Contraceptive steroids increase hepatic uptake of chylomicron remnants in healthy young women[1]

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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 HDL_2/HDL_3 cholesterol ratio ($P < 0.05$), implying impaired peripheral lipoprotein triglyceride hydrolysis and/or increased HDL_2 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. — **Berr, F., R. H. Eckel, and F. Kern, Jr.** Contraceptive steroids increase hepatic uptake of chylomicron remnants in healthy young women. *J. Lipid Res.* 1986. **27:** 645–651.

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Female sex steroid hormones used as oral contraceptives produce clinically important alterations in lipid and lipoprotein metabolism (1, 2). For example, plasma triglyceride as well as total (3–6) and LDL (7, 8) cholesterol concentrations are increased and the secretion of cholesterol into the bile is also increased (9, 10). The mechanisms of these effects are incompletely understood.

We report here the effect of contraceptive steroids on the plasma clearance of chylomicron remnants, the major carrier of exogenous cholesterol to the liver. Lipoproteins are removed from the plasma by the liver by receptor-mediated processes dependent upon recognition of the apolipoproteins on their surface (11). Estrogens induce hepatocyte receptors for the apolipoproteins of low density lipoproteins (LDL) and high density lipoproteins (HDL) and increase their uptake by the liver (12–14). Most studies in experimental animals suggest that estrogens do not affect the hepatic removal of chylomicron remnants (13, 15) but, in man with type III hyperlipidemia (16) and in rabbits (17), estrogen administration increases hepatic uptake of chylomicron and very low density lipoprotein (VLDL) remnants.

In this study, we employed a newly developed method to estimate chylomicron remnant clearance that uses autologous plasma containing chylomicrons endogenously labeled with retinyl palmitate (18). With this method, removal of remnants from the plasma in healthy fasting individuals is not limited by the rate of remnant formation (19). To understand better the effects of contraceptive steroids, we also measured postheparin plasma lipoprotein lipase and hepatic triglyceride lipase activities and HDL cholesterol and its subfractions.

METHODS

Subjects

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

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-ethinyl-estradiol 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/m^2 body surface area as retinol equivalent) was taken orally with cream (39% fat) (100 ml/m^2 body surface area), and 5-6 hr later 2 units of lipemic plasma were obtained by plasmapheresis (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 for 200 min to measure the disappearance of the administered retinyl palmitate. The distribution of retinyl palmitate in the various lipoprotein fractions was measured in each plasma unit that was injected and in the plasma of two control and three contraceptive steroid studies at 4 and 75 min after administering the retinyl palmitate-labeled plasma.

The volume of plasma given was adjusted so that similar doses of retinyl palmitate could be given in control (1.31 ± 0.68) and contraceptive steroid studies (1.47 ± 0.63 μmol) (*P*, not significant). The mean plasma volume injected was 315 ml in the control study and 399 ml in the contraceptive steroid study (*P*, not significant). The amount of triglyceride injected was 32% ($P = 0.20$) more during contraceptive steroid studies (323 mg vs. 245 mg). In control studies, 83 ± 4% of the retinyl palmitate in the plasma injected was in lipoproteins of d < 1.006 g/ml (chylomicrons and VLDL), and 81 ± 5% in contraceptive steroids studies (*P*, not significant). The remainder had been transferred to lipoproteins of d > 1.006 g/ml, primarily LDL. Prior to each study, fasting serum levels of triglycerides, cholesterol, total HDL, and HDL_3 cholesterol were determined.

The next day, heparin (100 IU/kg) was given intravenously in a bolus to five subjects and total plasma lipase and hepatic triglyceride lipase activities were determined.

