in the HRT-treated monkeys were 61±15 pmol/L. Plasma levonorgestrel concentrations were 55±10 pmol/L.

Discussion

The major finding of this study was that current OC and HRT regimens given to nonhuman primates do not increase the susceptibility of the artery wall to develop an injury-induced thrombus in this model. In fact, arteries from OC-treated animals had a reduced incidence of arterial thrombosis after injury.

OCs and Thrombosis

Since the introduction of OCs, there has been controversy regarding their use and the association with thrombotic cardiovascular events. Several retrospective and prospective studies have linked OC use to increased thrombotic complications.21–23 Other studies have disagreed, indicating that the increased risk could be explained by factors such as smoking.8–9 As low-dose OCs became widely used, studies indicated a lower thrombotic risk.21–23 It is now generally accepted that the use of low-dose OCs decreases the thrombotic risk compared with higher-dose compounds. This finding is particularly true for deep vein thrombosis22,24 but may not be as well defined for arterial events such as stroke and myocardial infarction. Studies have shown that both low- and high-dose OCs increase levels of several coagulation factors (II, VII, VIII, IX, and X; fibrinogen; and soluble fibrin). These studies have been previously reviewed.18 It has been suggested that the increase in coagulation factors may produce a “hypercoagulation state.” However, there is no direct evidence that this results in thrombosis in vivo. Any changes in coagulation may be balanced by changes in fibrinolytic activity. OCs have been reported to

**TABLE 2. Coagulation Parameters**

<table>
<thead>
<tr>
<th></th>
<th>OC (n=15)</th>
<th>No OC (n=13)</th>
<th>HRT (n=24)</th>
<th>No HRT (n=15)</th>
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<tbody>
<tr>
<td>% von Willebrand factor antigen* 26-28 mo after diet</td>
<td>98.9±4.2†</td>
<td>114.2±2.8</td>
<td>99.0±1.5</td>
<td>103.0±2.0</td>
</tr>
<tr>
<td>% Protein C activity* 26-28 mo after diet</td>
<td>110.7±2.7</td>
<td>112.2±3.1</td>
<td>117.0±3.2†</td>
<td>110.4±3.3†</td>
</tr>
<tr>
<td>Platelet count, ×1000/mm 26-28 mo after diet</td>
<td>351±25</td>
<td>365±30</td>
<td>432±27</td>
<td>351±24</td>
</tr>
<tr>
<td>Slope of platelet aggregation curve 26-28 mo after diet</td>
<td>3.85±0.26</td>
<td>3.75±0.30</td>
<td>6.51±0.33</td>
<td>5.76±0.26</td>
</tr>
<tr>
<td>Lp(a) before diet, mg/dL</td>
<td>43.3±6.9</td>
<td>44.5±7.5</td>
<td>36.1±3.8</td>
<td>37.2±5.8</td>
</tr>
<tr>
<td>Lp(a) 1 year after diet, mg/dL</td>
<td>33.1±5.6</td>
<td>33.9±5.8</td>
<td>40.3±4.3</td>
<td>32.9±4.2</td>
</tr>
</tbody>
</table>

*Expressed as a percent of a standard plasma pool.
†Mean±SEM.
‡n=9.
increase factor XII–dependent fibrinolysis. In addition, if such a hypercoagulable state resulted in vascular thrombosis, then markers of in vivo clotting activity would be expected to increase. However, OCs have not been shown to increase thrombin–antithrombin III complexes or prothrombin 1 and 2, indicators of elevated levels of factor Xa generation.

This study focused on arterial thrombotic effects of OCs and HRT rather than coagulation parameters or venous thrombosis. In this model, OC administration did not increase the incidence of arterial thrombosis and, in fact, may have lowered the incidence.

When all groups were considered, those with the most atherosclerosis had the highest incidence of arterial thrombosis. The OC group had both the lowest incidence of thrombosis and the least amount of atherosclerosis. The reduced atherosclerosis seen in the OC group could indicate a slowing of atherogenesis and a more stable plaque, resulting in less thrombosis. A previous study examining the effects of contraceptives on atherosclerosis showed that OCs of similar formulation, although not identical to those in this present study, were not associated with increased coronary plaque size; in fact, at the intermediate total cholesterol and HDL cholesterol levels, these OCs were associated with decreased plaque size. In that study, it was suggested that the ethinyl estradiol in the OCs neutralized the atherogenic influence of decreased HDL caused by the contraceptive progestin levonorgestrel through estrogenic influences on cellular proliferation and metabolism in arterial tissue.

