Contraceptive steroids increase cholesterol in bile: mechanisms of action

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Abstract *Contraceptive steroids increase the risk of acquiring* cholesterol gallstones. The factors responsible include an increase in cholesterol saturation of bile and an increase in rate of secretion of cholesterol into bile. The goal of this study was to investigate the mechanism(s) of these increases in biliary cholesterol. During the use of contraceptive steroids, cholesterol saturation of gallbladder bile and the amount of cholesterol secreted per mole of bile acid increased ($P < 0.05$ and $P < 0.02$, respectively). Cholesterol absorption, cholesterol synthesis, chylomicron remnant clearance, and the concentration of plasma and lipoprotein lipids were not altered by contraceptive steroids. Despite this apparent lack of effect, important correlations were present during steroid use. LDL (low density lipoprotein) cholesterol increased as dietary cholesterol increased ($r = 0.58$, $P < 0.025$). Cholesterol synthesis correlated directly with VLDL cholesterol concentration ($r = 0.64$, $P < 0.01$), *biliary cholesterol secretion* ($r = 0.68$, $P < 0.01$) *and with molar* percent cholesterol in bile ($r = 0.49$, $P = 0.06$). Chylomicron remnant clearance also correlated with cholesterol secretion ($r = 0.85$, $P < 0.001$). As either remnant uptake or synthesis increased, the effect of the other source of hepatic cholesterol on biliary cholesterol secretion diminished. These relationships were not observed in the same subjects when they were not taking the hormones. The findings suggest that both newly synthesized and dietary cholesterol contribute to the cholesterol secreted in bile. This is consistent with the hypothesis that cholesterol for secretion into bile and VLDL is derived from a common metabolic pool of free cholesterol. It is proposed that contraceptive steroids exert their effect on biliary cholesterol by increasing cholesterol entering the pool and/or by inhibiting hepatic ACAT (acylcoenzyme A:cholesterol acyltransferase) activity, a known effect of progesterone, so that an increase in free cholesterol entering the pool leads to an increase in output. — **Kern, F., Jr., and G. T. Everson.** Contraceptive steroids increase cholesterol in bile: mechanisms of action. *J. Lipid Res.* 1987. **28:** 828–839.

Supplementary key words cholesterol absorption • cholesterol synthesis • chylomicron remnants • retinyl palmitate • mononuclear leukocytes

The risk of developing cholesterol gallstones is higher in women during their child-bearing years than in men (1). This risk is increased by pregnancy (2, 3), and is directly related to parity (4). Most (5–7) but not all (8) studies show that exposure to oral contraceptive steroids and estrogens (9, 10) also increases the risk of gallstones. Pregnancy (11) and the use of estrogens (10, 12, 13), synthetic progestins (14), and contraceptive steroid mixtures (15, 16) are associated with increases in cholesterol saturation of fasting gallbladder bile and in the rate of cholesterol secretion, especially in relation to bile acid secretion. These metabolic changes are necessary for cholesterol gallstone formation (17). The purpose of the present investigation was to determine the mechanisms of the increased biliary cholesterol saturation and secretion induced by contraceptive steroids.

Biliary secretion of cholesterol could be increased by any or all of several mechanisms: increased absorption of cholesterol, increased hepatic uptake of dietary cholesterol in chylomicron remnants or of endogenous cholesterol in other lipoproteins, increased hepatic synthesis of cholesterol, inhibition of hepatic cholesterol esterification, or alteration in physicochemical coupling of cholesterol to lecithin and/or bile acids during secretion. In this study, we report the effects of contraceptive steroids on chylomicron remnant clearance, intestinal absorption of cholesterol, and cholesterol synthesis. Biliary bile acid composition and bile acid kinetics were also measured in these studies, but will be reported separately.

METHODS

Subjects

Sixteen healthy women were studied on and off contraceptive steroids. All had normal tests of liver function, normal levels of plasma cholesterol and triglyceride, nega-

Abbreviations: ACAT, acylcoenzyme A:cholesterol acyltransferase; GLC, gas–liquid chromatography; HMG-CoA, hydroxymethylglutaryl CoA; HPLC, high performance liquid chromatography; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

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tive HCG tests for pregnancy, and normal blood glucose and thyroid function. Their mean age was 25.5 ± 3.4 yr, body weight 61.0 ± 5.5 kg, and percent ideal body weight 109 ± 16.9 (18) (**Table 1**). The subjects had used contraceptive steroids for 1 to 48 months prior to their participation in the study. The specific hormone preparations, prescribed by their personal physicians, varied but most contained 35 μg of estradiol and 1 mg of norethindrone (Table 1).

Subjects were paid volunteers. They gave written informed consent for the study which was approved by the Human Subject Committee of the University of Colorado School of Medicine.

Procedures

Studies were performed after 6 weeks of discontinuing medication and during the 3rd week of a cycle of contraceptive steroid use. In eight subjects the control study was performed first and in eight the treatment study was performed first. Most procedures, except as noted, were performed as outpatients. Every study was not completed in each subject because of various procedural and technical problems.

Diet

The Clinical Research Center dietician instructed the subjects in a regular American diet containing 400 to 500 mg of cholesterol per day and in the proper maintenance of a daily food diary. The diet and the diary were periodically reviewed by the dietician who estimated the daily cholesterol intake throughout the study period.

Plasma lipids

The concentrations of cholesterol and triglyceride in total plasma and in VLDL, LDL, and HDL fractions were measured in the laboratory of Dr. Seymour Sabesin at the University of Tennessee. Plasma was separated immediately after the blood was drawn and stored at 4°C until mailed to Tennessee. Lipoprotein fractions were separated by ultracentrifugation. The techniques used for separation of lipoproteins and analysis of lipids have been described (19).

