Oral Contraceptive Treatment Decreases Arterial Low Density Lipoprotein Degradation in Female Cynomolagus Monkeys

Janice D. Wagner, Michael R. Adams, Dawn C. Schwenke, and Thomas B. Clarkson

The effect of oral contraceptive therapy on early events in atherogenesis was studied in female cynomolgus monkeys. After a 1-month dietary challenge, monkeys were randomized into three groups stratified by total plasma cholesterol and high density lipoprotein cholesterol concentrations. The monkeys were then fed a cholesterol-containing diet for 16 weeks. This relatively short period ensured that studies were done before any treatment-induced differences in arterial morphology occurred. Monkeys were treated with either diet alone (control group), with the addition of a monophasic oral contraceptive (equivalent to a human dose of 50 μg ethinyl estradiol and 500 μg norgestrel per day), or with a triphasic oral contraceptive (equivalent to a human dose of 30–40 μg ethinyl estradiol and 50–125 μg levonorgestrel per day). Twenty-four hours before necropsy, low density lipoproteins (LDLs) labeled with 131I and LDLs labeled with the residualizing label [125]I-tyramine cellobiose were injected into the animals. The arterial LDL degradation rate, amount of undegraded LDLs, and total LDL accumulation were then determined. Although there were regional differences in LDL metabolism, both treatments decreased the rate of LDL degradation and LDL accumulation in the coronary arteries and other arterial sites. Treatment also resulted in significantly lower LDL molecular weights. Despite a trend toward a more atherogenic lipid profile (decreased high density lipoprotein cholesterol and increased total plasma/high density lipoprotein cholesterol ratio), oral contraceptive treatment may inhibit atherogenesis by decreasing arterial LDL degradation. (Circulation Research 1993;72:1300–1307)

Key Words: atherosclerosis • cynomolgus monkeys • estrogen • low density lipoprotein metabolism • oral contraceptives • females

There continues to be concern that oral contraceptive use causes an increased risk of atherosclerosis-related coronary heart disease. This concern is based almost exclusively on the observation that some oral contraceptives have theoretically adverse effects on plasma lipoproteins; e.g., they increase plasma concentrations of low density lipoprotein (LDL) cholesterol and decrease plasma concentrations of high density lipoprotein cholesterol (HDL). However, there is no convincing evidence that oral contraceptives promote the progression of atherosclerosis. Although there is a well-known increase in the risk of coronary heart disease associated with oral contraceptive use as currently formulated, the increase is statistically much lower than pregnancy-associated morbidity and mortality risks and disproportionate to the effects on plasma lipoproteins. In addition, current evidence indicates that this increased risk is largely confined to users of older high-dose formulations who smoke. Also, the increased risk disappears after oral contraceptive use ceases, suggesting that a nonatherogenic mechanism such as thrombosis or vasospasm is the real cause of the increased risk. Furthermore, some studies actually suggested a decreased risk among past users of oral contraceptives, whereas none has provided compelling evidence for an increased risk of coronary heart disease.

Experimental evidence from our laboratory supports the hypothesis that combination oral contraceptives inhibit atherosclerosis, despite theoretically atherogenic changes in plasma lipoprotein profiles. We hypothesize that this is due to direct inhibitory effects of ethinyl estradiol, a potent estrogen, on atherogenesis. In support of this hypothesis is our finding that endogenous hyperestrogenism (i.e., pregnancy) inhibits atherosclerosis progression in ovariectomized monkeys. Furthermore, physiological estrogen replacement therapy, with or without added progesterone, inhibits atherosclerosis progression in ovariectomized monkeys. Both effects occur independent of changes in plasma lipoproteins. How this hypothesized direct beneficial effect of exogenous estrogen may be mediated remains unclear. We report here evidence that administration of combination oral
contraceptives to monkeys fed an atherogenic diet results in markedly decreased arterial uptake and degradation of plasma LDL, an effect that may, at least in part, explain an inhibitory effect of exogenous estrogen on atherosclerosis progression.