**HRT and Thrombosis**

In this study, HRT had no apparent effect on the incidence of arterial thrombosis in the postmenopausal monkeys. Much of the concern for HRT and thrombosis comes from the use of OCs. The studies examining HRT and thrombosis are limited and show no evidence of increased thromboembolism in women taking HRT. Platelet adhesion to fibronectin, collagen I, and collagen III has been shown to be increased in women taking OCs, but women receiving HRT had platelet adhesion similar to that of premenopausal women, postmenopausal women not receiving HRT, and men. Recently, it has been shown that HRT does not alter the plasma levels of prothrombin fragment 1 and 2 or thrombin-antithrombin III complex, indicating that HRT does not increase in vivo clotting activity. In addition, it has been shown that transdermal estradiol does not alter platelet numbers, plasma concentrations of a number of coagulation factors, or fibrinolytic activity.

HRT is associated with a reduction of coronary heart disease partially because of its beneficial effects on lipoproteins. In this study, the amount of atherosclerosis in the injured/stenosed vessels and incidence of thrombosis were similar in both postmenopausal groups. Thus, the beneficial effects of HRT in postmenopausal women may involve mechanisms not detectable with this model.

**Coagulation Parameters**

This study did not attempt to measure all the coagulation factors that have been shown to change with estrogen and/or progestin intake. Two parameters that have previously been shown to be important in this injury/stenosis model are vWF and Lp(a). In this model, the absence of vWF prevents arterial thrombosis, whereas high levels of Lp(a) have been shown to be associated with an increase in arterial thrombosis. In this study, neither the plasma concentrations of vWF nor those of Lp(a) were associated with occlusive arterial thrombosis. In the previous studies, vWF was absent, whereas in this study, even the lowest levels would be sufficient to support arterial thrombosis. The formation of neointima during atherogenesis has been associated with a local increase in vWF in the vessel wall. VWF was clearly present in the damaged arterial wall and most likely serves as an adhesive protein to anchor the forming thrombus. The concentration of vWF was lower in the OC group. Although plasma vWF concentrations increase during pregnancy, it is unclear why they should be lower in the OC group in this study.

In this study, high plasma concentrations of Lp(a) were not associated with occlusive arterial thrombi. The previous study examining the effect of plasma Lp(a) was done in the absence of atherosclerosis, which seems to be an important contributor to the arterial thrombotic process. In addition, other studies have shown a decrease in Lp(a) with HRT and a correlation of Lp(a) to the amount of atherosclerosis. Neither of these was observed in this study. This finding could reflect that the last Lp(a) measurement was done at 1-year after diet feeding rather than at the termination of the study.

Studies looking at platelet function and hormonal status have had varied results. HRT has been associated with decreased platelet aggregation, and OCs have been associated with increased platelet adhesion. This study demonstrated an increased slope of the aggregation curve in the postmenopausal monkeys and an increase in platelet count in the HRT group, again showing an effect of hormonal status on platelets. However, these changes did not appear to affect arterial thrombosis.

Arterial thrombosis is dependent on multiple factors influenced by various components, including the vessel wall, coagulation factors, platelets, and the fibrinolytic system. Therefore, it is unlikely that any one factor alone in the complicated setting of atherosclerosis will account for all changes in arterial thrombosis.

**Relationship of OCs, HRT, and Coronary Heart Disease**

Coronary heart disease results when myocardial blood flow is reduced by occlusion of the coronary arteries. This condition can occur when atherosclerotic plaque grows into the lumen and creates a physical blockage to blood flow. Atherosclerotic arteries are prone to spasm, which can also reduce coronary blood flow. Finally, a thrombus can form in a segment of a coronary artery, creating a physical blockage of the artery. The pathogenesis of coronary artery thrombosis is unclear but may be related to areas of atherosclerotic plaque that rupture, exposing underlying thrombogenic material to circulating blood.

There is ample evidence that estrogens reduce the risk of coronary heart disease, possibly by inhibiting the progression of atherosclerotic plaque or by protecting atherosclerotic arteries against vasospasm. Although progestins may ameliorate some of these beneficial effects of estrogens on atherogenesis and vasospasm, the net result seems to be that exogenously administered hormones at new lower doses reduces the risk of coronary heart disease in women.
The effect of exogenously administered hormones on risk of thrombosis has been unclear. It seems logical that these hormones must not have an overwhelmingly harmful effect on thrombosis risk or they would be associated with a more decisive increased risk of coronary heart disease. The results of the present experiment suggest that these hormones do not increase the risk of thrombosis once the plaque is disrupted. The present study does not address, however, whether or not exogenously administered hormones reduce the risk of the plaque rupturing.