Cholesterol absorption

The isotope ratio method was used for estimating the percent cholesterol absorbed from the intestine (20, 21). [4-^{14}C]Cholesterol (57.4 Ci/mmol) and [1,2-^{3}H]cholesterol (47.9 Ci/mmol) (both obtained from New England Nuclear Corp., Boston, MA) were checked for reliability as recommended by Davidson et al. (22). Subjects came to the laboratory after an overnight fast. Blood was drawn for measurement of cholesterol synthesis (see below) and the cholesterol absorption study was initiated. They drank 100 ml of milk containing 2 μCi of [^{14}C]cholesterol dissolved in 1.0 ml of ethanol. The vessel was rinsed several times with small amounts of milk and the rinses were also drunk. At the same time 2 μCi [^{3}H]cholesterol, also in 1.0 ml of ethanol mixed with 50 ml of 0.9% saline, was given intravenously. The tubing and all containers were rinsed with ethanol and Budgetsolve (Research Products International, Mount Prospect, IL), the radioactivity was assayed, and the net amount of radioactivity administered was calculated.

TABLE 1. Characteristics of subjects and composition of contraceptive steroids used

Subject	Age	Weight	Ideal Body Weight[a]	Estrogen		Progestin	
				Drug[b]	Dose	Drug[b]	Dose
	yr	*kg*	*%*	*μg/day*		*mg/day*	
1	28	63.4	109	EE	35	N	1.0
2	25	72.3	141	EE	50	N	1.0
3	28	52.7	108	EE	50	N	1.0
4	25	46.4	95	M	50	N	1.0
5	22	72.8	150	M	50	N	1.0
6	28	66.6	105	EE	30	NG	0.3
7	31	65.5	115	EE	35	N	0.5-1.0
8	22	61.2	112	EE	35	N	0.5-1.0
9	30	57.3	96	EE	35	N	0.5-1.0
10	30	47.7	93	EE	35	N	1.0
11	22	61.6	110	EE	35	N	1.0
12	23	62.0	101	EE	35	N	1.0
13	22	66.4	100	EE	35	N	1.0
14	28	60.0	93	EE	35	N	1.0
15	22	57.3	112	EE	35	N	1.0
16	22	62.7	124	EE	35	N	1.0
Mean ± SD	25.5 ± 3.4	61.0 ± 7.5	109.9 ± 16.9				

[a] Reference 18.
[b] EE, ethinyl estradiol; N, norethindrone; NG, norgestrel; M, mestranol.

Two and again 3 days after isotope administration, when the decay slopes of the [^{14}C]- and [^{3}H]cholesterol were parallel (21), blood was taken and 5.0 ml of plasma was saponified in 95% ethanolic KOH, and extracted with petroleum ether. Cholesterol was eluted from an aluminum oxide column with diethyl ether and acetone (1:1, by vol) and radioactivity was measured in a scintillation spectrometer. Percent absorption was calculated as follows:

$$\% \text{ absorption} = \frac{\text{DPM } ^{14}\text{C in sample}}{\text{DPM } ^{3}\text{H in sample}} \times \frac{\text{DPM } ^{3}\text{H administered}}{\text{DPM } ^{14}\text{C administered}} \times 100.$$

The means of the values for the 2 days were used. The mean variation in the two daily measurements was 3.7 ± 1.1%.

In preliminary studies the "cholesterol" fraction eluted from the alumina column was subjected to thin-layer chromatography (22). Although not all radioactivity migrated with cholesterol, the distribution of ^{14}C and tritium was identical. Accordingly, the specific activity of cholesterol labeled with each isotope was not determined.

Cholesterol synthesis

Sterol synthesis was measured in peripheral blood mononuclear cells using [2-^{14}C]acetate (51 mCi/mmol, New England Nuclear) as substrate essentially as described by McNamara, Davidson, and Fernandez (23). Mononuclear cells (monocytes and lymphocytes) were separated from 15 ml of blood by centrifuging in Ficoll solution with sodium metrizoate (Lymphoprep, Nyegaard & Co., Oslo) and incubated with [^{14}C]acetate (15 dpm/pmol), final concentration of acetate 2.5 mM, in autologous serum for 4 hr. After termination of the incubation with 50% KOH, [1,2-^{3}H]cholesterol was added as internal standard. The incubation mixture was saponified, extracted with petroleum ether, and the sterols were eluted from an aluminum oxide column, as described above, and radioactivity was assayed. All incubations were done in duplicate. The mean variation in the duplicates was 6.2 ± 3.3%. Although several sterols in addition to cholesterol are synthesized by these cells (24), all sterols eluted from the column will be referred to collectively as "cholesterol."

A 0.1-ml sample of the original cell suspension was diluted for a cell count in a hemocytometer and the number of cells was counted three times. Since monocytes synthesize more cholesterol than lymphocytes (25), the percentage of monocytes was determined by counting the esterase-positive cells on a smear of the cell suspension (26). The mean percent of monocytes was 25.4 ± 10.9 during contraceptive steroid use and 26.8 ± 10.4 during the control period.

Sterol synthesis was expressed as pmoles of acetate converted to sterols per hr per 10^7 mononuclear cells.

