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EFFECTS OF SHORT-TERM TREATMENT WITH A COMBINED OESTROGEN–PROGESTIN ORAL CONTRACEPTIVE ON BILIARY LIPIDS AND CHOLESTEROL SATURATION INDEX IN YOUNG WOMEN

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Summary—The effect of daily ingestion for 7 days of ethinyloestradiol (30 μg) plus DL-norgestrel [0.5 mg] (Eugynon-30) on the lipid composition of duodenal bile in 8 healthy young women was investigated from the fifth day after onset of menstrual bleeding. This treatment did not significantly affect the concentrations of cholesterol, phospholipid and total bile acids expressed as mmol/l, nor the mean molar percentage of phospholipid. However, the treatment caused a significant increase in the mean molar percentage of cholesterol which was accompanied by a significant decrease in the mean molar percentage of total bile acids. The cholesterol saturation index of the bile of 7 subjects was elevated after treatment while both serum cholesterol and testosterone were significantly reduced.

The results show that administration to healthy young women, not previously exposed to oral contraceptives, with a low oestrogen–progestin preparation for only 7 days produces a more lithogenic bile, accompanied by a decrease in serum cholesterol and plasma testosterone concentrations.

INTRODUCTION

Administration of 30 μg of ethinyloestradiol to healthy young men for only 7 days caused a significant increase in the cholesterol saturation index (CSI) of the bile of those subjects; plasma testosterone levels were decreased whereas serum cholesterol remained unchanged after treatement [1]. The purpose of the present investigation was to ascertain whether or not administration of a combined oestrogen–progestin oral contraceptive for only 7 days to a carefully selected group of healthy young women would cause changes in the CSI of the bile, and plasma testosterone and serum cholesterol concentrations of the subjects within that short space of time.

EXPERIMENTAL

Subjects

These were 8 healthy, nulliparous, Caucasian female medical students who has no history of liver disease or endocrine abnormalities. None of the women had ever used oral contraceptives or other hormonal agents prior to this study. They were matched as closely as possible with respect to age (mean, 22 y, 11 months; SE, 6 months), and they had had regular menstrual cycles (mean, 28 days; SE, 3 days) for at least 2 years previously. All subjects were within 10% of ideal body weight. They abstained from alcohol and aspirin for 2 weeks before and during the investigation, but no other dietary restrictions were imposed.

The study was carried out with informed consent, and with the approval of the Ethical Committees of this Medical School and Area Health Authority, with the proviso that no subject should be intubated more than twice.

Design of experiment

Each woman was studied from the fifth day after onset of menstruation. They abstained from food and drink from 20.00 h on the evening before duodenal intubation. At 08.45 h on the following morning 10 ml of blood were withdrawn from the antecubital vein, and the serum obtained was stored at −20°C. Duodenal contents were then withdrawn using a single lumen tube which was radio-opaque along its whole length and weighted at the tip with mercury. Under X-ray fluoroscopy the end of the tube was placed in the duodenum as close as possible to the sphincter of Oddi by reference to the ligament of Treitz. After residual duodenal contents had been aspirated the subjects were given an i.v. dose (1 u/kg) of cholecystokinin–pancreozymin (CCK-PZ; Boots Pure Drug Co., Nottingham, England). Duodenal samples were withdrawn at 1–2 min intervals; usually 6 samples were obtained and these were placed in separate tubes. The least pigmented samples, usually the first and the last, were put to one side. The other samples were combined and mixed, and 2 ml were

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taken for analysis. The total residual duodenal aspirates were then returned to the duodenum through the original tube in order to minimise alteration in bile acid pool size.

At 09.30 h on that day, and at 09.00 h for the next 6 days each subject ingested one tablet of Eugynon-30 (Schering Chemicals Ltd, Berlin) which contains 30 μg of ethinyloestradiol and 0.5 mg of DL-norgestrel (i.e. 0.25 mg of the biologically active D-form of 17α-ethinyl-18-methyl-4-oestren-17β-ol-3-one). Blood samples and duodenal aspirates were again taken on the morning after the last tablet had been taken.

