

Contraceptive steroids alter the steady-state kinetics of bile acids

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Abstract Contraceptive steroids increase the ratio of cholic acid to chenodeoxycholic acid in bile. This alteration may contribute to the development of cholesterol gallstones. The objective of this study was to measure the effects of contraceptive steroids on bile acid kinetics and to relate them to changes in cholesterol metabolism. Steady-state kinetics of bile acids were measured in 15 healthy women, on and off contraceptive steroids. Cholic acid synthesis increased 30.3% ($P < 0.025$) and its pool increased by 37.4% ($P < 0.025$). Chenodeoxycholic acid synthesis decreased 6.4% ($P = 0.08$) and its pool decreased by 11.8% ($P < 0.05$) during use of contraceptive steroids. The fractional turnover rates of both primary bile acids did not change. The changes in kinetics of the primary bile acids were related to alterations in biliary lipid and cholesterol metabolism, separately reported (*J. Lipid Res.* 1987. **28**: 828–839). During use of contraceptive steroids, total bile acid pool and total bile acid synthesis correlated directly with cholesterol synthesis, assayed in mononuclear leukocytes ($r = 0.50$ and $r = 0.54$, respectively) but not with the plasma clearance of chylomicron remnants, measured with retinyl palmitate. The data indicate that contraceptive steroids directly alter the hepatic synthesis of bile acids and suggest that newly synthesized cholesterol may be a preferred substrate for bile acid synthesis during use of contraceptive steroids.—**Everson, G. T., P. Fennessey, and F. Kern, Jr.** Contraceptive steroids alter the steady-state kinetics of bile acids. *J. Lipid Res.* 1988. **29**: 68–76.

Supplementary key words female steroid hormones • gas-liquid chromatography-mass spectrometry • stable isotopes • cholesterol gallstones • biliary lipid metabolism

Women are at increased risk of developing cholesterol gallstones compared to men (1, 2). This risk is increased further by pregnancy (3), use of contraceptive steroids (CS), conjugated estrogen, diethylstilbesterol, and ethinyl estradiol (4–7). The overall goal of our studies has been to elucidate mechanisms whereby female steroid hormones increase the risk of gallstones.

A prerequisite for cholesterol gallstone formation is the hepatic secretion of bile that is supersaturated with cholesterol (8, 9). Although nucleation of cholesterol crystals, gallbladder mucin secretion, stasis of bile within the gallbladder, and agglomeration of crystals may pro-

mote stone formation (10), the secretion of excess cholesterol in bile is a critical initial step.

Recent studies have shown that contraceptive steroids (CS) (11, 12) and conjugated estrogen (13) increase biliary cholesterol secretion. Contraceptive steroids may increase biliary cholesterol by reducing the rate of secretion of bile acid, altering bile acid composition (14–18), increasing the uptake of lipoprotein cholesterol (19, 20), inhibiting cholesteryl ester formation (21–23), or increasing the hepatic synthesis of cholesterol (24). Studies from our laboratory (11) and those of Bennion et al. (6) suggest that CS increase the ratio of cholic acid to chenodeoxycholic acid in bile, and that this increase may be related to increased biliary cholesterol secretion.

The specific aims of this study were to determine the effects of CS on the steady-state kinetics of bile acids and to relate them to changes in biliary lipid and cholesterol metabolism.

METHODS

The study was approved by the Human Subjects Committee of the University of Colorado School of Medicine. Studies were conducted in the NIH-sponsored Clinical Research Center of University Hospital, University of Colorado School of Medicine. All subjects were paid volunteers and gave written informed consent.

Subjects

Fifteen healthy women between the ages of 21 to 32 yr (25.6 ± 3.7 yr) and % ideal weight of 93 to 185% ($115 \pm 23\%$), completed paired studies of bile acid kinetics. Three additional subjects were studied, but two of the stud-

Abbreviations: CDCA, chenodeoxycholic acid; CA, cholic acid; DCA, deoxycholic acid; GLC-MS, gas-liquid chromatography-mass spectrometry; IR, isotope ratio; CS, contraceptive steroids; FTR, fractional turnover rate.

ies could not be analyzed because of technical difficulties with the sample preparation. Kinetics in the other subject could not be accurately measured due to an exceedingly high bile acid turnover rate and low isotopic enrichment of the bile acid pool. The characteristics of these patients as well as the effects of CS on biliary lipid secretion, cholesterol synthesis and absorption, and the clearance of chylomicron remnants are reported separately (25). Most subjects used CS containing 35 μg of ethinyl estradiol plus 1 mg of norethindrone. The specific contraceptive preparations used were Norinyl 1 + 35 ($n = 5$, Syntex Laboratories Inc., Palo Alto, CA), Norinyl 1 + 50 ($n = 1$, Syntex Laboratories Inc., Palo Alto, CA), Lo-Ovral ($n = 1$, Wyeth Laboratories, Philadelphia, PA), Ortho-Novum 1/35 ($n = 4$, Ortho Pharmaceutical, Raritan, NJ), Ortho-Novum 10/11 ($n = 2$, Ortho Pharmaceutical, Raritan, NJ), Ovcon-50 ($n = 1$, Mead Johnson Laboratories, Evansville, IN) and Modicon EE ($n = 1$, Ortho Pharmaceutical, Raritan, NJ).