Biliary lipid composition of gallbladder bile and biliary lipid secretion

Subjects were admitted to the Clinical Research Center for this study and for the study of the hepatic uptake of chylomicron remnants (see below). The procedures and analytical techniques, which have been described in detail (11, 27), were modified only slightly. After placement of a double-lumen tube in the duodenum, a sample of fasting or hepatic bile was collected from the proximal opening in the tube. Gallbladder contraction was then stimulated by intravenous bolus injection of cholecystokinin octapeptide (CCK-OP) (E. R. Squibb, Princeton, NJ) 10 ng/kg in 5 ml of saline. A sample of the darkest bile, called "gallbladder bile," was taken for analysis. A liquid formula mixture was infused into the duodenum through the distal opening in the tube at the rate of 4 ml/min for 8 hr. The mixture contained skim milk powder, safflower or corn oil, and either Polycose® (Ross Laboratories, Columbus, OH) or Summacal® (Organon Pharmaceuticals, West Orange, NJ) as carbohydrate (10 g of protein, 27 g of fat, 15 g of carbohydrate, 82 kcal per 100 ml) with 6 μCi of [^{14}C]PEG 4000 (New England Nuclear, 0.82 mCi/g) as nonabsorbable marker. Duodenal samples were collected continuously and an aliquot of each hourly pooled sample was taken for analysis. Since biliary lipid secretion fluctuates widely during the first 2 hr of the infusion (27, 28), data for the last 6 hr were used in calculating lipid secretion. All duodenal fluid not used for analysis was returned to the subject through the tube.

Radioactivity and the concentrations of bile acid, phospholipid, and cholesterol were determined in all duodenal bile samples. Lipid phosphorus was assayed by the method of Bartlett (29) and bile acids and cholesterol were assayed by gas–liquid chromatography essentially as described previously (11) except that a capillary instead of a glass column was used. The hourly output of each lipid was calculated by standard equations (27, 30) and the molar percent of each lipid and the cholesterol saturation index were calculated (31, 32).

Hepatic uptake of chylomicron remnants

Retinyl palmitate was given by mouth and its plasma concentration was measured for 24 hr. Its clearance was used to estimate the hepatic uptake of chylomicron remnants (33).

The evening before, study subjects were fed a vitamin A-free dinner and fasted thereafter. The next morning they were given a gelatin capsule containing retinyl palmitate (10,000 Iu {3 mg} retinol equivalent/m^2 body surface area) (Roche Chemical Co., Nutley, NJ) dissolved in 0.15 ml of corn oil, with 100 ml of Ensure R (Ross Laboratories, Columbus, OH) (F. Berr and F. Kern, Jr., unpublished data). A standard breakfast was given 1 hr later and followed at appropriate intervals by lunch and

dinner. All meals contained less than 100 Iu of vitamin A. Blood was collected in EDTA before the retinyl palmitate was administered, 1 and 2 hr afterwards, every 30 min for 3 to 7 hr, hourly for 7 to 14 hr, and at 24 hr after administration. Retinyl esters were extracted from plasma, retinyl undecanoate was added as internal standard, and retinyl esters were separated and quantitated by reverse phase high pressure liquid chromatography (33). During the collection, separation, and processing of plasma, care was taken to prevent its exposure to light and oxygen.

The concentrations of retinyl palmitate were plotted against time for 24 hr and, after subtraction of the fasting value, the area under the curve (AUC) was determined by the trapezoidal method. The rate of clearance was calculated as:

$$\text{clearance (ml/min)} = \text{dose/AUC}$$

and was corrected for the mean percent absorption of the administered retinol (92.7%) (F. Berr and F. Kern, Jr., unpublished data) and for the fact that only 71% of the absorbed retinol is esterified with palmitic acid (33).

Statistical methods

The Wilcoxon signed-ranks test for matched pairs was used to compare results obtained on and off contraceptive steroids. A two-tailed test of significance was always used. Some results are presented as means ± one standard deviation. Spearman correlation coefficients and linear regression analyses for single and multiple variables were calculated (34).

Since both cholesterol synthesis and uptake of chylomicron remnant uptake correlated with biliary cholesterol secretion, the interaction between these variables as determinants of biliary cholesterol secretion was investigated by multiple linear regression and the coefficients were used in a restricted second degree equation. This equation was fit to the data by using a cross product term as an additional independent variable.

RESULTS

Dietary cholesterol

The mean intake of cholesterol off contraceptive steroids was 474 ± 84 mg per day (8.14 ± 1.55 mg/kg per day) and on steroids it was 486 ± 75 mg/d (8.16 ± 1.89 mg/kg per day). The range was wide in both study periods. It was 361 to 632 mg/day (5.2 to 10.5 mg/kg per day) off and 333 to 602 (4.6 to 11.8 mg/kg per day) on steroids.

Plasma lipids

Contraceptive steroids did not significantly affect the concentration of cholesterol or triglycerides in whole plasma or in any lipoprotein fraction (**Table 2**), but during their use, LDL cholesterol concentration was correlated with total cholesterol intake ($r = 0.58$, $P < 0.025$), with cholesterol intake adjusted for body weight ($r = 0.71$, $P < 0.005$) and with actual amount of cholesterol absorbed (dietary cholesterol multiplied by the percent absorbed) ($r = 0.71$, $P < 0.005$). Off contraceptive steroids none of these correlations was significant.

Biliary lipid composition and secretion (Figs. 1 and 2)

In the ten subjects in whom these studies were completed on and off medication, gallbladder bile was more saturated with cholesterol on medication ($P < 0.05$). The rate of secretion of bile acid was slightly reduced on contraceptive steroids (39.0 ± 15.4 off and 32.5 ± 12.0 on, $P = 0.06$). The rate of phospholipid secretion was not changed, but the rate of cholesterol secretion was increased in most subjects (1.72 ± 0.67 off steroids to 2.05 ± 0.86 μmoles/kg per hr on steroids, P = ns). The relative molar percent cholesterol was increased from 3.83 ± 0.75 to 5.16 ± 1.48 ($P < 0.05$). The molar percent phospholipid was increased slightly (14.2 ± 1.7 vs. 16.6 ± 2.1, $P < 0.05$), and the relative molar percent of bile acids was reduced (81.6 ± 2.6 vs. 77.6 ± 3.3, $P < 0.03$). The cholesterol secreted per mole of bile acid (the calculated slope of the rate of cholesterol secretion plotted against the rate of bile acid secretion) increased from 0.034 ± 0.009 to 0.052 ± 0.022 ($P < 0.02$).

