Oral Contraceptives Decrease Hepatic Cholesterol Independent of the LDL Receptor in Nonhuman Primates

Perry L. Colvin, Jr, Janice D. Wagner, Mark D. Heuser, Mary G. Sorci-Thomas

Pharmacological doses of estrogens have been reported to increase hepatic catabolism of low-density lipoprotein (LDL) by the LDL receptor (LDL-R) pathway and to increase the concentration of mRNA for the LDL receptor. The induction of LDL-Rs by large doses of estrogen may not be relevant to the role of estrogens under physiological conditions. Furthermore, the mechanisms by which oral contraceptives, a combination of synthetic estrogen and progestin, may modulate LDL metabolism remain largely unexplored. Adult female cynomolgus monkeys were given combination ethinyl estradiol/norgestrel preparations \((n=16)\) for 16 weeks and were compared with a control group that did not receive exogenous sex hormones \((n=7)\). All animals consumed a diet containing 0.25 mg cholesterol/kcal with 40% of calories from saturated fats. After 16 weeks of treatment there was no significant difference in LDL cholesterol \((LDL-C, 242\pm113 \text{ mg/dL})\) and hepatic LDL-R mRNA concentration between oral contraceptive-treated animals \((LDL-R \text{ mRNA, } 0.60\pm0.31 \text{ pg/µg RNA})\) and control animals \((LDL-C, 277\pm100 \text{ mg/dL}; \text{LDL-R mRNA, } 0.51\pm0.21 \text{ pg/µg RNA})\). In contrast, the hepatic cholesteryl ester concentration was significantly lower in the oral contraceptive-treated animals \((7.28\pm3.59 \text{ mg/g liver})\) compared with the control animals \((16.07\pm11.86 \text{ mg/g liver}; P=.01)\) with no significant difference in hepatic free cholesterol concentration between the groups. Thus, oral contraceptives decrease hepatic cholesterol concentration independent of LDL-R expression. These data support the hypothesis that the increase in LDL-R mRNA abundance and activity observed with pharmacological doses of estrogen may be secondary to depletion of hepatic cholesterol.

Key Words • oral contraceptives • LDL • LDL receptor • hepatic cholesterol • hepatic cholesteryl ester • estrogen

Estrogen may mediate the sex differential in longevity and atherosclerosis. Much of our understanding of how estrogen modulates lipoprotein metabolism is derived from studies in which pharmacological doses of estrogens are administered. Estrogens in pharmacological doses have been reported to increase catabolism of low-density lipoprotein (LDL), increase LDL receptor activity, and increase the concentration of mRNA for the LDL receptor. The induction of LDL receptor activity by pharmacological doses of estrogen may have little relevance to the role of estrogen under physiological conditions because other concomitant changes in cholesterol metabolism occur with the administration of pharmacological doses of estrogen that may secondarily give rise to the observed increase in LDL receptor activity.

Observations from cell culture studies provide evidence of the complex intracellular changes induced by estrogen. Estrogens at pharmacological concentrations increase binding and degradation of LDL by the LDL receptor–dependent pathway in a human hepatoma cell line (HepG2) grown in the absence of LDL. The increase in LDL receptor activity in HepG2 cells is accompanied by a decrease in the cell content of free cholesterol, suggesting that stimulation of LDL receptor activity by pharmacological doses of estrogen is secondary to depletion of the regulatory pool of cholesterol in the hepatocyte.

In contrast with studies of pharmacological doses of estrogen, in studies using physiological concentrations of estrogen, the extent that estrogen affects LDL receptor activity remains unclear. Furthermore, the mechanisms by which oral contraceptives, a combination of synthetic estrogen and progestin, may modulate LDL metabolism remain largely unexplored. Orally administered progestins increase plasma LDL cholesterol (LDL-C) concentration and may negate the beneficial effect of oral estrogen on LDL-C concentration. It is also known that estrogens and oral contraceptives increase biliary cholesterol secretion. This study was undertaken to examine the effect of oral contraceptives on potential regulatory pools of hepatic cholesterol and mRNA for the LDL receptor.