Analysis of biliary lipids

Total bile acids and cholesterol were determined by GLC and phospholipid by conversion to inorganic phosphate as previously described [2, 3]. Total bile acids, phospholipid and cholesterol were expressed as mmol/l and as a molar percentage of their sum total concentration in each duodenal aspirate. Individual bile acids were expressed as a percentage of the total bile acid concentration (mmol/l) in each sample. The CSI was calculated by the method of Thomas and Hofmann[4] using the pooled cholesterol solubility data of Hegardt and Dam[5] and Holzbach *et al.*[6]. Correction for the total lipid concentration [7] could not be made because the bile samples had been diluted to an unknown extent by pancreatic and, possibly, gastric juices.

Serum cholesterol and plasma testosterone

Cholesterol was determined by Autoanalyser and testosterone by radioimmunoassay.

Statistical analyses

Results are expressed as means $\pm$ 1 SE. Significance of difference determined by a 2-tailed paired Student's t-test was set at $P < 0.05$.

RESULTS

None of the subjects complained of any side effects as a result of ingesting Eugynon-30.

Biliary lipids

The concentrations (mmol/l) of total bile acids, phospholipid and cholesterol in the duodenal bile samples of the 8 subjects before and after treatment are given in Table 1. No significant changes in the mean concentrations of any of these lipids were caused by treatment with the contraceptive. However, when the results were expressed as a molar percentage of total biliary lipids (Table 2, Fig. 1) the molar percentage of cholesterol was raised in all subjects, resulting in a significant increase in the mean from 9.66 ± 0.9 before to 14.85 ± 1.7 moles percent after treatment. In contrast, the molar percentage of total bile acids was decreased in all subjects so that the mean decreased significantly from the pre-treatment value of 78.46 ± 1.8 to 72.12 ± 2.4 post treatment. There was no significant difference in the mean molar percentage of phospholipid before (11.86 ± 1.3) and after (13.03 ± 1.5) treatment.

The overall effect of these changes was to cause an increase in the CSI of the duodenal bile of 7 of the subjects; the mean increased significantly from 2.1 ± 0.2 before to 3.0 ± 0.4 after treatment with the contraceptive for only 7 days.

The mean values for the percentages of the individual bile acids before and after treatment are shown in Table 3. The primary bile acids, cholic and chenodeoxycholic acids, accounted for about 85% of the total, and the secondary bile acid, deoxycholic acid, for the remainder. In some samples small peaks corresponding to minor secondary bile acids were observed on GLC traces. As these never amounted to more than 1% of the total bile acids they were not included in the calculations. Minor bile acids, also observed in bile of male subjects before and after treatment with ethinyloestradiol [1], were not related to steroid treatment.

Serum cholesterol and testosterone

Treatment caused a decrease in the serum cholesterol of each subject, and the mean value for the group fell from 4.0 ± 0.12 to 3.7 ± 0.14 mmol/l ($P = 0.002$). Serum testosterone concentrations were

Table 1. Cholesterol, phospholipid and total bile acids in duodenal bile of eight women before and after treatment with Eugynon-30 for 7 days

	Cholesterol (mmol/l)		Phospholipid (mmol/l)		Total bile acids (mmol/l)	
	Before	After	Before	After	Before	After
Subject						
1	4.4	8.1	7.4	8.2	35.2	27.7
2	2.8	2.4	2.6	1.5	12.6	7.1
3	8.3	10.5	11.5	12.3	82.5	79.5
4	5.1	5.3	8.3	6.6	42.6	36.1
5	8.3	10.5	12.7	10.9	76.9	42.6
6	4.3	6.6	7.0	11.1	43.7	56.3
7	7.0	9.2	4.6	4.1	79.3	60.5
8	9.9	7.2	7.1	3.2	72.0	26.6
Mean	6.3	7.5*	7.6	7.2*	55.6	42.1*
SE mean	0.87	0.97	1.17	1.43	9.05	8.07

*Values after treatment not significantly different from value before treatment (two-tailed paired Student's t-test).

