Contraceptive steroids increase hepatic uptake of chylomicron remnants in healthy young women

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Abstract In an investigation of alterations in cholesterol metabolism during contraceptive steroid use, we studied plasma clearance of chylomicron remnants. Six healthy women were studied on and off contraceptive steroid therapy. Remnant clearance was measured from the disappearance of retinyl palmitate administered intravenously in plasma endogenously labeled with retinyl palmitate. We also measured cholesterol in HDL and its subfractions and postheparin lipoprotein lipase and hepatic triglyceride lipase activities. Plasma decay of retinyl palmitate was biexponential. The rapid component, reflecting chylomicron remnant removal, accounted for about 90% of the total clearance in all studies. During contraceptive steroid intake, both rapid and slow decay constants and the calculated plasma clearance rates were significantly increased (mean values: rapid decay constant, control 0.048 versus treated 0.101 min⁻¹, P < 0.05; slow decay constant, 0.004 versus 0.014 min⁻¹, P < 0.01; plasma clearance 74 versus 115 ml/min, P < 0.025) indicating enhanced hepatic uptake of chylomicron remnants and probably an increased hepatic uptake of higher density lipoproteins (d > 1.006 g/ml). Total postheparin lipolytic activity and lipoprotein lipase activity were depressed in all six women (P < 0.05) and hepatic triglyceride lipase activity was increased in four of five subjects. Contraceptive steroids also caused a decrease in the HDL₄/HDL₃ cholesterol ratio (P < 0.05), implying impaired peripheral lipoprotein triglyceride hydrolysis and/or increased HDL₂ clearance by hepatic triglyceride lipase. In conclusion, during intake of contraceptive steroids, the plasma clearance of chylomicron remnants and higher density lipoproteins was increased. Since plasma clearance of these particles is largely dependent upon hepatocyte uptake, it is likely that hepatic metabolism of cholesterol is also altered.

METHODS

The protocol was approved by the Human Subjects Committee of the University of Colorado Medical School and written consent was obtained prior to study.

Subjects

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Abbreviations: LDL, low density lipoproteins; HDL, high density lipoproteins; VLDL, very low density lipoproteins; RP, retinyl palmitate; HPLC, high performance liquid chromatography.


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Six women were studied. All were healthy, paid volunteers, age 25 to 40 with normal plasma lipid levels and stable body weight. To exclude pregnancy, control studies were done during days 4 to 7 of the ovulatory cycle and a rapid HCG test was negative. Each subject was studied twice, once prior to contraceptive steroid use or after discontinuing contraceptive steroids for at least 6 weeks (control study), and again 21-24 days after beginning a new cycle of contraceptive steroids. The contraceptive steroids contained either 35 or 50 μg of 17α-ethinylestradiol or mestranol, two synthetic estrogens of similar estrogenic potency (20), and 1 mg of norethindrone. Characteristics of study subjects and the composition of contraceptive steroids are given in Table 1.

Study protocol
The same design was used for control and contraceptive steroid studies. On day 1, a large dose of retinyl palmitate (60 mg/m² body surface area as retinol equivalent) was taken orally with cream (39% fat) (100 ml/m² body surface area), and 5-6 hr later 2 units of lipemic plasma were obtained for 200 min to measure the disappearance of the retinyl palmitate in the various lipoprotein fractions (obtained by ultracentrifugation) (18). The plasma was stored in ACD buffer and light-shielded, at room temperature for 42 hr. At that time all retinyl palmitate given on day 1 had been cleared from the subject's circulation. On the morning of the third day, the autologous stored plasma was pulse-injected (in about 3 min) into an antecubital vein and frequent blood samples were obtained by plasmapheresis (18). The plasma was prepared by sequential flotation on a discontinuous salt gradient using a Beckman L 5-75 ultracentrifuge and an SW 40 rotor. After the last spin, the remaining gradient was fractionated into an intermediate density fraction (approximate d 1.010-1.020 g/ml), a visible LDL band (approximate d 1.021-1.060 g/ml), and an infranatant (approximate d 1.10 g/ml) according to Redgrave and Carlson (18, 21).