Study protocol

Each subject was studied during the third week of a 3-week cycle on CS and 1 to 2 months after CS were discontinued. Eight subjects were first studied off CS and seven were first studied on CS. They had been using CS for 1 month to 4 yr. Each study was 6 days. *Day 1*: fasting blood samples were obtained for measurement of bilirubin, alkaline phosphatase, aspartate aminotransferase, complete blood count, thyroid function tests, and serum lipids. A 2-hr postprandial blood sample was obtained for measurement of the natural abundance of the isotope ratios of serum bile acids. Once the initial blood samples were obtained, stable isotope-labeled bile acids were administered orally.¹ *Days 2 to 6*: 10-ml blood samples were obtained 2 hr after ingestion of a fatty meal for subsequent measurement of bile acid isotope ratios. During this period of time subjects also underwent studies of cholesterol synthesis by peripheral blood monocytes (26), oral retinyl palmitate clearance (25), and cholesterol absorption by a dual isotope technique (27). Gallbladder bile composition and biliary lipid secretion were usually measured (28, 29) on the final day to avoid altering the steady state.

Analytical methods and calculations

Serum bile acid kinetics. A recently published method was used (30). In brief, bile acids were extracted from serum

¹The stable isotope-labeled bile acids used in this study included [24-¹³C]chenodeoxycholic acid (CDCA), [11,12-²H₂]chenodeoxycholic acid, [24-¹³C]cholic acid (CA), and [2,2,4,4-²H₄]cholic acid. Each subject took one isotope of CDCA and one isotope of CA. The same isotopes were used for both "on" and "off" studies in a given subject. In addition, samples from both on and off studies for a given patient were always simultaneously processed and analyzed by GLC-MS.

using C18-liquid chromatographic cartridges (Sep-Pak C18®, Waters Associates, Milford, MA). After elution from the C18-cartridges, samples underwent acid solvolysis using dimethoxypropane, enzymatic hydrolysis using cholyglycine hydrolase, methylation using dimethoxypropane, methanol, and concentrated HCl. Further purification was achieved using a C8-liquid chromatographic cartridge (Baker 10, J. T. Baker Chemical Company, Phillipsburg, NJ). Bile acid methyl esters were then derivatized to their trimethylsilyl ethers. The isotope ratios of CDCA and CA were determined by selected ion monitoring using a benchtop capillary GLC-MS system (Model #HP #5790/5970B, Hewlett Packard, Englewood, CO).

The molar ratio of labeled to unlabeled bile acid in each sample (MR_t) was determined from the enrichment in isotope ratio above natural abundance by use of appropriate standard curves. MR_t exhibited monoexponential decay in all studies such that

$$\text{MR}_t = \text{MR}_0 e^{-kt} \quad (\text{Eq. 1})$$

where MR_0 is the molar ratio of labeled to unlabeled compound at $t = 0$, and k is the fractional turnover rate (FTR). Thus, for CDCA and CA, the FTR was calculated from Ln/linear regression of MR_t versus t . Pool size was calculated from MR_0 , and steady-state synthesis was the product of pool and FTR. DCA pool was estimated from the same GLC-MS runs by comparison of the ion intensity of its 370 ion to the intensity of the 370 ion of CDCA.

Statistical methods. The significance of differences between on and off studies was evaluated by Wilcoxon non-parametric tests for paired data (31). The plots of LnMR_t versus t were fit by single linear regression analysis using the method of least squares. Relationships between measured variables were assessed by both single and multiple linear regression analysis (32).