Table 2. Molar percentages of cholesterol, phospholipids, total bile acids and the CSI of healthy young women before and after treatment with Eugynon-30

	Cholesterol		Phospholipid		Total Bile Acids		CSI*	
Subject	Before	After	Before	After	Before	After	Before	After
1	9.4	18.4	15.7	18.6	74.9	63.0	1.7	2.8
2	15.6	21.8	14.4	13.6	70.0	64.5	2.9	4.0
3	8.1	10.3	11.2	12.0	80.6	77.7	1.9	2.2
4	9.1	11.0	14.8	13.8	76.1	75.2	1.8	2.2
5	8.5	16.4	13.0	17.0	78.5	66.6	1.8	2.7
6	7.8	8.9	12.7	15.0	79.5	76.1	1.7	1.7
7	7.7	12.5	5.1	5.6	87.2	82.0	2.3	3.6
8	11.1	19.5	8.0	8.6	80.9	71.9	2.9	4.8
Mean	9.66	14.85	11.86	13.03	78.46	72.12	2.1	3.0
SE	0.9	1.7	1.3	1.5	1.8	2.4	0.2	0.4
P†	0.02		NS		0.004		0.004	

*Calculated from the pooled cholesterol solubility data of Hegardt and Dam [8] and Holzbach *et al.*[9].
†2-Tailed paired Student's t-test.

also decreased; the mean value for the group fell from 2.05 ± 0.07 before to 1.30 ± 0.05 mmol/l after treatment ($P = 0.002$).

DISCUSSION

The results presented here clearly show that ingestion of a low oestrogen–progestin mixed type oral contraceptive (Eugynon-30) causes an increase in the cholesterol saturation of bile of young women treated for only 7 days. As in male subjects treated with ethinyloestradiol for 7 days [1], plasma testosterone levels were significantly reduced (see Results), but in contrast to male subjects in whom serum cholesterol levels were unchanged, the serum cholesterol concentrations were significantly reduced. Although other workers have shown that exogenous sex steroids and oral contraceptives increase biliary cholesterol saturation, this is the first study to demonstrate such an effect in a well-defined population chosen to minimise possible variations in biliary lipids due to ethnic origin, obesity, menstrual history and drugs affecting the liver. Furthermore, other workers have not determined plasma testosterone and serum cholesterol in treated subjects. Treatment with Eugynon-30 was begun on the fifth day of menstrual bleeding and therefore extended up to the twelfth day of the ovarian cycle, a period over which oestrogen secretion from the developing follicle is normally rising. Therefore it is possible that the increase in the CSI of the subjects (Fig. 1) might have been caused by a rise in secretion of endogenous oestrogens. However, this possibility appears to be most unlikely. Oral contraceptives exert at least part of their effect by inhibition of secretion of anterior pituitary gonadotrophins and hence of ovarian steroids. The significant decrease in serum testosterone caused by Eugynon-30 (see Results) supports this view, since part of this circulating androgen in women is of ovarian origin [8].

There have been several studies of the effects of oestrogens and oral contraceptives on cholesterol saturation of bile. Bennion *et al.*[9] showed that the CSI increased in women taking oral contraceptives for relatively long periods of 2–13 months, and that in another group of women the high CSI of oral

Table 3. Bile acid composition (percent of total bile acids) of duodenal bile before and after treatment with Eugynon 30 for 7 days

	Before treatment	After treatment*
Cholic acid		
Mean	52.7	55.0
SE	2.74	2.41
Chenodeoxycholic acid		
Mean	30.7	30.7
SE	3.41	3.14
Deoxycholic acid		
Mean	16.3	14.4
SE	5.76	4.73

*Differences not significant.