**Materials and Methods**

**Animal Study**

Twenty-four adult female monkeys (*Macaca fascicularis*) were imported directly from CV Primates, Indonesia, and quarantined for 3 months, during which time they ate monkey chow (High Protein Monkey Chow, Ralston Purina Co., St. Louis, Mo.). The monkeys were then fed a moderately atherogenic diet containing 0.06 mg cholesterol/kJ for a 1-month dietary challenge to stratify the animals into three groups with similar total plasma cholesterol (TPC) and HDL concentrations. During the challenge period, the animals were fed monkey chow until the treatment part of the study began to allow their plasma cholesterol concentrations to return to normal. One monkey from each group started the treatment part of the study every week for 8 weeks (after a minimum postchallenge period of 2 weeks). During the 16-week experimental period, monkeys were fed the same atherogenic diet as during the challenge period with 1) no oral contraceptive (control group), 2) the atherogenic diet plus a monophasic oral contraceptive (MOC) (Ovral, Wyeth-Ayerst, Philadelphia, Pa.) added to the diet, equivalent to a human dose of 50 μg ethinyl estradiol and 500 μg norgestrel per day, or 3) the atherogenic diet plus a triphasic oral contraceptive (TOC) (Triphasil, Wyeth-Ayerst) added to the diet. The TOC dose was equivalent to a human dose varying from 30 to 40 μg ethinyl estradiol and from 50 to 125 μg levonorgestrel per day to simulate the varying estrogen and progesterone levels of the menstrual cycle. As in women taking oral contraceptives, the doses were given for 21 days; a placebo was administered to the monkeys for the remaining 7 days of the month. Oral contraceptive dosages were calculated on a caloric basis to approximate those for women consuming 7,560 kJ and one oral contraceptive pill per day.

All procedures involving animals were conducted in compliance with state and federal laws, standards of the Department of Health and Human Services, and guidelines established by the Institutional Animal Care and Use Committee. Femoral catheterizations were done while the animals were anesthetized with ketamine hydrochloride (10 mg/kg i.m.) and butorphanol tartrate (0.05 mg/kg i.m.), and blood sampling was done while the animals were sedated with ketamine hydrochloride (15 mg/kg i.m.).

**Plasma Lipids and Lipoproteins**

Blood samples were collected into tubes containing EDTA (final concentration, 1 mg/ml) after the animals were fasted overnight. TPC and HDL were determined once during the quarantine period, at weeks 3 and 4 of the dietary challenge, and at weeks 3, 7, 11, 15, and 16 of the experimental period. High density lipoprotein (HDL) and plasma triglyceride concentrations were determined at weeks 3, 7, 11, and 15. LDL cholesterol was estimated as the difference between TPC and HDL concentrations.

Analyses for TPC, triglycerides, and HDL are in full standardization with the Centers for Disease Control–National Heart, Lung, and Blood Institute Lipid Standardization Program. Apo(protein) A-I, apo B, LDL molecular weight, and lipoprotein(a) [Lp(a)] concentrations were determined at weeks 3, 11, and 15. Lp(a) concentrations were measured by enzyme-linked immunosorbert assay using techniques similar to those for apo A-I. Polyclonal anti–rhescus monkey apo(a) antibody was used as the capture antibody, and polyclonal anti–cynomolgus monkey apo B100 antibody conjugated to horseradish peroxidase was used for detection. The assay was standardized against cynomolgus monkey Lp(a).