RESULTS

Evaluation of the serum method for measuring bile acid kinetics

A prerequisite for the accurate measurement of bile acid kinetics is that the measurements of the natural abundance isotope ratios from baseline sera must equal the isotope ratios of pure bile acid standards. As shown in **Table 1**, the isotope ratios of bile acids isolated from the serum of study subjects equaled those of pure standards. In accordance with the initial observation of Lindstedt (33) and subsequent studies by others (34), the administered labeled bile acids disappeared from the bile acid pool in a monoexponential fashion in all subjects both on and off CS. The correlation coefficients of all plots of $\text{Ln}(\text{MR})$ versus t were greater than 0.87 (CDCA:

TABLE 1. Isotope ratios of chenodeoxycholic acid and cholic acid from sera (natural abundance) compared to those of pure standards (n = 31 samples for each isotope ratio)

	Isotope Ratios			
	Chenodeoxycholic Acid		Cholic Acid	
	371/370	372/370	459/458	462/458
Basal sera, mean ± SD	0.3116 ± 0.0023	0.0512 ± 0.0017	0.3595 ± 0.0036	0.0037 ± 0.0004
Standards, mean ± SD	0.3114 ± 0.0016	0.0510 ± 0.0007	0.3621 ± 0.0017	0.0037 ± 0.0002

0.98 ± 0.03 (on) vs. 0.98 ± 0.01 ± 0.01 (off); CA: 0.94 ± 0.09 (on) vs. 0.97 ± 0.03 (off).

Bile acid pool size (Fig. 1). Total bile acid pool (μmol/kg) increased slightly but not significantly on CS (58.0 ± 18.7 (off) vs. 69.1 ± 29.9 (on), *P*, NS). However, there were significant alterations in individual bile acid pools. CDCA pool (μmol/kg) decreased (20.7 ± 6.6 (off) vs. 18.3 ± 6.1 (on), *P* < 0.05), CA pool (μmol/kg) increased (20.2 ± 9.5 (off) vs. 27.7 ± 13.5 (on), *P* < 0.025), and DCA pool (μmol/kg) increased, but not significantly (17.1 ± 5.7 (off) vs. 23.0 ± 13.4 (on)). Thus, contraceptive steroids increased the CA:CDCA ratio of pools by 52% (1.00 ± 0.50 (off) vs. 1.52 ± 0.55 (on), *P* < 0.01) and the [CA+DCA]:CDCA ratio of pools by 51% (1.86 ± 0.73 (off) vs. 2.75 ± 0.90 (on), *P* < 0.01).

Bile acid synthesis (Fig. 2). Total bile acid synthesis (μmol/kg per day) increased slightly on CS (12.5 ± 4.4 (off) vs. 14.5 ± 5.0 (on), *P* < 0.05). CDCA synthesis (μmol/kg per day) decreased (4.8 ± 1.5 (off) vs. 4.5 ± 1.0 (on), *P* = 0.08) and CA synthesis (μmol/kg per day) increased (7.7 ± 3.4 (off) vs. 10.0 ± 4.5 (on), *P* < 0.025). The magnitude of the mean decrease in synthesis of CDCA (6.4%) was similar to the mean decrease in CDCA pool (11.8%); likewise the increase in CA synthesis (30.3%) paralleled the increase in CA pool (37.4%).

Bile acid turnover. The turnover rates of CDCA and CA were not altered by contraceptive steroids: CDCA (d⁻¹): 0.27 ± 0.12 (off) versus 0.27 ± 0.10 (on), *P*, NS; CA (d⁻¹): 0.48 ± 0.28 (off) versus 0.41 ± 0.17 (on), *P*, NS.

Lipid composition of gallbladder bile. Contraceptive steroids increased the relative biliary cholesterol concentration of gallbladder bile whether expressed as molar % cholesterol (3.83 ± 0.75 (off) vs. 5.16 ± 1.48 (on), *P* < 0.05) or lithogenic index (0.94 ± 0.16 (off) vs. 1.09 ± 0.16 (on), *P* < 0.03). In addition, the relative percent of CDCA in bile decreased (38.8 ± 5.6 (off) vs. 33.4 ± 5.7 (on), *P* < 0.05), % CA increased (33.9 ± 5.0 (off) vs. 41.5 ± 7.5 (on), *P* < 0.05), and % DCA did not change (27.2 ± 7.1 (off) vs. 25.0 ± 7.7 (on), *P*, NS). The CA:CDCA and [CA + DCA]:CDCA ratios as calculated from the composition of gallbladder bile increased on CS (1.12 ± 0.36 (off) vs. 1.41 ± 0.25 (on), *P* < 0.01, and 1.91 ± 0.74 (off) vs. 2.29 ± 0.41 (on), *P* < 0.03, respectively). The changes in these ratios as calculated from biliary

composition are similar to those obtained from pools (see above).

Biliary lipid secretion (μmol/kg per hr). Contraceptive steroids reduced bile acid secretion (39.0 ± 15.4 (off) vs. 32.5 ± 12.0 (on), *P* < 0.05), increased cholesterol secretion (1.72 ± 0.67 (off) vs. 2.05 ± 0.86 (on), *P* = 0.08), and did not alter rates of phospholipid secretion. Changes in the secretion rates of individual bile acids reflected both the decrease in overall bile acid secretion and the divergent alterations in individual pools. Thus, the rate of secretion of CDCA, whose pool was reduced, was markedly lowered by CS (14.5 ± 7.3 (off) vs. 10.1 ± 5.3 (on), *P* < 0.005). The rate of secretion of CA whose pool increased during use of CS, was unchanged. The rate of secretion of DCA, whose pool did not change during use of CS, tended to decrease (9.9 ± 5.1 (off) vs. 7.3 ± 3.4 (on), *P* = < 0.08).