Methods

Study Design

This study was conducted in 24 adult, sexually intact, premenopausal female cynomolgus monkeys (Macaca fascicularis) imported from Indonesia (CV Primates,
Bogor, Indonesia). The animals were fed monkey chow (High Protein Monkey Chow, Ralston Purina Company, St Louis, Mo) during a 3-month quarantine and then were stratified into groups by their total plasma cholesterol and high-density lipoprotein cholesterol (HDL-C) concentration response to a 1-month challenge diet (0.25 mg cholesterol/kcal) to control for factors that determine individual variability in response to the atherogenic diet. The groups did not differ in their LDL-C response to the atherogenic diet. After the 1-month challenge period, the animals were fed monkey chow until they entered the experimental protocol. The groups were assigned randomly to receive an atherogenic diet with oral contraceptive mixed into the diet (n=16) or to receive an atherogenic diet without oral contraceptive (control group, n=8).

The study occurred over 16 weeks, during which all animals were fed the challenge diet (0.25 mg cholesterol/kcal). The control group received no exogenous hormone (n=8). The monkeys in the oral contraceptive–treated group received a monophasic oral contraceptive preparation (n=8) at a dose equivalent, by caloric intake, to 50 µg ethinyl estradiol and 500 µg norgestrel per day (Ovral, Wyeth Ayerst, Philadelphia, Pa) or a triphasic oral contraceptive preparation (n=8) at a dose equivalent, by caloric intake, to 30 to 40 µg ethinyl estradiol and 50 to 125 µg norgestrel per day (Triphasil, Wyeth Ayerst). As in women taking oral contraceptives, the estradiol and norgestrel were administered for 21 days; a placebo was administered to the monkeys for the remaining 7 days of a 28-day cycle. The dose of oral contraceptive was calculated on a caloric basis to approximate that of women consuming 1800 kcal and one oral contraceptive pill per day.

During week 15 of the experiment, LDL-C concentrations were determined. One week later, liver samples were collected at necropsy, immediately frozen with liquid nitrogen, and stored at -70°C for later measurement of mRNA and hepatic cholesterol concentration. The dose of oral contraceptive was calculated on a caloric basis to approximate that of women consuming 1800 kcal and one oral contraceptive pill per day.

Lipoprotein Analysis

Blood samples were collected from the femoral vein of animals after sedation with ketamine hydrochloride (15 mg/kg, IM). Venous blood samples were collected into Vacutainer tubes (Becton Dickinson, Rutherford, NJ) containing dry sodium EDTA (1.0 mg/mL blood) from the femoral vein of animals fasted overnight (18 hours). Blood was immediately centrifuged at 1500g for 30 minutes at 4°C, and plasma was separated. Cholesterol concentration was measured by autoanalyzer (Technicon, Tarrytown, NY) in a central laboratory in full standardization with the Centers for Disease Control and Prevention–National Heart, Lung, and Blood Institute Standardization Program using the standard Lipid Research Clinics methodology. Cholesterol concentration was determined for whole plasma. HDL-C concentration was quantified after precipitation of the apoB-containing lipoproteins with heparin-manganese. Very-low-density lipoprotein (VLDL) cholesterol concentration is negligible in nonhuman primates on this diet. ApoB was measured by enzyme-linked immunosorbent assay using purified monospecific goat antibodies.

RNA Isolation and Quantification of mRNA

Total cellular RNA was purified from the liver tissue by using previously published procedures. Purified RNA was dissolved in diethyl pyrocarbonate–treated water, and the concentration was determined by the absorbance at 260 nm. The integrity of the purified RNA was determined by formaldehyde–agarose gel electrophoresis as previously described. Cellular LDL receptor and 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase mRNA content was measured with a DNA-excess solution hybridization assay that employed a single-stranded DNA probe prepared from the human LDL receptor and HMG-CoA reductase cDNA.