Biliary lipid secretion was not affected by dietary cholesterol.

Cholesterol absorption and synthesis (Fig. 3)

The absorption of cholesterol varied from 24 to 82% (mean 53.2 ± 12.9) off and from 41 to 67% (55.7 ± 8.0) on contraceptive steroids, unaffected by treatment. Biliary lipid secretion was not significantly associated with percent cholesterol absorbed or the amount of cholesterol absorbed, i.e., dietary cholesterol multiplied by the percent absorbed. Further, cholesterol absorbed did not correlate significantly with cholesterol synthesis rate.

Cholesterol synthesis by mononuclear cells ranged from 27 to 86 (53.2 ± 18.6) picomoles of acetate into sterols/10^7 cells/hr off drug and from 36 to 77 (54.0 ± 15.3) on drug. It was not significantly altered by contraceptive steroids. Despite the apparent lack of contraceptive steroid effect however, several statistically significant correlations were present in subjects on contraceptive steroids that were not present in their absence. The rate of cholesterol synthesis was directly related to the rate of biliary cholesterol secretion ($r = 0.68$, $P < 0.01$) (**Fig. 4**) and was weakly correlated with the molar percent cholesterol in the bile ($r = 0.49$, $P < 0.06$). Cholesterol synthesis was also directly correlated with the concentration of cholesterol in

TABLE 2. Cholesterol and triglycerides in plasma and plasma lipoprotein fractions of subjects off and on contraceptive steroids

	Plasma		Lipoprotein Fraction			
Treatment	Chol	TGL	VLDL Chol	LDL Chol	HDL Chol	VLDL TGL
Off contraceptive steroids						
Mean ± SD	159.2 ± 28.7	54.4 ± 27.4	17.7 ± 11.2	78.8 ± 31.6	50.3 ± 12.8	28.0 ± 18.7
n	15	15	15	13	13	15
On contraceptive steroids						
Mean ± SD	153.8 ± 33.7	66.8 ± 17.4	10.4 ± 6.6	87.3 ± 31.4	47.0 ± 13.4	29.0 ± 10.1
n	15	15	15	12	12	15

Chol, cholesterol; TGL, triglyceride.

VLDL ($r = 0.64$, $P < 0.01$) (**Fig. 5**). Cholesterol synthesis and absorption were not significantly related.

Retinyl palmitate clearance

Contraceptive steroids did not significantly affect the rate of plasma clearance of the orally administered retinyl palmitate (**Fig. 6**). The mean clearance rate was 80.5 ± 24.5 ml/min off and 93 ± 30 on steroids. The rate of retinyl palmitate clearance however, was highly correlated with the rate of biliary cholesterol secretion ($r = 0.85$, $P < 0.001$) (**Fig. 7**) on, but not off, contraceptive steroids.

Interaction between cholesterol synthesis and retinyl palmitate clearance as determinants of biliary cholesterol secretion

Using rates of cholesterol synthesis and retinyl palmitate clearance as independent variables and biliary cholesterol secretion as the dependent variable, the equation below was derived by multiple linear regression:

$$y = -3.229 + 0.0556a - 0.00039ab + 0.0465b$$

where y = rate of cholesterol secretion, a = rate of cholesterol synthesis and b = rate of retinyl palmitate clearance. Each coefficient was significantly different from zero and the fit was better for the second degree equation ($r = 0.97$) than for either first degree equation. As illustrated in **Fig. 8,** the effect of retinyl palmitate clearance on biliary cholesterol secretion diminishes as the rate of cholesterol synthesis increases.

DISCUSSION

The basic goal of this study was to gain insight into the mechanism(s) of the increase in biliary cholesterol that occurs in women exposed to contraceptive steroid hormones. Since there is no ideal experimental animal model of sex steroid-induced cholesterol gallstones that might have allowed a more in-depth exploration of this problem, we examined several aspects of the regulation of cholesterol metabolism in women on and off contraceptive steroid mixtures. The study yielded important information about the effect of these hormones on cholesterol homeostasis and cholesterol secretion into bile. Even though there were no significant effects on total plasma or lipoprotein lipid concentrations, probably because of the small amount and limited potency of the hormones used, contraceptive steroid administration had important effects on the relationships between lipoproteins, cholesterol synthesis, and other features of cholesterol metabolism. Specifically, during the use of contraceptive steroids containing small amounts of estrogen and relatively more progestin, an increase in either cholesterol synthesis or chylomicron remnant uptake by the liver was associated with an increase in biliary cholesterol secretion (Figs. 4 and 7). Furthermore, changes in either synthesis or uptake lead to a reciprocal change in effect of the other on biliary cholesterol (Fig. 8). An increasing rate of cholesterol synthesis was also associated with increasing VLDL cholesterol (Fig. 5).

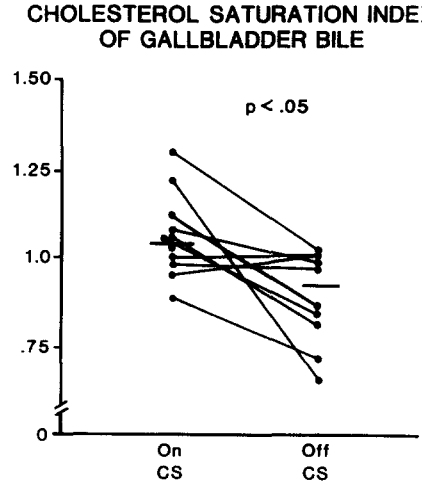

Fig. 1. The cholesterol saturation index in subjects on and off of contraceptive steroids (CS). In this and similar figures the horizontal lines represent the means.