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 1.02-1.06 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

TABLE 1. Characteristics of study volunteers and composition of contraceptive steroids

Subject[a]	Age	Body Weight	Ideal Body Weight[b]	Serum Triglyceride[c]	Serum Cholesterol[c]	Contraceptive Steroid[d]	
						Ethinyl Estradiol	Mestranol
	yr	*kg*	*%*	*mg/dl*	*mg/dl*	*μg*	*μg*
A	26	55	99	48	173		35
B	40	50	92	67	159		50
C	30	61	111	82	186		50
D	40	51	97	70	179		50
E	25	69.5	125	62	175	50	
F	28	54.5	109	57	108	50	

[a]All subjects were Caucasian, except E who was black.
[b]Statist. Bull. #40, Metropolitan Life Insurance Co., Nov.-Dec., 1959.
[c]Serum lipids were measured at the time of the control study (at least 6 weeks after using contraceptive steroids).
[d]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 HDL_3 cholesterol levels were determined using a two-step dextran sulfate–magnesium precipitation procedure prior to cholesterol measurement (25, 26). HDL_2 cholesterol was determined by subtracting HDL_3 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 (18). Final fit of the data to the resulting biexponential function was obtained using a nonlinear least squares 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 HDL_2/HDL_3 cholesterol ratio was lower ($P < 0.05$) during contraceptive steroid use (Table 4).

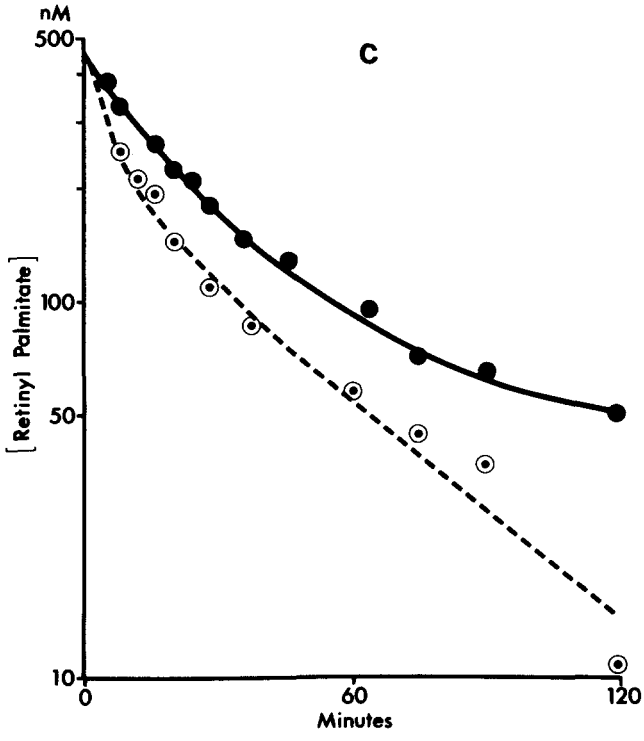

Fig. 1. Plasma decay of retinyl palmitate in a single subject (subject C) during the control period (●—●) and while taking contraceptive steroids for 22 days (○- -○). The rate of decay is clearly enhanced by the drugs.

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

Subject	Clearance[a]		
	Control	CS	Change
	ml/min		*%*
A	47	100	+ 113
B	61	120	+ 97
C	70	126	+ 80
D	57	96	+ 68
E	140	159	+ 14
F	68	87	+ 28
Mean	74	115	+ 67
P	< 0.025		

[a]Clearance, dose of retinyl palmitate administered divided by the area under the plasma decay curve, determined by the trapezoidal method.

Total postheparin lipolytic activity and lipoprotein lipase activity decreased ($P < 0.025$) in all five subjects during intake of contraceptive steroids, and hepatic triglyceride lipase activity increased in four of the five subjects (*P*, not significant) (**Fig. 2**). There were no significant correlations between either component of the retinyl palmitate decay and change in either lipoprotein or hepatic triglyceride lipase activity. Further, changes in lipase activity did not correlate significantly with changes in either HDL cholesterol subfraction or with the HDL_2/HDL_3 cholesterol ratio.