**Studies of LDL Metabolism**

LDL particles for labeling and reinjection were isolated from pooled plasma obtained from 15 female monkeys fed the same atherogenic diet. Blood was collected in tubes containing Na2-EDTA, aprotinin, and d-phenylalanyl-1-prolylarginine chloromethylketone at final concentrations of 1 mg/ml, 25 kallikrein inhibitory units/ml, and 1 μM, respectively. Phenylmethylsulfonyl fluoride was added to isolated plasma at a final concentration of 0.5 mM. The LDL (1.020–1.063 g/ml) was isolated by differential ultracentrifugation followed by exhaustive dialysis against buffer (0.9% NaCl, 0.01% EDTA, pH 7.4). LDL protein was determined using bovine serum albumin as a standard. One aliquot of the LDL preparation (10–20 mg) was coupled to [125]tyraine cellobiose (TC)\(^*\)\(^{19,20}\) to a second aliquot (10–20 mg) of each LDL preparation was labeled with \(^{131}\)I using 1,3,4,6-tetrachloro-3,6-diphenylglycoluril (lodogen) as described. Specific activities averaged 744±187 and 194±47 cpm/ng protein for \(^{125}\)I–TC-LDL and \(^{131}\)I–LDL, respectively (mean±SEM) for eight preparations. For \(^{125}\)I–TC-LDL and \(^{131}\)I–LDL, trichloroacetic acid–soluble radioactivities (10% final concentration of trichloroacetic acid) averaged 1.5±0.4% and 3.9±0.71%, respectively, and radioactivities extractable in chloroform/methanol\(^{23}\) averaged 7.7±0.9% and 14.3±2.0%, respectively. Just before injection, the two aliquots of each LDL preparation were combined and sterilized by filtration (0.45-μm filter, Millipore Corp., Bedford, Mass.). LDL preparations were used within 1 week of labeling.

After the monkeys had consumed the atherogenic diet for 16 weeks, radiolabeled LDL preparations (5.60±0.84×10\(^6\) cpm \(^{125}\)I and 7.12±1.07×10\(^6\) cpm \(^{131}\)I) were injected through an indwelling femoral venous catheter 24 hours before necropsy. Subsequent blood samples were collected in tubes containing EDTA (final concentration, 1 mg/ml) from the arterial catheter at 5, 15, 30, and 60 minutes and 3, 7, 13, and 24 hours after injection to determine the plasma decay of labeled LDL. The fractional catabolic rate (FCR) of LDL by the whole body was calculated from coefficients and exponents determined by the biexponential equation fitted from the decay of protein-bound radioactivity in the plasma. \(^{24}\) [\(^{3}H\)]Thymidine (New England Nuclear, Boston) was also injected intravenously (0.5 mCi/kg body wt per injection) at three time points (17, 9, and 1 hour before necropsy) to label replicating endothelial cells.
Necropsy and Measurement of Atherosclerosis

After collecting the final (24-hour) blood sample, the animals were anesthetized with sodium pentobarbital (80 mg/kg body wt i.v.). The cardiovascular system was flushed via the left ventricle with 1 l lactated Ringer’s solution and then perfused with a modified Karnovsky’s solution at 100 mm Hg for an additional 15 minutes to provide adequate fixation for electron microscopy and radiolabeled LDL. The following arteries and their adventitia were removed: thoracic and abdominal aorta, common carotid arteries and carotid bifurcations, left main coronary artery, and left anterior descending and left circumflex coronary arteries (Figure 1).

All arterial samples were fixed in modified Karnovsky’s solution for an additional 24 hours. Arterial samples for histological study (Figure 1, segment A) were dehydrated, embedded in paraffin, and stained with Verhoeff–van Gieson stain. Sections were projected onto a screen, and the area occupied by intima was measured with a digitizer. The extent of atherosclerosis was expressed as the mean intimal area (in square millimeters). Segments for scanning electron microscopy (Figure 1, segment B) were prepared as described previously. Two hundred contiguous fields from each arterial segment (0.006 mm² each) were examined at a magnification of ×1,250. Endothelial cell turnover rates were expressed as the number of labeled nuclei per square millimeter of endothelial surface, and the number of adherent leukocytes was reported as the number of cells per square millimeter. LDL metabolism (¹²⁵I-TC accumulation, rates of LDL degradation, and calculated concentration of undegraded LDL) was evaluated in the arteries (Figure 1, segment C; segments ranged from approximately 10 to 200 mg). Radioactivities in all samples were corrected for overlap of the energy spectra of the two isotopes, for background radioactivity, and for isotopic decay. Samples were counted for approximately 30 minutes, giving a 2σ counting error of <1.0% for ¹²⁵I and <3.0% for ¹³¹I. Background was counted until a minimum of 10,000 counts was accumulated, resulting in a 2σ counting error of <2%.