For assay of lipolytic activities, blood specimens were immediately cooled on ice, and the heparinized plasma was separated by centrifugation. Lipolytic activity was extracted on heparin–Sepharose 6B (Pharmacia) eluted in 0.02 M Na barbital buffer, 0.3 M NaCl, pH 7.4, containing 6 mg/ml Na heparin, and analyzed by an enzymatic assay.

Analyses
Retinyl palmitate levels in plasma and in individual lipoprotein fractions (obtained by ultracentrifugation) were analyzed by reverse phase HPLC (18). Plasma lipoprotein fractions [chylomicrons of d < 0.90 g/ml, VLDL fraction A (approximate d 0.933 g/ml), B (approximate d 0.967 g/ml), C (approximate d 0.984 g/ml)] were prepared by sequential flotation on a discontinuous salt gradient using a Beckman L 5-75 ultracentrifuge and an SW 40 rotor. After the last spin, the remaining gradient was fractionated into an intermediate density fraction (approximate d 1.010-1.020 g/ml), a visible LDL band (approximate d 1.021-1.060 g/ml), and an infranatant (approximate d 1.10 g/ml) according to Redgrave and Carlson (18, 21).

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**Table 1.** Characteristics of study volunteers and composition of contraceptive steroids

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Body Weight</th>
<th>Ideal Body Weight</th>
<th>Serum Triglyceride</th>
<th>Serum Cholesterol</th>
<th>Ethinyl Estradiol</th>
<th>Mestranol</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>26</td>
<td>55</td>
<td>99</td>
<td>48</td>
<td>173</td>
<td>315</td>
<td>35</td>
</tr>
<tr>
<td>B</td>
<td>40</td>
<td>50</td>
<td>92</td>
<td>67</td>
<td>159</td>
<td>399</td>
<td>50</td>
</tr>
<tr>
<td>C</td>
<td>30</td>
<td>61</td>
<td>111</td>
<td>82</td>
<td>186</td>
<td>81</td>
<td>50</td>
</tr>
<tr>
<td>D</td>
<td>40</td>
<td>31</td>
<td>97</td>
<td>70</td>
<td>197</td>
<td>323</td>
<td>50</td>
</tr>
<tr>
<td>E</td>
<td>25</td>
<td>69.5</td>
<td>125</td>
<td>62</td>
<td>175</td>
<td>83</td>
<td>50</td>
</tr>
<tr>
<td>F</td>
<td>28</td>
<td>54.5</td>
<td>109</td>
<td>57</td>
<td>108</td>
<td>36</td>
<td>50</td>
</tr>
</tbody>
</table>

*All subjects were Caucasian, except E who was black.
*Serum lipids were measured at the time of the control study (at least 6 weeks after using contraceptive steroids).
*All contraceptive steroids contained 1.0 mg of norethindrone.
using a radiolabeled triolein substrate at pH 8.2 (22, 23). Hepatic triglyceride lipase activity was assayed in the absence of serum in the substrate and in the presence of 1.0 M NaCl (pH 8.6), conditions which completely inhibit lipoprotein lipase (24). Lipoprotein lipase activity was calculated as the difference between total plasma lipolytic activity and hepatic triglyceride lipase activity. Serum triglyceride and cholesterol were measured by standard techniques, and HDL and HDL₃ cholesterol levels were determined using a two-step dextran sulfate-magnesium precipitation procedure prior to cholesterol measurement (25, 26). HDL₄ cholesterol was determined by subtracting HDL₃ cholesterol from total HDL cholesterol.

Calculations and statistical procedures

Plasma clearance of retinyl palmitate was calculated by dividing the dose of retinyl palmitate administered by the total area under the plasma decay curve, determined by the trapezoidal method. In addition, plasma decay data were tested for fit to a mono-, bi-, or triexponential function by an exponential stripping program as described (18). The apparent volume of distribution of retinyl palmitate was estimated by dividing the retinyl palmitate dose injected by the y-intercept of the decay curve.