Enterohepatic cycling frequency (daily secretion rate/pool). The number of enterohepatic cycles per day of the total bile acid pool and of each bile acid decreased on contraceptive steroids (total: 15.4 ± 6.8 (off) vs. 11.7 ± 5.4 (on), *P* < 0.05; CDCA: 16.9 ± 6.1 (off) vs. 13.6 ± 7.7 (on), *P*,

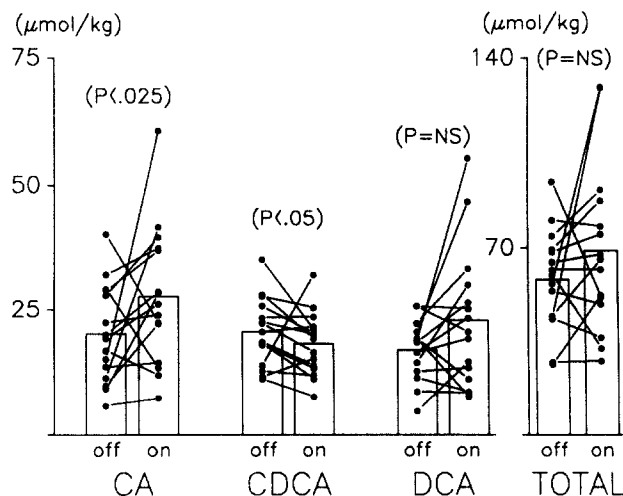


Fig. 1. Bile acid pool size off and on contraceptive steroids. CS increased the CA pool and decreased the CDCA pool; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; total, CA + CDCA + DCA.

NS; CA: 17.0 ± 6.1 (off) vs. 13.6 ± 7.7 (on), P , NS; DCA: 15.0 ± 6.6 (off) vs. 8.2 ± 3.2 (on), $P < 0.02$.

Cholesterol synthesis, cholesterol absorption and retinyl palmitate clearance were not altered by contraceptive steroids (25). The means \pm SD of these measurements are given: cholesterol synthesis pmol of [14 C]acetate incorporated into sterols per hr per 10^7 mononuclear cells, 53.2 ± 18.6 (off) versus 54.0 ± 15.3 (on); cholesterol absorption, %, 53.2 ± 12.9 (off) versus 55.7 ± 8.0 (on); retinyl palmitate clearance, ml/min, 80.5 ± 24.5 (off) versus 93.0 ± 30.0 (on).

Relationships of bile acid kinetics to other measurements of biliary lipid metabolism: relationships on and off CS

During use of contraceptive steroids, cholesterol synthesis was significantly related to total bile acid pool, CA pool, and [CA+DCA] pool (Fig. 3 A-C). In addition, cholesterol synthesis was directly related to total bile acid (Fig. 4A) and cholic acid synthesis (Fig. 4B). There were no significant relationships between bile acid pool or synthesis and the clearance of orally administered retinyl palmitate (Table 2).

The increase in [CA + DCA]:CDCA ratio on contraceptive steroids was inversely related to the duration of use of CS (Fig. 5), suggesting that the change in bile acid composition was greatest during initial use of CS. On the other hand, biliary cholesterol was not related to duration of use of CS. There was no significant relationship between the increase in biliary cholesterol and the increase in either CA:CDCA or [CA + DCA]:CDCA ratios that occurred with use of CS.

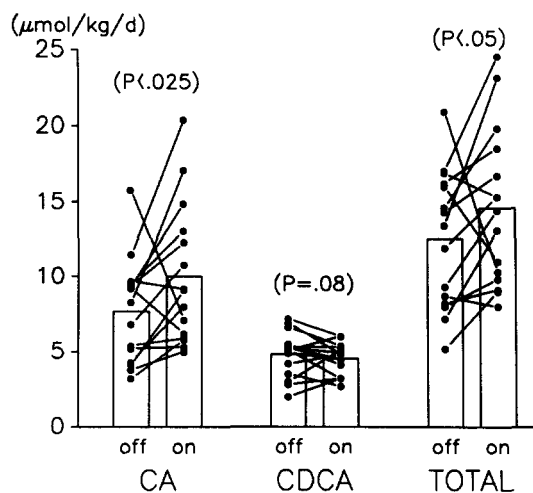


Fig. 2. Bile acid synthesis off and on contraceptive steroids (CS). CS significantly increased CA synthesis. Although slight, the decrease in CDCA synthesis was also significant. CA, cholic acid; CDCA, chenodeoxycholic acid; total, CA + CDCA.