Analysis of LDL Studies

Arterial samples were fixed in modified Karnovsky’s solution, which preserves products of ¹²⁵I-TC-LDL degradation as well as undegraded ¹²⁵I-TC-LDL and ¹³¹I-LDL. Thus, accumulation of radioactivity from ¹²⁵I-TC represents both undegraded LDL and products of LDL degradation. The arterial ¹³¹I-TC radioactivity (in counts per minute per gram) was normalized by the area under the curve of protein-bound ¹²⁵I-TC radioactivity in plasma during the metabolic experiment (in counts per minute per microliter) × hours) to express the arterial ¹²⁵I-TC radioactivity in a form (in microliters per gram per hour) independent of the plasma LDL concentration and amount of labeled LDL injected.

The rates of LDL degradation and the calculated concentration of undegraded LDL were determined as described previously. Since the [¹³¹I]iodotyrosine released during cellular degradation is leached from arteries during fixation in modified Karnovsky’s solution, the remaining ¹³¹I radioactivity represents undegraded LDL. The ¹²⁵I-TC representing LDL degraded by the artery can then be determined by subtracting the arterial ¹³¹I radioactivity from the total arterial ¹²⁵I-TC radioactivity, taking into account the relative activities of these two isotopes in plasma LDL at the time of necropsy.

Arterial LDL degradation was first calculated in fractional terms and expressed as a fraction of the plasma LDL pool degraded per hour per gram of artery (FCRartery). FCRartery was calculated as the product of the whole-body FCR determined from the plasma decay curve and the ratio of LDL degradation products per gram of artery to the LDL degraded by the whole body (dose injected multiplied by the fraction of the dose irreversibly degraded by the whole body).

Degradation of LDL in absolute terms (in micrograms LDL cholesterol per gram artery per hour) was calculated by multiplying the fractional rate of LDL degradation (FCRartery) by the total amount of LDL cholesterol in plasma, which was calculated as the product of the plasma LDL cholesterol concentration and the plasma volume.

The amount of arterial undegraded LDL (in micrograms LDL cholesterol per gram) was calculated as the ratio of ¹³¹I counts in the artery (in counts per minute per gram) to that in plasma (in counts per minute per milliliter) at the time of necropsy multiplied by the plasma LDL cholesterol concentration of individual animals.

Statistical Analysis

To reduce skewness and equalize group variances, data for arterial morphology were analyzed after
### Table 1. Baseline Measurements of Plasma Lipids, Lipoproteins, and Body Weight

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=7)</th>
<th>MOC (n=8)</th>
<th>TOC (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before dietary challenge</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPC (mg/dl)</td>
<td>135±9.1</td>
<td>131±11.2</td>
<td>119±8.4</td>
</tr>
<tr>
<td>HDLc (mg/dl)</td>
<td>52.3±4.9</td>
<td>52.9±2.7</td>
<td>47.5±4.1</td>
</tr>
<tr>
<td>TPC/HDLc ratio</td>
<td>2.7±0.2</td>
<td>2.5±0.2</td>
<td>2.6±0.2</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>83±6.6</td>
<td>78±10</td>
<td>72±6.3</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>2.82±0.15</td>
<td>2.94±0.17</td>
<td>2.78±0.10</td>
</tr>
<tr>
<td><strong>During dietary challenge</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPC (mg/dl)</td>
<td>346±27</td>
<td>301±37</td>
<td>303±33</td>
</tr>
<tr>
<td>HDLc (mg/dl)</td>
<td>45.0±5.0</td>
<td>44.5±5.0</td>
<td>45.7±6.2</td>
</tr>
<tr>
<td>TPC/HDLc ratio</td>
<td>8.8±1.7</td>
<td>8.1±1.9</td>
<td>8.1±1.7</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>301±30</td>
<td>257±40</td>
<td>257±36</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>2.88±0.13</td>
<td>2.96±0.15</td>
<td>2.95±0.14</td>
</tr>
</tbody>
</table>

Control, no oral contraceptive; MOC, monophasic oral contraceptive; TOC, triphasic oral contraceptive; TPC, total plasma cholesterol; HDLc, high density lipoprotein cholesterol; LDL, low density lipoprotein. Values are mean±SEM.