Hepatic Cholesterol Quantification

Lipids were extracted from a known mass of liver tissue with trichloromethane/methanol (2:1, vol/vol) according to the method of Folch et al. Total plasma cholesterol and triglyceride, LDL-C, HDL-C, HDL subfractions (HDL 2 and HDL 3), and apolipoprotein (apo) A-I concentrations were determined during the experimental period. The fractional catabolic rate of LDL apoB was determined during week 16 of the study. 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 Animal Care and Use Committee of Bowman Gray School of Medicine.

Statistical Methods

All values in this report are the mean±SD for each group. The sample size used in this study (control group, n=7; treatment group, n=16) provided a power of 95% to detect a biologically significant difference (40%) in mRNA concentration for the LDL receptor between the two groups. The inclusion of different oral contraceptive preparations into a single group for analysis is a method that has been used previously, and in this case it has biological plausibility due to the small differences in the dose of estradiol and norgestrel between these two preparations. A preanalysis did not show a significant difference between the groups receiving the two oral contraceptive preparations. The t test was used to assess treatment differences in normally distributed variables with homogeneous variances. Variables with nonhomogeneous variances (hepatic total and esterified cholesterol concentrations) as determined by the variance ratio test were log transformed before application of the t test. All t tests were two tailed, with α=5% considered statistically significant.
Results

Sixteen monkeys in the oral contraceptive–treated group and seven monkeys in the control group completed the study protocol and were included in the analysis. One monkey in the control group died during the study period of causes unrelated to the experiment and procedures. The small difference in the dose of estradiol and norgestrel between these two preparations should not produce a significant difference in lipoprotein concentration. Detailed analysis of plasma lipids, lipoproteins, and apoproteins in these animals were published previously. The administration of oral contraceptives did not alter the total plasma cholesterol and triglyceride, LDL-C, HDL-C, HDL fractions (HDL1 and HDL2), and apoA-I concentrations nor the fractional catabolic rate of LDL apoB. After the monkeys had consumed the atherogenic diet for 15 weeks there were no significant differences in LDL-C concentration, apoB concentration, or apoB/LDL ratio between the control and the oral contraceptive–treated groups (Table 1).

Medications other than exogenous sex hormones that significantly increase LDL receptor abundance and activity in vivo produce at least an 80% increase in mRNA concentration. The present study had a power of 95% to detect an increase of at least one half this magnitude (40%) in mRNA for the LDL receptor at P < .05. After 16 weeks of the study there were no significant differences in concentrations of hepatic mRNA for the LDL receptor or HMG-CoA reductase between the control and the oral contraceptive–treated groups (Table 2).

The administration of oral contraceptives had a significant effect on hepatic cholesterol concentration (Figure). The total hepatic cholesterol concentration at 11.98 ± 4.92 mg/g liver in the oral contraceptive–treated group was significantly lower than the 20.82 ± 12.40 mg/g liver in the control group (P < .03). The lower total hepatic cholesterol concentration observed with oral contraceptive administration was entirely accounted for by lower hepatic cholesteryl ester concentration. The hepatic cholesteryl ester concentration at 7.28 ± 3.59 mg/g liver in the oral contraceptive–treated group was significantly lower than the 16.07 ± 11.86 mg/g liver in the control group (P = .01). There was no significant difference in hepatic free cholesterol concentration between the control and the oral contraceptive–treated groups (Table 2).

Discussion

Pharmacological doses of synthetic estrogens in men during hormonal treatment of prostatic carcinoma (oral ethinyl estradiol 1 mg/d) lower plasma cholesterol concentration and induce a more rapid fractional catabolic rate of autologous radiolabeled native LDL. LDLs isolated from these patients have reduced LDL receptor affinity in tissue-culture studies, which is hypothesized to be the consequence of increased expression of hepatic LDL receptors in vivo. The increase in LDL receptor activity induced by pharmacological doses of estrogen is accompanied by an increase in mRNA for the LDL receptor. However, men with prostatic carcinoma treated with pharmacological doses of estrogen have an enhanced rate of gallstone formation and a 40% higher biliary secretion rate of cholesterol during estrogen treatment. This marked increase in biliary secretion of cholesterol may decrease intrahepatocyte cholesterol concentration and thus, secondarily, upregulate LDL receptor activity.