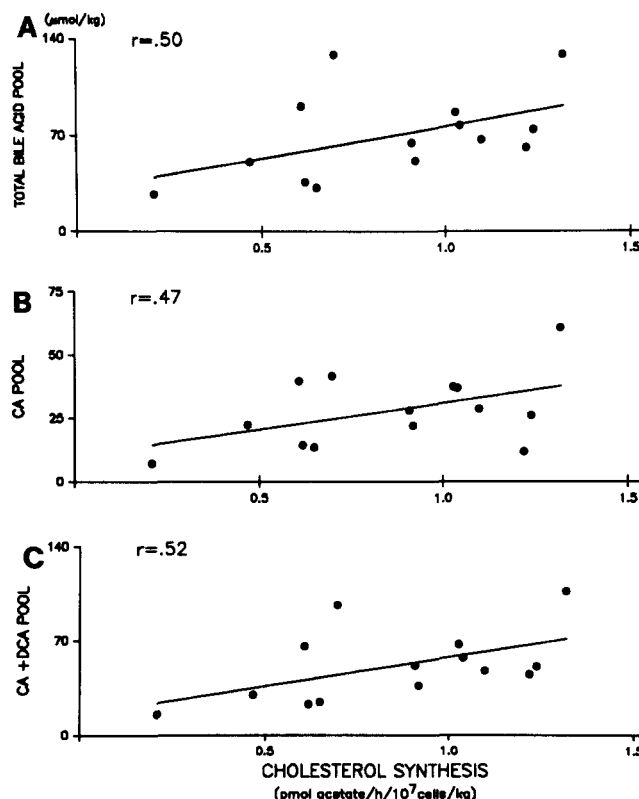


Fig. 3. Correlations of bile acid pool during use of CS with cholesterol synthesis by peripheral blood monocytes. Total bile acid pool (A), CA pool (B), and the sum of CA + DCA pools (C), directly correlated with the rate of cholesterol synthesis; CA, cholic acid; DCA, deoxycholic acid; r , correlation coefficient.

DISCUSSION

Evaluation of study and methods

We used a paired design in this study to measure changes in bile acid and biliary lipid metabolism induced by contraceptive steroids. A paired design is desirable since interindividual variations in the measurements of steady-kinetics of bile acids may be marked (34). For example, the small decreases in CDCA synthesis and pool observed in the present study were not detected in our previous study (11) using an unpaired design.

Several other features of the present study are noteworthy. The number of subjects studied is greater than that in any previous study of the effects of contraceptive steroids on bile acid kinetics. We previously reported results from eight women, Down et al. (12) reported results from ten women, Bennion, Mott, and Howard (35) studied five women, and Pertsemliadis, Panveliwalla, and Ahrens (4) evaluated only two women. Unlike other studies (4, 12, 35) the composition and dose of both the estrogen and progestin in the oral contraceptives used in this study were relatively uniform. Finally, and most im-

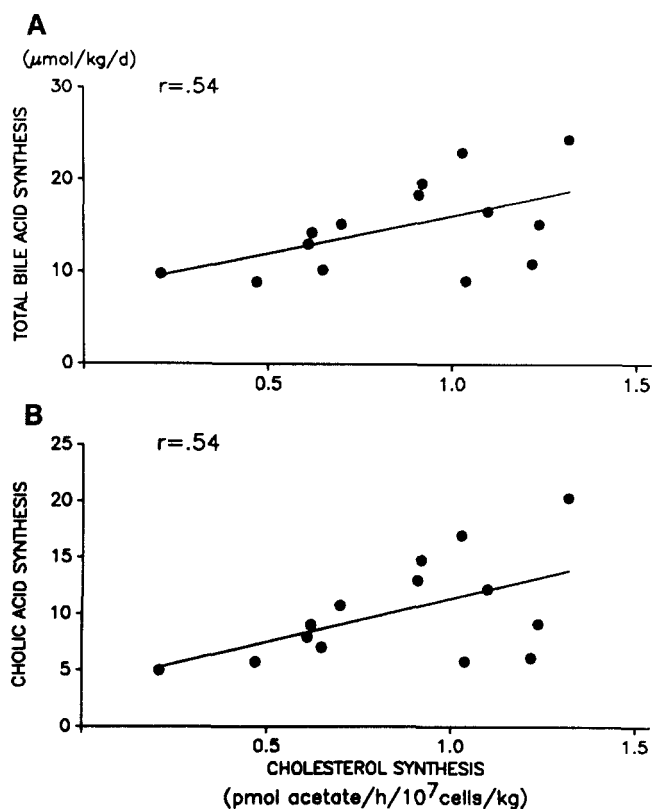


Fig. 4. Correlations of bile acid synthesis during use of CS with cholesterol synthesis by peripheral blood monocytes. Total bile acid synthesis (A) and cholic acid synthesis (B) directly correlated with cholesterol synthesis.

portantly, the present study is the only one that has attempted to correlate changes in bile acid metabolism induced by CS to other parameters of biliary lipid metabolism in the same human subjects (biliary lipid secretion, bile composition, cholesterol synthesis, cholesterol absorption and the clearance of retinyl palmitate).