Square-root transformation, whereas data for LDL metabolism were analyzed after logarithmic transformation. Treatment groups were compared statistically by using analysis of variance (ANOVA) as well as repeated-measures ANOVA. Post hoc analyses were done by the method of Tukey when significant differences were found for individual treatment differences. Pearson product–moment correlations were used to assess the relation between dependent variables. In all cases, two-sided tests were used to assess statistical significance. Analyses were performed with the BMDP statistical package (programs 2v, 7d, and 8d, BMDP Statistical Software, Los Angeles). Means presented in the text are reported with SEM.

One animal in the control group died during the treatment period of causes unrelated to the experiment. Data from this animal were excluded from all analyses.

### Results

#### Plasma Lipoproteins

No significant differences in baseline lipid or lipoprotein concentrations or body weight measurements were found before or during dietary challenge (for all values, p>0.58; Table 1). The atherogenic diet induced in all groups moderate hypercholesterolemia that was not affected by oral contraceptive treatment (Table 2). Although there was a trend toward lower HDLc and increased triglyceride concentrations and an increased TPC/HDLc ratio with oral contraceptive treatment, these changes were not statistically significant. Oral contraceptive treatment also had no effect on plasma apo or Lp(a) concentrations (p<0.05). LDL molecular weight did not differ between MOC and TOC treatment groups but was significantly decreased compared with that of control animals (for MOC, p<0.05; for TOC, p<0.01). The whole-body FCR of LDL was not significantly affected by oral contraceptive treatment (p=0.50, Table 2).

#### Arterial LDL Metabolism

Arterial LDL metabolism was analyzed by repeated measures (across all arterial sites) as well as separately for each arterial site (Table 3). Repeated-measures analyses revealed significant treatment differences for absolute degradation rate and 125I-TC accumulation (p≤0.05) and borderline differences for fractional degradation rate and concentration of undegraded LDL (p≤0.10). The greatest effect of oral contraceptive treatment was on the rates of LDL degradation. MOC decreased the absolute and fractional rates of arterial LDL degradation by an average of 86% and 80%, respectively, whereas TOC decreased the absolute and fractional rates of arterial LDL degradation by an average of 50% and 60%, respectively. The greater decrease in the absolute versus fractional rates of degradation with MOC is due to the slightly lower plasma LDL cholesterol concentrations in MOC-treated animals. The concentration of undegraded LDL was decreased an average of 45% by MOC treatment and 22% by TOC treatment. The total accumulation of 125I-TC (representing both degraded and undegraded LDL) was decreased an average of 46% and 32% with MOC and TOC, respectively.

There were significant regional differences for all indexes of LDL metabolism (effect of site for all, p<0.01). Greater rates of LDL metabolism were found in the carotid bifurcation and thoracic aorta, moderate amounts were found in the coronary arteries, and lesser amounts were found in the common carotid arteries and abdominal aorta. Although there were regional differences in LDL metabolism, the effect of oral contraceptive treatment did not vary by arterial site (treatment×site interaction for all, p≥0.32).
### Table 3. Effect of Hormone Treatment on Indexes of Low Density Lipoprotein Metabolism

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=7)</th>
<th>MOC (n=8)</th>
<th>TOC (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute degradation (µg LDL cholesterol/g wet wt/hr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary arteries</td>
<td>1.73±0.92</td>
<td>0.30±0.16*</td>
<td>0.39±0.30*</td>
</tr>
<tr>
<td>Carotid bifurcation</td>
<td>2.12±0.57</td>
<td>0.30±0.08*</td>
<td>1.37±0.50</td>
</tr>
<tr>
<td>Carotid arteries</td>
<td>1.18±0.51</td>
<td>0.12±0.05</td>
<td>0.83±0.47</td>
</tr>
<tr>
<td>Thoracic aorta</td>
<td>2.44±0.81</td>
<td>0.33±0.12</td>
<td>1.16±0.56</td>
</tr>
<tr>
<td>Abdominal aorta</td>
<td>0.53±0.16</td>
<td>0.09±0.03*</td>
<td>0.22±0.11</td>
</tr>
</tbody>
</table>

Effect of treatment, p<0.05
Effect of site, p<0.01
Treatment by site interaction, p=0.59