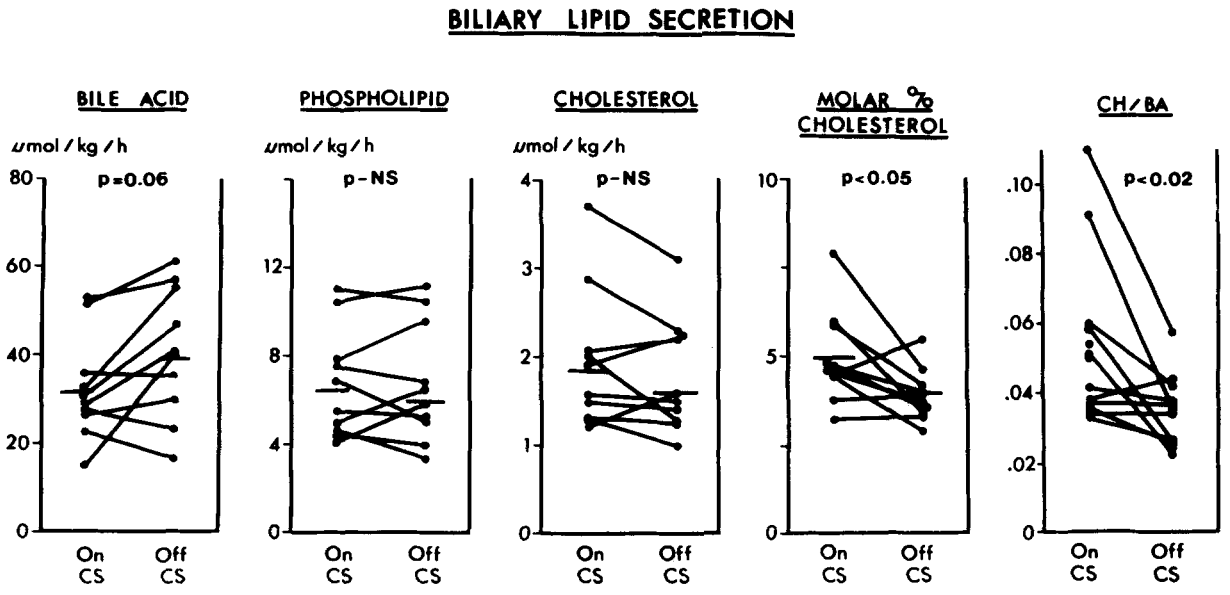

Fig. 2. The first three panels on the left show the rate of secretion of bile acids, phospholipids, and cholesterol in subjects on and off contraceptive steroids (CS). The effect of contraceptive steroids on molar percent cholesterol and the molar ratio of cholesterol to bile acids during the secretion study is shown in the two panels on the right.

Our ability to examine these regulatory mechanisms of cholesterol metabolism in ambulatory, healthy volunteers in an acceptable, safe, and accurate manner is the result of the recent development and validation of appropriate methods for the study of cholesterol absorption, cholesterol synthesis, and hepatic chylomicron remnant uptake.

The isotope ratio method for measuring cholesterol absorption was introduced in 1972 (20), but the more recent demonstration that the decay slopes of orally and intravenously administered labeled cholesterol become parallel in only 2 to 3 days (21) made the method ideal for this study. Measurement of cholesterol synthesis in freshly isolated blood mononuclear leukocytes is also a suitable method for this type of study. Although the activity of HMG-CoA reductase in freshly isolated mononuclear leukocytes is about one-fourth of its activity in human liver (35), cholesterol synthesis by these cells varies in a manner parallel to hepatic cholesterol synthesis. It is inhibited by cholesterol feeding, starvation, and exposure to LDL, and increased by cholestyramine (23, 36). Freshly isolated monocytes synthesize cholesterol more actively than lymphocytes (25), have twice the activity of HMG-CoA reductase (35), and exhibit LDL receptor activity, that is down-regulated by plasma LDL (37). It is not known whether female sex hormones affect sterol synthesis in either type of cell, but these hormones have many effects on other functions of both monocyte/macrophages and lymphocytes in man and animals (38, 39).

A suitable method for measuring chylomicron remnant uptake using an orally administered label was developed in our laboratory (33, 40) for this and related studies. The validity of the assumptions that form the basis for the procedure has been established by studies in several laboratories and has been recently summarized (F. Berr and F. Kern. Jr., unpublished data).

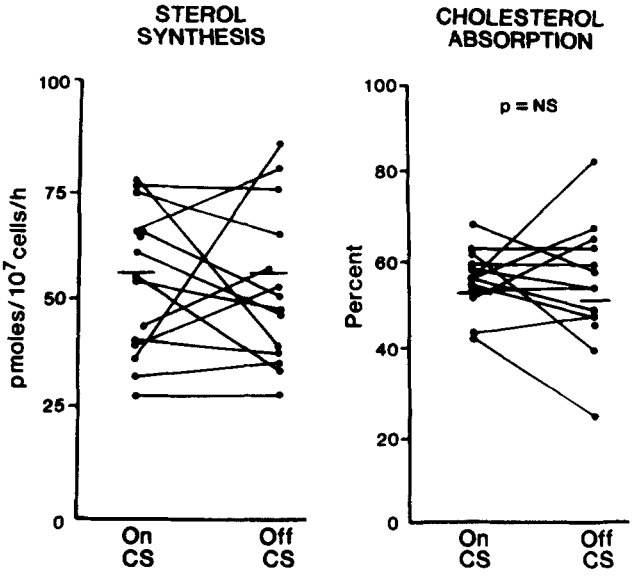

Fig. 3. Lack of effect of contraceptive steroids (CS) on cholesterol synthesis by peripheral blood mononuclear cells and on intestinal cholesterol absorption.