The serum technique for measuring bile acid kinetics greatly simplified the procedures required of subjects par-

ticipating in the study. Essentially ten venipunctures replaced ten nasoduodenal intubations. This technique has recently been published in detail (30) and is similar to that of Stellaard et al. (36) and de Mark et al. (37). It yields highly purified samples of serum bile acids, gives highly precise and accurate measurements of isotope ratios, and yields kinetic parameters from serum that equal those from bile. Only 2 of 36 kinetic studies failed as a result of analytical problems related to the technique. The accuracy of the measurements of isotope ratios is demonstrated by the data in Table 1. Measurements of the natural abundance of isotope ratios from baseline sera equaled the isotope ratios of pure standards. In addition, all disappearance curves of isotopes from the bile acid pool were monoexponential both off and on CS. The latter observation implies that the size of the bile acid pool remained relatively constant during the period of sample gathering for the kinetic study.

Changes in bile acid kinetics induced by contraceptive steroids

Pertsemlidis et al. (4) first measured the effects of a combination of estrogen-progestin on bile acid kinetics in two women status postcholecystectomy for cholelithiasis. The two women took 2.5 mg of conjugated equine estrogens (Premarin®) plus 10 mg of medroxyprogesterone (Provera®). Cholic acid kinetics were measured by standard Lindstedt technique (33). Although the study is quite limited, this combination of estrogen and progestin did not produce significant changes in either CA pool, synthesis or turnover. Bennion et al. (35) used a paired design to measure the pool sizes of bile acids in five women taking contraceptive preparations containing a variety of estrogens and progestins of varying dose. Although the total pool did not change, the CA pool increased in four of five (mean = +26%) and the CDCA pool decreased in all five (mean = -18%). These results are similar to those in our

TABLE 2. Relationships of bile acid pool and synthesis to cholesterol synthesis and retinyl palmitate clearance

Independent Variable	Dependent Variable	Off Contraceptive Steroids		On Contraceptive Steroids	
		n	r	n	r
Cholesterol synthesis	Total BA pool	14	0.01	14	0.50
	CA pool	14	0.00	14	0.47
	DCA + CA pool	14	0.01	14	0.52
	Total BA synthesis	14	0.14	14	0.54
	CA synthesis	14	0.09	14	0.54
Retinyl palmitate clearance	Total BA pool	12	0.03	11	0.05
	CA pool	12	0.03	11	0.04
	DCA + CA pool	12	0.01	11	0.02
	Total BA synthesis	12	0.06	11	0.06
	CA synthesis	12	0.10	11	0.05

Abbreviations: BA, bile acid; CA, cholic acid; DCA, deoxycholic acid; n, number; r, correlation coefficient.

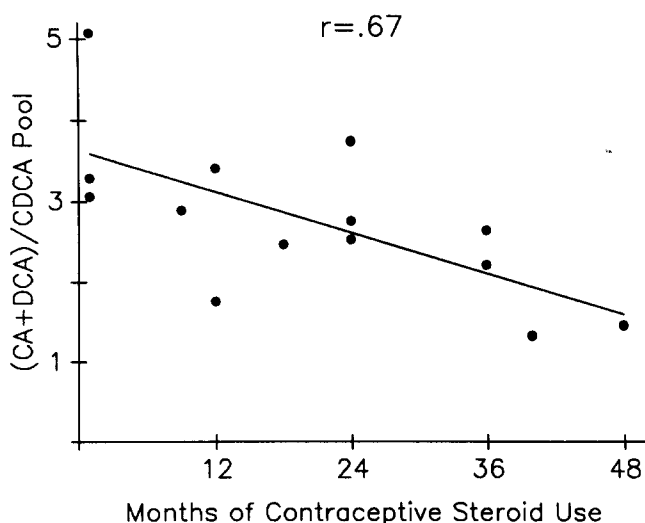


Fig. 5. The ratio of [CA + DCA]:CDCA inversely correlated with duration of CS use. Those subjects with the shortest exposure to CS had the greatest increase in the [CA + DCA]:CDCA ratio. The duration of use of CS was not recorded in one subject (thus, $n = 14$ instead of 15).