### Table 4. Effect of Hormone Treatment on Morphological Indexes of Atherosclerosis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=7)</th>
<th>MOC (n=8)</th>
<th>TOC (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intimal area (mm²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary arteries</td>
<td>0.007±0.002</td>
<td>0.004±0.002</td>
<td>0.006±0.003</td>
</tr>
<tr>
<td>Thoracic aorta</td>
<td>0.028±0.007</td>
<td>0.006±0.002*</td>
<td>0.017±0.006</td>
</tr>
<tr>
<td>Abdominal aorta</td>
<td>0.020±0.006</td>
<td>0.012±0.003</td>
<td>0.011±0.004</td>
</tr>
<tr>
<td>Carotid arteries</td>
<td>0.003±0.002</td>
<td>0.002±0.001</td>
<td>0.005±0.002</td>
</tr>
</tbody>
</table>

Effect of treatment, p=0.21
Effect of site, p<0.0001
Treatment by site interaction, p=0.04

Endothelial cell turnover rate (cells/mm²)

- Coronary arteries: 1.27±0.52 vs. MOC: 1.25±0.57 vs. TOC: 0.77±0.31

Adherent cells (cells/mm²)

- Coronary arteries: 2.9±0.6 vs. MOC: 2.7±0.4 vs. TOC: 3.0±0.6

Analysis of the individual arterial sites showed that the absolute and fractional LDL degradation rates in coronary arteries were decreased by both MOC and TOC treatment (p<0.05, Table 3). In addition, the absolute and fractional LDL degradation rates were lower in the carotid bifurcation and abdominal aorta of monkeys treated with MOC. The concentration of undegraded LDL and ¹²⁵I-TC accumulation were significantly decreased (p<0.05 and p<0.01, respectively) in the carotid bifurcation because of MOC treatment. Post hoc analysis revealed no significant differences between MOC- and TOC-treated monkeys for any index of LDL metabolism.

**Morphological Analyses**

The effects of oral contraceptive treatment on intimal area, endothelial cell turnover, and adherent leukocytes are shown in Table 4. Repeated-measures analyses showed no overall treatment effect for intimal area (p>0.05). However, histological characteristics differed significantly among arterial sites (effect of site, p<0.0001), ranging from little to no intimal thickening in the coronary and common carotid arteries to varying amounts of foam cell accumulation in the thoracic and abdominal aorta. A significant treatment by site interaction was found. This was mainly due to the decreased intimal thickening seen in the thoracic aorta of the MOC group (p<0.05). There was no effect of oral contraceptive treatment on intimal area in the coronary arteries, common carotid arteries, or the abdominal aorta. Ultrastructural analyses of the coronary arteries indicated minimal endothelial injury, i.e., a low endothelial cell turnover rate and few adherent leukocytes with no effect of oral contraceptive treatment.
TABLE 5. Correlation Coefficients Between indexes of Arterial Low Density Lipoprotein Degradation and Total Plasma Cholesterol, Low Density Lipoprotein Molecular Weight, and Intimal Area

<table>
<thead>
<tr>
<th>Correlation coefficients</th>
<th>Treatment group</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Control (n=7)</td>
</tr>
<tr>
<td>TPC vs. LDL degradation</td>
<td></td>
</tr>
<tr>
<td>Coronary arteries</td>
<td>0.61</td>
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<tr>
<td>Carotid bifurcation</td>
<td>0.89‡</td>
</tr>
<tr>
<td>Carotid arteries</td>
<td>0.83†</td>
</tr>
<tr>
<td>Thoracic aorta</td>
<td>0.49</td>
</tr>
<tr>
<td>Abdominal aorta</td>
<td>0.56</td>
</tr>
<tr>
<td>LDL molecular weight vs. LDL degradation</td>
<td>0.67‡</td>
</tr>
<tr>
<td>Coronary arteries</td>
<td>0.70‡</td>
</tr>
<tr>
<td>Carotid arteries</td>
<td>0.69‡</td>
</tr>
<tr>
<td>Thoracic aorta</td>
<td>0.24</td>
</tr>
<tr>
<td>Abdominal aorta</td>
<td>0.91†</td>
</tr>
<tr>
<td>Arterial intimal area vs. LDL degradation</td>
<td>0.83*</td>
</tr>
<tr>
<td>Coronary arteries</td>
<td>0.87†</td>
</tr>
<tr>
<td>Carotid arteries</td>
<td>0.15</td>
</tr>
<tr>
<td>Thoracic aorta</td>
<td>0.72*</td>
</tr>
</tbody>
</table>

*p<0.05, †p<0.01, and ‡p<0.10 by Pearson product-moment correlation.