This study was not designed to examine the mechanism by which OCs or HRT regimens affect thrombosis. Circulating coagulation factors measured in this experiment were not greatly affected by treatment or associated with the formation of occlusive arterial thrombi. The amount of atherosclerotic plaque seemed to play an important role in regulating the incidence of thrombosis. If exogenously administered sex hormones do reduce thrombosis risk, they may do so by affecting atherogenesis or having other effects on the artery wall that directly or indirectly act on thrombotic/thrombolysis regulation.

**Cynomolgus Monkey Model**

Cynomolgus monkey females were used because they have reproductive characteristics similar to those of women. These include a 28-day menstrual cycle, the occurrence of natural menopause, and cyclic changes in plasma concentrations of estradiol, progesterone, follicle-stimulating hormone, and luteinizing hormone that are similar both qualitatively and quantitatively to those of women. Like premenopausal women, premenopausal female cynomolgus monkeys have significantly higher plasma concentrations of HDL cholesterol than their male counterparts, and sex differences in the extent of coronary artery atherosclerosis are like those of human beings. Delivery of OCs in an experimental diet based on caloric requirements and body weight has proved effective in previous studies with female cynomolgus monkeys in preventing ovulation as determined by menstrual cycles and plasma hormonal concentrations. Postmenopausal cynomolgus monkeys, like women, have reduced plasma concentrations of HDL cholesterol and increased amounts of coronary atherosclerosis compared with premenopausal females fed the same atherosclerotic diet for the same length of time. Thus, both premenopausal and postmenopausal (ovariectomized) female cynomolgus monkeys share with women many of the same risk factors for the development of coronary artery disease. Furthermore, estrogen replacement therapy inhibits progression of coronary artery atherosclerosis, suggesting that, like postmenopausal women, ovariectomized cynomolgus females respond similarly to hormone treatment.

In this cynomolgus monkey model, a combination of arterial stenosis and injury was used to induce occlusive arterial thrombus formation measured as blood flow reductions. It has been shown in pigs that a combination of both injury and stenosis is necessary to induce occlusive arterial thrombosis. Evidence indicates that reductions in blood flow are due to the interactions of platelets with the vessel wall and with each other, leading to vascular occlusion. The technique, developed originally in dogs, has been used in other species, including monkeys. Although this model does not require the presence of atherosclerotic plaque and does not directly mimic what happens during plaque rupture, it, like other injury thrombosis models, causes intimal injury and exposes subintimal components to circulating blood in a setting of altered blood flow.

**Experimental Considerations**

The present report examines only one section of the carotid artery at the injury/stenosis site and may not reflect the overall amount of atherosclerosis in these monkeys. When all animals were considered, there was a significant correlation of atherosclerosis and thrombosis. This finding is not necessarily surprising, because arterial thrombosis is usually associated with the rupture of plaque. Under the experimental conditions of this study, external injury to the vessel wall and stenosis were used to initiate the thrombotic events. Thus, interpretations about atherosclerosis and thrombosis in this study should be done cautiously, and conclusions about plaque stability cannot be determined.

The lower incidence of arterial thrombosis in the OC group should also be interpreted cautiously, because the conditions of injury and stenosis were chosen to enhance thrombotic effects. Generally, OCs are taken by young women who have limited arterial disease and are at low risk of arterial thrombosis anyway. It was originally anticipated from previous studies with monkeys and other species that the overall incidence of flow reductions would be higher and that qualitative differences in flow reductions (CFR versus PCF) may be observed. Qualitative differences were not observed, and the overall incidence of thrombosis in this study allowed for detection of differences between groups of \( \approx 25\% \) at a confidence level of 95%.

**Conclusions**

It is generally thought that administration of exogenous sex hormones (whether as OCs or HRT) increases the risk of thrombotic disease. Much of the concern is founded in much higher doses of estrogens and progestins prescribed in the 1960s and 1970s. Additionally, risk of venous thrombosis and arterial thrombosis often were not differentiated. Concern about the thrombotic effects of sex hormones has promoted the concept of stopping all forms of exogenous estrogen/progestin therapies in women at increased risk of thrombosis. The actual risk of the newer OC and HRT regimens on arterial thrombosis has yet to be delineated. Therefore, the goal of the present study was to use a well-established nonhuman primate model of atherosclerosis to examine the effects of present day OC and HRT regimens on incidence of arterial thrombosis after arterial injury. Results indicate that neither triphasic OCs nor HRT (CEE plus MPA) increases the risk of arterial thrombosis and that arterial thrombosis may be particularly dependent on the amount of atherosclerosis present. These data may provide some reassurance that current OC and HRT regimens do not adversely affect the risk of arterial thrombosis in women.

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**References**


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