present study. In an unpaired study (11) of eight women taking a single contraceptive preparation containing 50 μg of ethinyl estradiol plus 1 mg of norethindrone, the CA pool increased and the CDCA pool did not change. CA synthesis tended to increase. Down et al. (12) measured total bile acid pool and bile composition in ten women taking varying doses of ethinyl estradiol with varying doses of either norgestrel or norethisterone. Total pool size did not change with any of the estrogen-progestin combinations but ethinyl estradiol given alone decreased the % CDCA of the bile acid pool. Finally, conjugated equine estrogens (Premarin) reduced both the pool and synthesis of CDCA without altering CA kinetics (13). Qualitatively, the above data suggest that estrogen and progestin may exert divergent effects on the kinetics of the primary bile acids, CDCA and CA. Quantitative differences between studies may reflect differences in estrogen-progestin combinations, subject populations, study design, or analytical techniques used to measure bile acid kinetics.

In the present study, we have shown that contraceptive steroids containing 35 μg of ethinyl estradiol and 1 mg of norethindrone reduced the CDCA pool, increased the CA pool, and did not alter the DCA pool. This preparation did not alter the turnover rates of the primary bile acids but increased CA synthesis, decreased CDCA synthesis, and induced a slight increase in both total pool and total synthesis of bile acids. These findings, as well those from the above studies, are most consistent with the conclusion that changes in the kinetics of the primary bile acids are due to direct effects of CS on the hepatic synthesis of bile acids.

Potential loci of the effect of CS on bile acid synthesis

Of the multiple enzymatic steps in bile acid biosynthesis, four may be sites of regulation by female steroid hor-

mones: cholesterol 7 α -hydroxylase, 7 α -OH-cholest-4-ene-3-one 12 α -hydroxylase, cholesterol 26-hydroxylase, and 5 β -cholestane-3 α ,7 α ,12 α -triol 25-hydroxylase. Ferreri and Naito (38) have shown that 25 $\mu\text{g}/\text{day}$ of 17 β -estradiol-3-benzoate given to rats for 20 days increases the activity of cholesterol 7 α -hydroxylase. On the other hand, Davis et al. (39) have shown that rats treated with 5 mg/kg per day of ethinyl estradiol for 5 days have reduced synthesis and secretion of bile acid. In the latter study, the activity of cholesterol 7 α -hydroxylase was reduced to the same degree as the reduction in bile acid synthesis. In spite of the divergent results from studies of the effects of estrogens, possibly related to dose, it is unlikely that effects of CS on cholesterol 7 α -hydroxylase could explain the results of the present study. One would anticipate that any increase or decrease in the activity of this enzyme would result in similar increases or decreases in the synthesis of both CDCA and CA. Thus, a change in cholesterol 7 α -hydroxylase activity alone would not account for the divergent effects on the primary bile acids. An increase in the activity of 7 α -OH-cholest-4-ene-3-one 12 α -hydroxylase, which irreversibly commits 7 α -OH-cholest-4-ene-3-one to cholic acid, could account for the observed increase in CA synthesis and the increase in the CA:CDCA and [CA + DCA]:CDCA ratios. Kuroki et al. (40) measured gallbladder bile acid composition and the activity of 7 α -OH-cholest-4-ene-3-one 12 α -hydroxylase in male and female hamsters. The CA:CDCA ratio of females was 2.74 ± 0.54 while that of males was 1.93 ± 0.39 . 12 α -Hydroxylase activity was two times greater in the microsomes of female hamsters and there was a positive correlation between the activity of 12 α -hydroxylase and the CA:CDCA ratio found in gallbladder bile. Thus, induction of the activity of 12 α -hydroxylase by CS could account for the increased CA pool, CA synthesis, CA:CDCA ratio, and [CA + DCA]:CDCA ratio.

Two additional enzymes represent potential sites for regulation by female steroid hormones: cholesterol 26-hydroxylase and 5 β -cholestane-3 α ,7 α ,12 α -triol 25-hydroxylase. Studies by Anderson, Kok, and Javitt (41) have shown that the initial 26-hydroxylation of cholesterol represents a preferential pathway for synthesis of CDCA. Salen et al. (42) have demonstrated that 25-hydroxylation of 5 β -cholestane-3 α ,7 α ,12 α -triol may represent a preferential pathway for the synthesis of CA. Thus, the increased CA:CDCA ratio induced by female steroid hormones could be due to either inhibition of cholesterol 26-hydroxylase or stimulation of 5 β -cholestane-3 α ,7 α ,12 α -triol 25-hydroxylase. At the present time, there are no studies of the effects of female steroid hormones on the activities of either of these two enzymes.