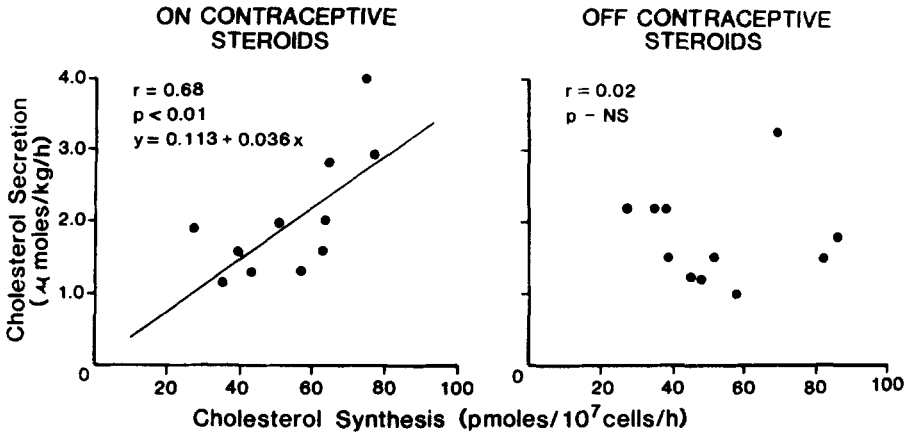

Fig. 4. The rate of biliary cholesterol secretion is plotted against the rate of cholesterol synthesis. A significant correlation is present on (left) but not off (right) contraceptive steroids.

Biliary lipids

During stimulated bile secretion, the rate of output of cholesterol increased in 9 of 12 subjects (P = not significant); the relative molar percent cholesterol and the molar ratio of cholesterol to bile acids increased significantly ($P < 0.05$ and < 0.02, respectively, Fig. 2). These changes and the increased saturation index of gallbladder bile ($P < 0.05$) are the initial and necessary metabolic disturbances in the formation of cholesterol gallstones. All of the changes in biliary lipids were less marked than they were in our earlier study (16) and in the studies of Bennion, Mott, and Howard (15). The mean saturation index of gallbladder bile increased only 12.5% in this study, compared with an increase of 34% in our first study. Biliary secretion of cholesterol increased 19% compared with 50%, and the cholesterol secreted per mole of bile acid rose 51% compared with 84% in the first study. The most probable reason for these differences is that in this study most subjects used contraceptive steroids containing less estrogen (35 μg) than in the previous studies (50 μg).

Regulation of cholesterol metabolism and its relationship to biliary cholesterol

Contraceptive steroids did not affect the rate of cholesterol synthesis in mononuclear leukocytes, but synthesis rate correlated directly with the biliary cholesterol secretion (Fig. 4). This finding is consistent with the

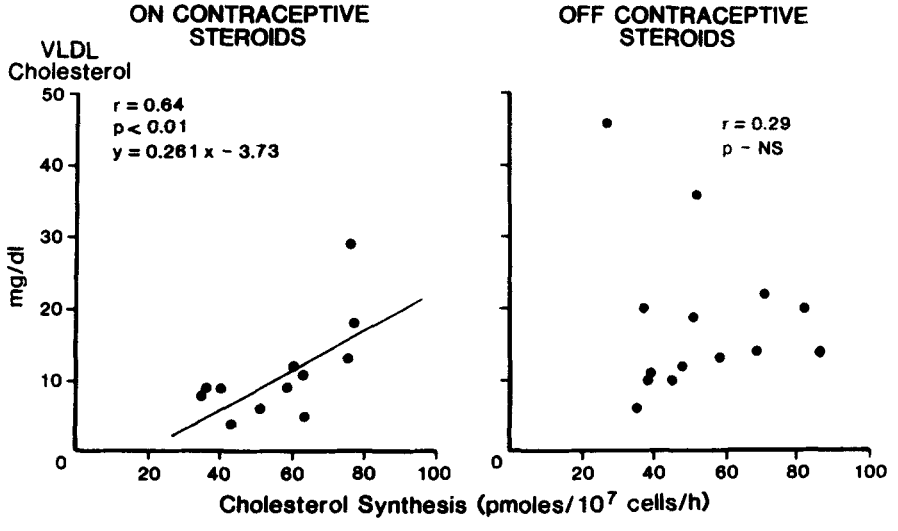

Fig. 5. VLDL cholesterol concentration is directly correlated with the rate of cholesterol secretion only during exposure to contraceptive steroids.

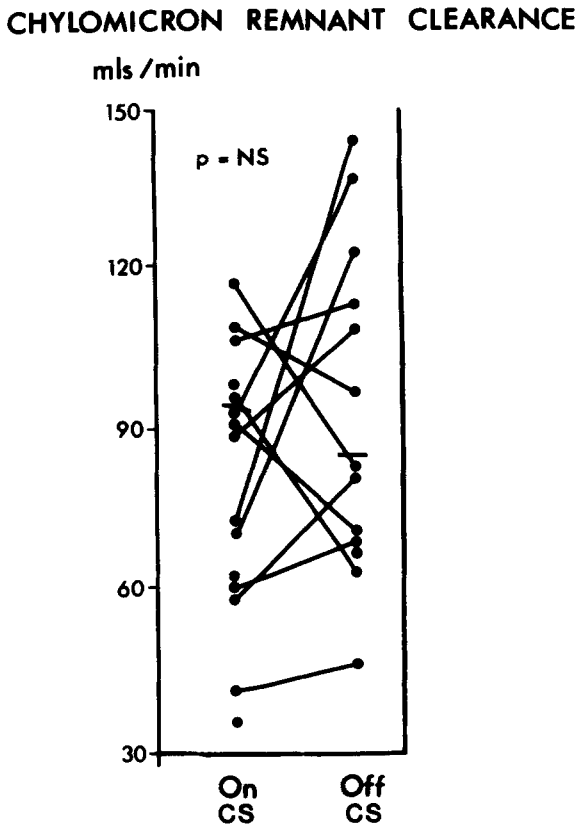

Fig. 6. The rate of chylomicron remnant clearance is not significantly affected by contraceptive steroids (CS).

proposal that the increased biliary cholesterol induced by female sex hormones is, at least in part, newly synthesized cholesterol. In addition, the significant relationship between cholesterol synthesis and cholesterol concentration in plasma VLDL during intake of contraceptive steroids (Fig. 6) suggests that newly synthesized cholesterol contributes to both VLDL and biliary secretion.