Significance of the differences between groups was calculated using the Student’s t-test for paired observations after testing the equality of variances by an F-test (27). P < 0.05 was considered statistically significant. All results, unless otherwise stated, are expressed as mean ± 1 standard deviation.

RESULTS

Plasma decay of retinyl palmitate-labeled chylomicrons

After 21 to 24 days of contraceptive steroid intake, the rate of plasma decay of retinyl palmitate was enhanced (Fig. 1) and retinyl palmitate clearance was increased in each subject (P < 0.01) (Table 2 and Table 3). The mean increase in clearance was 41%.

Decay of plasma retinyl palmitate significantly obeyed a biexponential first order function in all studies. The first, rapid kinetic component accounted for 89 ± 3% of clearance in control and 87 ± 4% in contraceptive steroid studies. The rapid decay constant was increased by contraceptive steroids from 0.053 ± 0.024 to 0.086 ± 0.012 min⁻¹ (P < 0.05) (Table 3) and the half-life was shortened from 18.3 ± 8.8 min to 8.7 ± 2.3 min. The slow decay constant also increased from 0.006 ± 0.004 to 0.012 ± 0.002 (P < 0.01) and its half-life decreased from 115 ± 87 min to 58 ± 12 min.

During the interval from 5 to 75 min after injection of the plasma, 89 ± 8% of the retinyl palmitate that was initially contained in chylomicrons and VLDL A was removed, whereas only 41 ± 2% was cleared from the smaller sized VLDL (fraction B + C) and 23 ± 7% from the LDL fraction. Furthermore, the percentage of plasma retinyl palmitate cleared by the slow kinetic process (Table 3) agreed reasonably well with the fraction of total plasma retinyl palmitate in d > 1.006 g/ml lipoproteins in the plasma injected (mean difference 9 ± 5%). The slow retinyl palmitate decay component, therefore, appears to represent, at least in part, retinyl palmitate transferred to lipoproteins of hepatic origin, whereas the rapid component reflects chylomicron remnant decay. Apparent volumes of distribution were similar in control and contraceptive steroid studies and were 96 ± 17% of the plasma volumes estimated from a standard table.

Plasma lipid levels and lipolytic activities

Plasma triglyceride levels increased slightly during contraceptive steroid intake (Table 4) but total serum cholesterol and total HDL cholesterol were unchanged. However, the HDL₄/HDL₃ cholesterol ratio was lower (P < 0.05) during contraceptive steroid use (Table 4).
**DISCUSSION**

In this study contraceptive steroids unequivocally accelerated the rate of disappearance of retinyl palmitate-labeled chylomicrons from the plasma in all subjects studied. The total plasma lipolytic activity and the activity of lipoprotein lipase as well as the HDL$_2$/HDL$_3$ cholesterol ratio decreased in all subjects. In four of five subjects, hepatic triglyceride lipase activity increased. These findings imply diminished chylomicron and VLDL triglyceride hydrolysis, decreased HDL$_2$ formation, and delayed chylomicron remnant formation, but increased remnant and HDL$_2$ clearance.

Each subject was studied as her own control. Control studies were done during the early follicular phase when serum estrogen and progesterone levels were low and were compared to identical studies performed after the subject took contraceptive steroids for 3 weeks. In each subject, the two plasma clearance studies were comparable. Plasma decay of the retinyl palmitate was biexponential with the first, rapid component, which probably reflects chylomicron remnant disappearance, accounting for more than 90% of the clearance in all studies. Both the major, rapid decay process and the minor, slower decay process were accelerated and plasma clearance was increased by intake of contraceptive steroids in each subject (Tables 2 and 3).