Control, no oral contraceptive; MOC, monophasic oral contraceptive; TOC, triphasic oral contraceptive; TPC, total plasma cholesterol; LDL, low density lipoprotein.

Discussion

The purpose of this study was to investigate mechanisms by which oral contraceptives may be protective against atherosclerosis (despite potentially adverse effects on plasma lipids and lipoproteins) and to determine whether two different oral contraceptives with different amounts of ethinyl estradiol and progestin differed in their effects on the early stages of atherogenesis. The results demonstrate that both oral contraceptive agents decreased arterial LDL metabolism similarly. Among the indexes of arterial LDL metabolism, oral contraceptives caused the largest percent decrease in the rate of LDL degradation (Table 3). These treatment-induced changes at the level of the arterial wall may explain the decreased extent of coronary artery atherosclerosis found in studies of animals treated with oral contraceptives in which advanced atherosclerosis was induced by long-term feeding of a cholesterol-containing diet.8

Although there was no statistical difference between the two treatment groups, there was a tendency toward a greater effect with MOC compared with TOC. For example, compared with the control group, overall arterial degradation was decreased by 86% in the MOC group compared with 50% in the TOC group. It is possible that this is due to the slightly greater amount of ethinyl estradiol in the MOC. However, both TOC and MOC decreased the LDL degradation rate and accumulation of LDL in the coronary arteries to a similar degree, suggesting that both oral contraceptives would decrease coronary artery atherosclerosis similarly.

The overall effects of oral contraceptives on plasma lipoproteins are dependent on both the dose and potency of the estrogen and progestin component.129 The oral contraceptives used in this study contained the identical estrogen, ethinyl estradiol, and the same active progestin, levonorgestrel (norgestrel is a 50:50 racemic mixture of the dextro [inactive component] and lev [active component] isomers). However, the doses of these compounds were quite different. The TOC contains 35% less ethinyl estradiol and 63% less levonorgestrel than the MOC. Despite the different amounts of estrogen and progestin in the MOC and TOC treatments, the changes seen in plasma lipids and lipoproteins were very similar (Table 2). Compared with no treatment, oral contraceptive treatment resulted in a trend toward lowered HDLC concentrations, increased triglyceride concentrations, and increased TPC/HDLC ratios. Also, both oral contraceptive treatments resulted in significantly lower LDL molecular weights. Although these changes in plasma lipids and lipoproteins are potentially more atherogenic, LDL particles with smaller molecular weights have been associated with the risk of atherosclerosis, and both oral contraceptive treatments may decrease the risk of atherosclerosis in women.
lessened coronary artery atherosclerosis in nonhuman primates.32,33 Consistent with studies in women,32 plasma Lp(a) concentrations were unaffected by oral contraceptive treatment. However, a significant correlation between Lp(a) and apo B concentrations was found in the current study, consistent with findings in human patients.33

The decreased LDL accumulation caused by hormone treatment in this study is consistent with previous studies.8,28 In surgically postmenopausal monkeys, hormone replacement therapy (17β-estradiol and progesterone) decreased the arterial LDL degradation rate and LDL accumulation while having no effect on plasma lipids and lipoproteins. As in this study, the percent decrease was greater for products of LDL degradation than for total arterial 3H-TDL.28 This finding is also consistent with a study of Hough and Zilversmit,34 in which the effects of estrogen treatment (using estradiol 17β-cypionate) on net arterial influx and hydrolysis of plasma cholesterol ester were determined in cholesterol-fed rabbits. Although there were no significant effects on plasma lipoprotein patterns, estrogen treatment resulted in decreased aortic atherosclerosis. Net cholesterol ester influx, which was positively correlated with the extent of atherosclerosis, was also decreased in estrogen-treated animals. In addition, the percentage of newly entering cholesterol ester hydrolyzed by the artery was reduced by estrogen treatment independent of the extent of atherosclerosis. Thus, in their study as in ours, estrogen seemed to inhibit the intracellular degradation and hydrolysis of lipoproteins.