Studies of the contribution of newly synthesized cholesterol to biliary cholesterol vary. In man (4) and rats (42) only 10–20% of biliary cholesterol is newly synthesized. However, the findings in the rat that newly synthesized cholesterol is preferentially secreted into bile (43) and that inhibition of HMG-CoA reductase with mevinolin reduces biliary cholesterol secretion (44) suggest that newly synthesized cholesterol might contribute more to biliary cholesterol than previously believed. On the other hand, since large variations in cholesterol synthesis have little effect on the secretion of cholesterol in the bile in the rat (45) or hamster (46), it is likely that only a small fraction of newly synthesized cholesterol is required to satisfy the requirements for biliary secretion. In two human studies, cholesterol synthesis and biliary secretion of cholesterol were significantly associated (47, 48).

Contraceptive steroids did not alter the rate of plasma clearance of chylomicron remnants, but remnant clearance was directly and strongly correlated with the rate of biliary cholesterol secretion (Fig. 7). This association, present only when the subjects were taking the hormones, suggests that part of the contraceptive steroid-induced increase in biliary cholesterol secretion is derived from the diet. Moreover, the reciprocal relationship between chylomicron remnant uptake and cholesterol synthesis in their stimulation of cholesterol secretion (Fig. 8) is consistent with the known inhibition of cholesterol synthesis by chylomicron remnant cholesterol (49). On the other hand, it is not clear how increases in cholesterol synthesis would decrease biliary cholesterol derived from remnant uptake. It could be postulated that the rate of de novo cholesterol

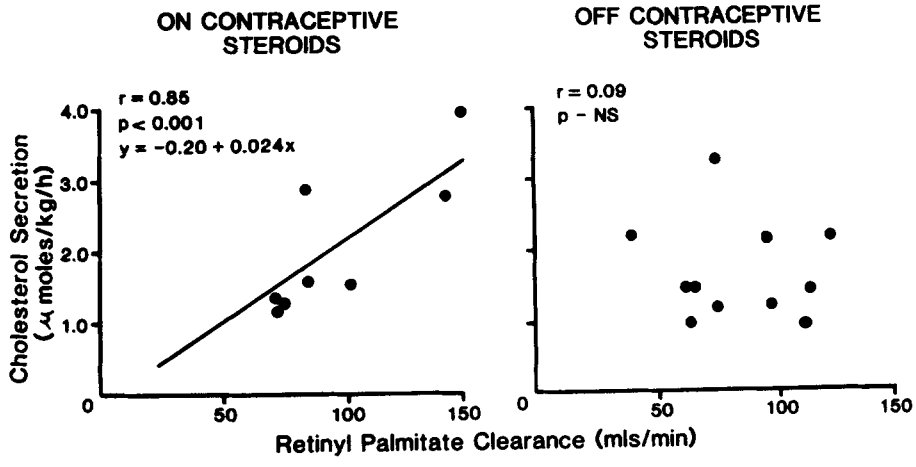

Fig. 7. Biliary cholesterol secretion is plotted against the rate of retinyl palmitate clearance in subjects on and off contraceptive steroids.

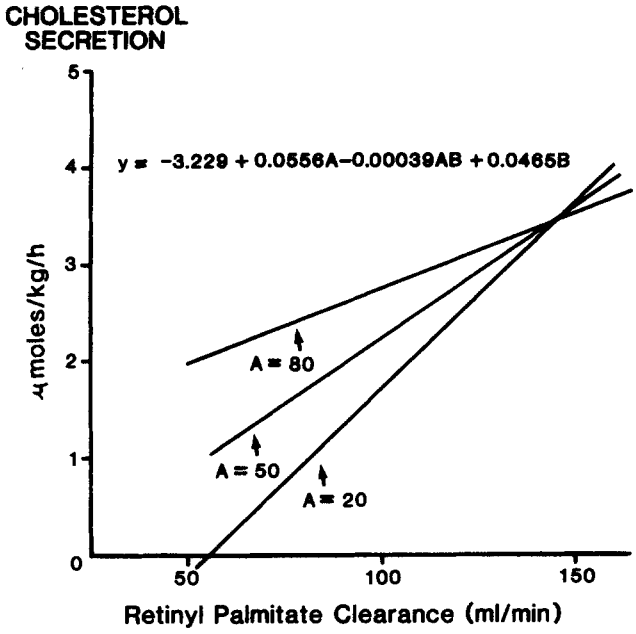

Fig. 8. This graph shows the estimated biliary cholesterol secretion rate (y) as a function of retinyl palmitate clearance (B) for several values of cholesterol synthesis (A). When A = 20 pmol of sterol synthesized per 10^7 mononuclear cells per hr, the slope of the regression line is 0.04 μmol of cholesterol secreted into the bile per ml of plasma cleared of retinyl palmitate per min. When A = 50, the slope is 0.03 and when A = 80, the slope is 0.02. The progressive decrease in the slope with increasing rates of cholesterol synthesis indicates that changes in hepatic uptake of chylomicron remnants (retinyl palmitate clearance) have less effect on biliary cholesterol secretion when cholesterol synthesis is high than when it is low.

synthesis regulates remnant uptake, but direct support for this proposal is lacking. Thus, cholesterol derived from either de novo synthesis or chylomicron remnant uptake contributes to biliary cholesterol in the expected reciprocal manner.