The increases in plasma clearance of retinyl palmitate were substantially greater ($P < 0.01$) in the four subjects

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**TABLE 2. Plasma clearance of retinyl palmitate in control studies and during contraceptive steroid intake (CS)**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Control</th>
<th>CS</th>
<th>Change</th>
<th>Clearance$^*$</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>47</td>
<td>100</td>
<td>+ 113</td>
<td>7.4</td>
<td>115</td>
</tr>
<tr>
<td>B</td>
<td>61</td>
<td>120</td>
<td>+ 97</td>
<td>6.8</td>
<td>126</td>
</tr>
<tr>
<td>C</td>
<td>70</td>
<td>126</td>
<td>+ 57</td>
<td>7.4</td>
<td>126</td>
</tr>
<tr>
<td>D</td>
<td>57</td>
<td>96</td>
<td>+ 68</td>
<td>6.8</td>
<td>96</td>
</tr>
<tr>
<td>E</td>
<td>140</td>
<td>159</td>
<td>+ 14</td>
<td>7.4</td>
<td>159</td>
</tr>
<tr>
<td>F</td>
<td>68</td>
<td>87</td>
<td>+ 28</td>
<td>6.8</td>
<td>87</td>
</tr>
<tr>
<td>Mean</td>
<td>74</td>
<td>115</td>
<td>+ 67</td>
<td>7.4</td>
<td>115</td>
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</tbody>
</table>

$^*$Clearance, dose of retinyl palmitate administered divided by the area under the plasma decay curve, determined by the trapezoidal method.

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**TABLE 3. Kinetic analysis of plasma retinyl palmitate decay in control studies (C) and during contraceptive steroid intake (CS)$^*$**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Apparent Volume of Distribution</th>
<th>Rapid Decay Constant$^a$</th>
<th>Slow Decay Constant$^b$</th>
<th>Fraction of Dose Cleared by the Rapid Process</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml/min</td>
<td>min$^{-1}$</td>
<td>min$^{-1}$</td>
<td>%</td>
</tr>
<tr>
<td>A</td>
<td>2204</td>
<td>0.043</td>
<td>0.010</td>
<td>80.0</td>
</tr>
<tr>
<td>B</td>
<td>1636</td>
<td>0.068</td>
<td>0.003</td>
<td>95.8</td>
</tr>
<tr>
<td>C</td>
<td>2298</td>
<td>0.040</td>
<td>0.003</td>
<td>93.0</td>
</tr>
<tr>
<td>D</td>
<td>2362</td>
<td>0.029</td>
<td>0.004</td>
<td>89.6</td>
</tr>
<tr>
<td>E</td>
<td>3127</td>
<td>0.094</td>
<td>0.011</td>
<td>89.7</td>
</tr>
<tr>
<td>F</td>
<td>2838</td>
<td>0.038</td>
<td>0.007</td>
<td>97.2</td>
</tr>
<tr>
<td>Mean</td>
<td>2414</td>
<td>0.053</td>
<td>0.006</td>
<td>89.7</td>
</tr>
<tr>
<td>± SD</td>
<td>524</td>
<td>0.024</td>
<td>0.004</td>
<td>6</td>
</tr>
<tr>
<td>$P$</td>
<td>NS</td>
<td>&lt; 0.05</td>
<td>&lt; 0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^*$Chylomicron-rich plasma was obtained by plasmapheresis 5–6 hr after a test meal (100 ml of cream/m² body surface area and 60 mg of retinol equivalent/m² body surface area); after 2 days, the plasma was reinjected into the donor.

$^a$The data were tested for a mono-, bi-, and triexponential decay function using a computer program and analyzed by an F-test to determine whether the fit of the data significantly ($P < 0.05$) improved by introducing an additional exponential. Final fit of the data to the resulting mono- or biexponential equation was performed with a nonlinear least squares program as described.

TABLE 4. Plasma lipid levels (mg/dl) during control period and during contraceptive steroid intake

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Contraceptive Steroids</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td>16.3 ± 11.6</td>
<td>7.6 ± 24.1</td>
<td>0.15 &lt; P &gt; 0.10</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>163.3 ± 18.6</td>
<td>159.7 ± 19.0</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>52.0 ± 8.0</td>
<td>52.5 ± 9.9</td>
<td>NS</td>
</tr>
<tr>
<td>HDL₄ cholesterol</td>
<td>15.5 ± 5.2</td>
<td>10.5 ± 5.6</td>
<td>0.06 &lt; P &gt; 0.05</td>
</tr>
<tr>
<td>HDL₂/HDL₃ ratio</td>
<td>0.42 ± 0.13</td>
<td>0.27 ± 0.15</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

Taking contraceptive steroids containing mestranol than in the two taking contraceptives containing ethinyl estradiol (Tables 1, 2). Since the known effects of these two estrogens are similar and since the variation in clearance within each group was large and the number of subjects was small, this difference may not reflect a true difference in pharmacologic effect.