Enterohepatic cycling of bile acids during use of CS

The present study confirmed our previous finding that CS reduced the rate of enterohepatic cycling of bile acids (11). Bennion et al. (35), however, did not observe a change in

the rate of enterohepatic cycling. Our two studies are similar in that the same progestin was used in the same dose (norethindrone, 1 mg). On the other hand, the types and amounts of both progestin and estrogen varied in the latter study. These data suggest that enterohepatic cycling frequency may be slowed by norethindrone.

The number of enterohepatic cycles (EHC) is equal to the 24-hr secretion rate of bile acid divided by the pool size. The reduction in EHC by CS was due to both a decrease in bile acid secretion and an increase in pool size. The increase in pool size is probably a direct effect of CS on the hepatic synthesis of bile acids. The reduction in bile acid secretion may be secondary to either a reduction in the hepatic secretion of bile acids or sequestration of bile acids within the enterohepatic circulation. Sequestration may occur within the gallbladder or in the intestine, due to slowed intestinal transit. Although we found that gallbladder emptying is complete and the gallbladder remains tonically contracted during ingestion of regular meals in women using CS (43), others have reported that contraceptive steroids may slow small bowel transit (44). Thus, the reduced cycling of bile acids is probably due to the effects of CS on the liver as well as on transit of bile acids within the enterohepatic circulation.

The EHC rates measured in the present study (mean = 15.4 (off), mean = 11.7 (on)) were greater than those measured in our previous study (11) (mean = 6.6 control, mean = 4.3 CS). This difference is due to a markedly higher bile acid secretion rate (39 $\mu\text{mol}/\text{kg}$ per hr (off) vs. 19 $\mu\text{mol}/\text{kg}$ per hr, (control)). Bile acid secretion rates were higher in the present study due to use of a liquid formula, containing 40% of calories as fat, to stimulate gallbladder contraction and intestinal transit (29). Bennion (35) used a similar liquid formula and obtained nearly identical rates of bile acid secretion (off CS, mean = 1260 mg/hr)² and enterohepatic cycling (mean = 14.4 (off), mean = 14.5 (on)).

Source of cholesterol for bile acid synthesis

Bile acids are synthesized from hepatic free cholesterol. Hepatic cholesterol is derived from either newly synthesized cholesterol, the uptake of lipoproteins, or the hydrolysis of hepatic cholesteryl ester. Hepatic cholesterol may be secreted into plasma as VLDL, secreted into bile as biliary cholesterol, esterified and stored within the liver as cholesteryl ester, or metabolized to bile acid. The positive relationship of bile acid synthesis to cholesterol synthesis only during use of CS suggest that CS alter homeostatic mechanisms for controlling the disposition of hepatic cholesterol. This is supported by the findings reported in the companion paper (25) to this one. Obviously all of the flux into and

²Weights of subjects are not given in the report by Bennion. Our secretion rate of 39 $\mu\text{mol}/\text{kg}$ per hr is equivalent to 1248 mg/hr to compare with the secretion rates measured by Bennion.

out of the hepatic cholesterol pool cannot be adequately measured in intact human subjects. However, bile acid synthesis did not correlate with either chylomicron remnant clearance or the intestinal absorption of cholesterol. Thus, the relative specificity of the correlation of bile acid synthesis and pool size with cholesterol synthesis suggests that during use of CS newly synthesized cholesterol may be a preferred substrate for bile acid synthesis.

In summary, this study has uncovered potential mechanisms of the effects of CS on biliary lipid metabolism. The CA:CDCA and [CA + DCA]:CDCA ratios in bile increase due to an expansion of the CA pool and a decrease in the CDCA pool. These changes are due to direct effects of CS on the hepatic synthesis of bile acids. A locus of this effect may include the enzyme 7 α -OH-cholest-4-ene-3-one 12 α -hydroxylase. The data suggest that newly synthesized cholesterol may be a preferred substrate for bile acid synthesis during use of CS. **■**

This studies were supported by grants (RO1 AM 31765 and RO1 AM 26356) from the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, and by the Clinical Research Center, University of Colorado School of Medicine, Grant #RR-00051 from the General Clinical Research Centers Research Program of the Division of Research Resources, National Institutes of Health. Additional support was from a USPHS grant #DK 34914 (University of Colorado Hepatobiliary Center). Dr. Everson was supported in part by a Clinical Investigator Award (KO8 AM 01156-02) and in part by a Research and Teaching Scholar Award from the American College of Physicians. Additional support was provided via the NIH-sponsored Mass Spectrometry Resource at the University of Colorado Medical School under grant 2 P41 RR 01152. The authors thank Carol McKinley, R.N. for assistance with the clinical studies; Lawrence Morse, Radene Showalter, and Elaine Butler for excellent technical assistance; and Mary Lou Strachan for help with dietary control. The authors also thank Drs. Craig Fausel, Michael Lawson, and Freider Berr for their participation in some of the studies.

Manuscript received 22 April 1987 and in revised form 10 August 1987.

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