In addition to sex hormone effects on arterial lipoprotein metabolism, there is also evidence that estrogen decreases arterial cholesterol accumulation. In studies by Haarbo et al,35 arterial cholesterol accumulation was reduced by one third in rabbits treated with 17β-estradiol, given either alone or in combination with the contraceptive progestins norethisterone and levonorgestrel. This effect was only partially explained by beneficial effects on plasma lipoproteins. Also, in a subsequent study, contraceptive progestins had no effect on cholesterol accumulation.36 This represents further evidence that estrogen is the hormonal component that affects arterial lipoprotein metabolism.

There was a small (but statistically significant) decrease in the LDL molecular weight in both TOC (p < 0.01) and MOC (p < 0.05) groups (Table 2). Whether these relatively small changes affect LDL receptor binding, the arterial metabolism of these particles, or both is unknown. However, in control monkeys, there was a correlation between LDL metabolism and LDL molecular weight (Table 5). A similar finding was noted in a previous study in which arterial LDL degradation correlated with LDL molecular weight in surgically postmenopausal monkeys treated with or without sex hormone replacement.28 Thus, subtle changes in LDL composition may be important modulators of LDL binding and/or metabolism in vivo as well as in vitro.37-39 Interestingly, although there was a correlation between LDL molecular weight and arterial LDL metabolism in control animals in this study, there was no correlation in oral contraceptive–treated animals. This suggests that the relation between lipoprotein characteristics (reflected by LDL molecular weight) and arterial metabolism is “uncoupled” by oral contraceptive treatment. This may reflect the inhibition of a form of LDL modification dependent on LDL particle characteristics. It is also possible that treatment causes a disruption in LDL–cell interaction independent of LDL characteristics. This is also suggested by the lack of correlation between TPC and LDL degradation in MOC-treated (but not TOC-treated) animals.

We chose a short period of study for this experiment (16 weeks) so that early events in atherogenesis would be stimulated but intimal thickening would be minimal and preferably not different among groups. Interestingly, although there were regional differences in both indexes of LDL metabolism and lesion extent, these did not necessarily parallel each other. For example, the coronary arteries, which metabolized LDL actively, had very little intimal thickening, whereas the abdominal aorta had substantially more intimal thickening (primarily foam cells) yet did not metabolize LDL as actively as other sites. Also, although there was a correlation between intimal area and LDL degradation rate in control monkeys, there was no correlation in MOC or TOC monkeys (Table 5). Again, this suggests some uncoupling of LDL metabolism consistent with blockage of intra-arterial LDL modification dependent on the state of atherogenesis (i.e., the relative preponderance of foam cells).

Although some oral contraceptives, primarily older “high-dose” formulations, have theoretically atherogenic effects on plasma lipoprotein profiles, there is no evidence of accelerated atherosclerosis among even long-term oral contraceptive users (i.e., use for 20 years or more).5,4 In fact, some epidemiological evidence indicates that long-term users are at decreased risk of developing coronary heart disease.4 Studies using a monkey model of diet-induced coronary artery atherosclerosis have shown that, despite a marked progestin-induced lowering of plasma HDL concentrations, atherosclerosis was retarded in monkeys treated with combination oral contraceptives.5 We have proposed that this unexpected response represents a potent and direct effect of ethinyl estradiol on the arterial wall, an effect overriding any adverse influences of oral contraceptive treatment on plasma lipoproteins. The results described here, taken together with other recent findings,5,35,36 support that hypothesis and suggest that estrogen may be acting to inhibit atherogenesis, at least in part, by inhibiting the arterial uptake and/or metabolism of plasma LDL particles.

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J D Wagner, M R Adams, D C Schwenke and T B Clarkson

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