Women are at increased risk of developing cholesterol gallstones compared with men. This risk is increased further by the use of oral contraceptives, which are a combination of synthetic estrogen and progestin. Oral contraceptives may not significantly affect the concentration of cholesterol or triglycerides in whole plasma or in any lipoprotein fraction in women, yet they increase biliary cholesterol secretion and the lithogenicity of bile.

**Table 1. Effect of Oral Contraceptives on LDL in Monkeys on an Atherogenic Diet**

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Control</th>
<th>Oral Contraceptive</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-C, mg/dL</td>
<td>277±100</td>
<td>242±113</td>
</tr>
<tr>
<td>ApoB, mg/dL</td>
<td>192±49</td>
<td>221±75</td>
</tr>
<tr>
<td>ApoB/LDL-C</td>
<td>0.78±0.32</td>
<td>1.00±0.32</td>
</tr>
</tbody>
</table>

**Table 2. Effect of Oral Contraceptives on Hepatic mRNA and Cholesterol Concentrations in Monkeys**

<table>
<thead>
<tr>
<th>mRNA CYT</th>
<th>Control</th>
<th>Oral Contraceptive</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL receptor mRNA, pg/µg RNA</td>
<td>0.507±0.212</td>
<td>0.604±0.311</td>
</tr>
<tr>
<td>HMG-CoA reductase mRNA, pg/µg RNA</td>
<td>1.031±0.421</td>
<td>1.078±0.508</td>
</tr>
<tr>
<td>Total hepatic cholesterol, mg/g liver</td>
<td>20.82±12.40</td>
<td>11.98±4.92</td>
</tr>
<tr>
<td>Hepatic free cholesterol, mg/g liver</td>
<td>4.76±1.44</td>
<td>4.69±1.91</td>
</tr>
<tr>
<td>Hepatic cholesteryl ester, mg/g liver</td>
<td>16.07±11.86</td>
<td>7.28±3.59</td>
</tr>
</tbody>
</table>

LDL indicates low-density lipoprotein; LDL-C, LDL cholesterol; and ApoB, apolipoprotein B. Data are mean±SD. No difference was found between control and oral contraceptive groups.
The effect of orally administered sex hormones on lipoprotein concentration and metabolism is mediated by the sex hormone concentration achieved in the portal circulation and by the first-pass effect on hepatic metabolism. Because the animals were not ovariectomized in this study, their lipoprotein concentrations would be affected by endogenous hormone production. All blood and liver tissue samples were collected from animals in the oral contraceptive–treated group at the same point in the treatment cycle, whereas blood and liver tissue samples were collected from animals in the control group at random points throughout the estrous cycle. This circumstance decreases the probability of detecting a difference between the control group and the oral contraceptive–treated group because some of the animals in the control group were presumably studied during a phase of high endogenous hormone production. Despite this, a clear difference between the control group and the oral contraceptive–treated group was found.

Both estrogen and oral contraceptives clearly increase biliary cholesterol secretion. The effect of estrogen on plasma cholesterol concentration, LDL receptor mRNA, and biliary secretion of cholesterol is dose dependent. We hypothesize that oral contraceptives induce an increase in biliary cholesterol secretion that results in depletion of hepatic cholesterol ester. This suggests a mechanism for the dose-dependent increase in concentration of LDL receptor mRNA and LDL receptor activity observed with administration of pharmacological doses of estrogen. We hypothesize that as the dose or potency of oral estrogen is increased, biliary secretion of cholesterol will increase, which may cause further depletion of hepatic cholesterol pools and, in turn, increase LDL receptor mRNA abundance and LDL receptor activity.

Acknowledgment

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References


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