The following hypothesis is proposed to explain the findings of this study (**Fig. 9**). Hepatic free cholesterol is the source of cholesterol for secretion into the bile and into VLDL. This cholesterol is derived from de novo synthesis, uptake of chylomicron and VLDL remnants and other lipoproteins, and hydrolysis of cholesteryl esters. Several processes compete for this pool: synthesis of bile acids, secretion of VLDL, secretion of cholesterol into the bile, and esterification, catalyzed by ACAT. (Since hepatocytes are normally quiescent, the amount of cholesterol needed for membrane biogenesis is small and is ignored in this discussion.) We propose that the size of the free cholesterol pool is insufficient to supply all of the cholesterol needed for these competing processes. Therefore, an increase in input to the pool should lead to a comparable increase in output until all of the processes become saturated with cholesterol. Contraceptive steroids affected hepatic cholesterol metabolism in such a manner that an increase in either synthesis or remnant uptake led to an increase in secretion into bile and into VLDL. We also propose that contraceptive steroids affect specific processes resulting in specific alterations in cholesterol flux.

Estrogens could affect entry of cholesterol into the pool by stimulating the uptake of LDL (50) and/or chylomicron remnants (51), and possibly by stimulating ACAT activity (52). The latter has been reported only with pharmacologic doses of estrogens (52). Although large doses of estrogens induce the specific hepatic LDL receptor (the B,E receptor), the effect of the small dose used here is not known. The LDL cholesterol level did not change in this or in another study of women given oral contraceptives containing 35 μg of ethinyl estradiol (53) and it did not significantly correlate with biliary cholesterol secretion. Even though 5 μg of ethinyl estradiol can stimulate synthesis of several hepatic proteins (54), there is no direct evidence supporting an increase in hepatic uptake of LDL cholesterol in this study.

It is generally believed that the chylomicron remnant receptor (the E receptor) is not induced by estrogens (50). However, since chylomicron remnants can be recognized by both the B,E and E receptors (55), induction of either should promote chylomicron clearance. Several studies support this concept. In rabbits (51) and in hypertriglyceridemic humans (56), modest doses of estrogens augmented chylomicron clearance. In an earlier study in our laboratory using intravenously administered autologous plasma containing chylomicrons labeled with retinyl palmitate, we found that contraceptive steroids (containing 50 μg of estrogens) caused a 67% increase in rate of clearance of chylomicron remnants and a 100% increase in rate of removal of the labeled LDL (57). The reason for the failure to find a similar effect of contraceptive steroids

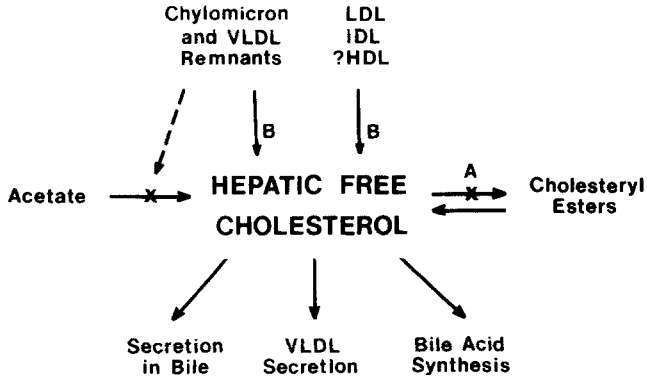

Fig. 9. Diagram illustrating the proposed effects of contraceptive steroids on hepatic cholesterol metabolism. "A" represents the possible effects of estrogen and "B," progestin. When ACAT activity is inhibited by progestin, an increase in cholesterol input to the free cholesterol pool either in chylomicron remnants or other lipoproteins causes a proportionate increase in output from the pool into biliary cholesterol or VLDL. The broken line shows the down-regulation of cholesterol synthesis by remnant cholesterol.

in this study is not certain, but it is probably due to the lower dose of estrogen used.

Progesterone inhibits hepatic ACAT activity (44, 58, 59) and in the rat increases biliary cholesterol secretion after both acute (44) and chronic (2 to 7 days) administration (59), consistent with the above hypothesis. Pharmacologic doses of progesterone were used in both studies. The effect of a small dose of a synthetic progestin is not known, but suppression of ACAT activity would decrease this pathway for disposition of cholesterol.

Although contraceptive steroid administration did not affect the rate of chylomicron remnant clearance or cholesterol synthesis, it altered hepatic cholesterol metabolism in a manner that uncovered the relationships between remnant uptake and cholesterol synthesis and biliary cholesterol secretion. Any increase in cholesterol to the pool from either synthesis or remnant uptake in the presence of inhibited ACAT activity would lead to increased output of cholesterol by secretion into the bile, incorporation into VLDL, and/or increased bile acid synthesis.

In summary, during exposure to female sex steroid hormones, even the small doses taken by most of these subjects, biliary secretion of cholesterol directly correlated with cholesterol synthesis and the hepatic removal of chylomicron remnants from the plasma, suggesting that both newly synthesized cholesterol and the hepatic uptake of dietary cholesterol contribute to the increased biliary cholesterol. As the contribution from either of these sources of biliary cholesterol increased, the contribution from the other source decreased. Thus, contraceptive steroids appear to regulate fluxes of cholesterol into and out of the hepatic pool of free cholesterol that supplies cholesterol for biliary secretion. These findings are consistent with known effects of estrogens and progestins.

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