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 (28), 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

TABLE 3. Kinetic analysis of plasma retinyl palmitate decay in control studies (C) and during contraceptive steroid intake (CS)[a]

Subject	Apparent Volume of Distribution		Rapid Decay Constant[b]		Slow Decay Constant[b]		Fraction of Dose Cleared by the Rapid Process	
	C	CS	C	CS	C	CS	C	CS
	ml		*min^{-1}*		*min^{-1}*		*%*	
A	2204	2184	0.043	0.100	0.010	0.013	80.0	88.0
B	1636	2273	0.068	0.085	0.003	0.014	95.8	83.0
C	2298	2168	0.040	0.090	0.003	0.015	93.0	85.0
D	2362	2309	0.029	0.044	0.004	0.011	89.6	80.1
E	3127	3247	0.094	0.110	0.011	0.016	89.7	90.1
F	2858	3430	0.038	0.085	0.007	0.009	97.2	88.2
Mean	2414	2602	0.053	0.086	0.006	0.012	89	87
± SD	524	576	0.024	0.023	0.004	0.002	6	4
P	NS		< 0.05		< 0.01		NS	

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

[b]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

	Control	Contraceptive Steroids	P
Triglycerides	64.3 ± 11.6	76.5 ± 24.1	$0.15 < P > 0.10$
Cholesterol	163.3 ± 18.6	159.7 ± 19.0	NS
HDL cholesterol	52.0 ± 8.0	52.5 ± 9.9	NS
HDL_2 cholesterol	15.5 ± 5.2	10.5 ± 5.6	$0.06 < P > 0.05$
HDL_2/HDL_3 ratio	0.42 ± 0.13	0.27 ± 0.15	< 0.05

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,

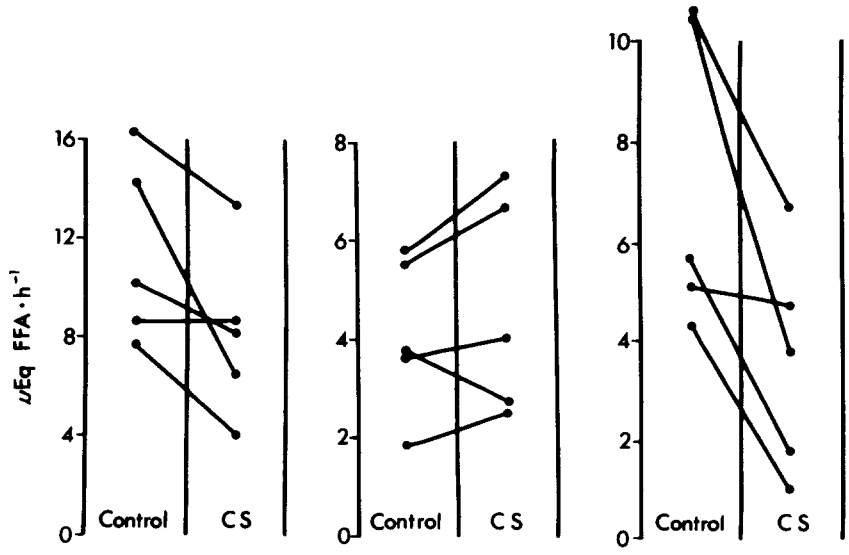

Fig. 2. Total postheparin lipolytic activity (left panel) and lipoprotein lipase (right panel) are decreased in all, and hepatic triglyceride lipase activity (middle panel) is increased in four of five women after 21–24 days of contraceptive steroid use. Note the differences in the vertical axes.

LDL, and especially VLDL (34, 36), and decrease postheparin lipolytic activity and hepatic triglyceride lipase (36, 37). Progestagens lower total HDL and HDL_2 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 HDL_2/HDL_3 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 HDL_2 lipoprotein fraction (28) as well as impaired hydrolysis of core triglycerides of chylomicrons and VLDL and/or increased clearance of HDL_2, 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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