Retinyl palmitate is quantitatively removed from the plasma by hepatocytes by endocytosis of chylomicron remnants and is not resecreted into the plasma (29). In earlier studies in healthy subjects, we examined the use of plasma endogenously labeled with retinyl palmitate to measure chylomicron remnant clearance and found that most retinyl palmitate remained in the chylomicron and VLDL fractions. It was not transferred to higher density lipoprotein fractions for at least 12 hr in vivo and 42 hr in vitro (18). Its rate of removal was not affected by heparin-induced lipolytic activity (19), and studies were reproducible (precision 6.4 ± 2.7% for intra-individual comparison). In untreated healthy subjects, the major rapid kinetic component of retinyl palmitate clearance from plasma was dose-dependent (30). We concluded that retinyl palmitate disappearance from the plasma in healthy normolipidemic subjects, as measured in this study, probably reflects receptor-mediated hepatic chylomicron remnant uptake.

Hepatocellular uptake of cholesterol-rich lipoproteins (chylomicron and VLDL remnants and LDL) is mediated by two independent types of receptors (31, 32). The first, the chylomicron remnant or apolipoprotein E receptor, selectively binds apolipoprotein E, while the second, the LDL or apolipoprotein B, E receptor, binds both apoB-100 and apoE (11, 15, 31, 32). These receptor mechanisms are believed to differ from each other in responsiveness to estrogens. Uptake of LDL by the isolated perfused liver is enhanced by pharmacologic doses of estrogens (12–14). Pretreatment with estrogens increases binding of LDL to isolated hepatocyte membranes. On the other hand, in most (15) but not all studies (17, 33), estrogen treatment has no such effect on chylomicron remnant uptake or binding to hepatocyte membranes. In a single human study, estrogen administration increased chylomicron remnant clearance by patients with familial type III hyperlipoproteinemia, a disorder of remnant metabolism (16).

The observed alterations in plasma lipids and lipase activities in our subjects are consistent with those reported in the literature and probably reflect the estrogen/progestin balance of the contraceptive steroid mixtures used. Estrogens increase serum triglyceride levels (34, 35), the triglyceride/cholesterol ratio of HDL,
LDL, and especially VLDL (34, 36), and decrease postheparin lipolytic activity and hepatic triglyceride lipase (36, 37). Progestagens lower total HDL and HDL₂ cholesterol levels, increase hepatic triglyceride lipase (4, 36), prevent the estrogen-induced rise in total HDL cholesterol (36, 38), and decrease plasma postheparin lipolytic activity (39).

In our studies, lipoprotein lipase activity and HDL₄/ HDL₂ cholesterol ratio were decreased, implying predominance of the progesterone effect (40). These changes suggest reduced transfer of surface components (phospholipid, free cholesterol, and apolipoprotein C) from triglyceride-rich lipoproteins to the HDL₂ lipoprotein fraction (28) as well as impaired hydrolysis of core triglycerides of chylomicrons and VLDL and/or increased clearance of HDL₂, probably mediated by hepatic triglyceride lipase (39).

In view of these findings, we propose that contraceptive steroid mixtures stimulate receptor-mediated hepatic uptake of chylomicron remnants in healthy women. Further, we suggest that hepatic removal of VLDL remnants is enhanced and this, in combination with decreased lipoprotein lipase activity, impairs conversion of endogenous VLDL to LDL, which might contribute to the decreased LDL cholesterol levels during estrogen intake that have been reported by others (36). The estrogen-stimulated hepatic uptake of LDL by the apolipoprotein B,E receptor would augment this effect. Thus, contraceptive steroids appear to stimulate both of the receptor-mediated pathways for hepatic cholesterol uptake, which probably alters the hepatic metabolism of cholesterol.

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References