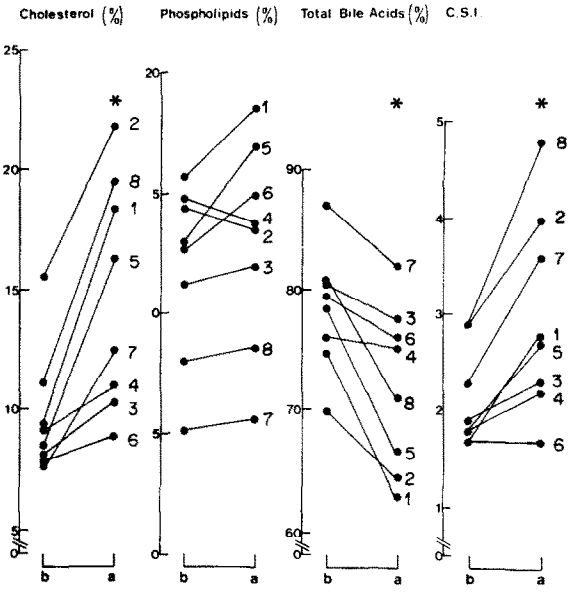

Fig. 1. Molar percentages of cholesterol, phospholipid and total bile acids, and cholesterol saturation index (CSI) of duodenal bile of eight young women before (b) and after (a) treatment with Eugynon-30 for 7 days (calculated from data in Table 1). The asterisks denote a significant difference (2-tailed paired Student's t-test) between the before and after treatment values.

contraceptive users returned to normal several months after cessation of treatment. However, the oral contraceptives used were mainly of the type with a high oestrogen content (at least 50 μg); the subjects had a wide age range (19–39) and some may have been parous [9]. Those authors also found that oral contraceptive users had a higher than normal proportion of biliary chenodeoxycholic acid, whereas no such change in the proportions of the major bile acids was observed in our subjects (Table 3).

It has been claimed that the CSI of women varies during the menstrual cycle [10], but this claim has not been confirmed by other workers. Six young women were investigated over 8 complete menstrual cycles; duodenal bile was obtained on days 3–6, 10–15 and 19–15 of the cycle and no significant changes in the biliary lipids were found amongst the samples taken at these different times in the cycle [11]. Similar studies were done in 9 women in whom the time of ovulation was assessed by plasma progesterone determinations. Duodenal bile was obtained at least 6 times throughout a complete cycle, but the CSI showed no dependence on any particular phase of the cycle [12]. In a more elaborate and definitive investigation involving the use of marker-perfusion techniques for collecting duodenal bile, and bile acid kinetics using labelled bile acids, it was found that the phase of the cycle had no effect on the CSI of female subjects, although changes were observed in the second and third trimesters of pregnancy [13].

Because of the very strong evidence cited above that the CSI of bile of women with normal menstrual cycles does not change in response to endogenous hormone levels during the cycle, it was not possible to obtain ethical permission to obtain bile from our subjects after no, or placebo, treatment. However, there is considerable evidence to support the view that the observed increases in the CSI of our subjects were caused by ingestion of Eugynon-30. This study provides no information about the mechanism(s) by which the oestrogen–progestin preparation caused an increase in biliary CSI, and was not designed for that purpose. However, no plausible explanation has been put forward to explain the effects of sex hormones on biliary lipid composition. The liver is now considered to be a target organ for sex hormones [14]. Treatment of young men with ethinyloestradiol for only 7 days caused a fall in plasma testosterone of about 50%, and we have put forward the hypothesis that one of the processes involved in the formation of a potentially lithogenic bile might be the result of disturbed hepatic lipid metabolism due to a reduction in the amount of testosterone available to exert an anabolic effect on the liver [1]. The administration of Eugynon-30 in the present study also caused a reduction in plasma testosterone of about 50% (see Results), so the effects of the oral contraceptive on biliary and serum lipids described here might also have been at least partly the result of the reduction in testosterone secretion caused by the oral contraceptive.

From the data available it is not possible to assert that the increase in biliary cholesterol (Table 2) is directly related to the decrease in serum cholesterol of the subjects (see Results). Cholesterol secreted into bile is derived from dietary and extrahepatic sources and also from newly synthesized hepatic cholesterol which contributes about 30% of the sterol in bile. Some cholesterol from extrahepatic pools could also be used for the synthesis of bile acids [15]. However, bile acid secretion was decreased by Eugynon-30 (Table 2; Fig. 1).

Recently it has been suggested that it is the synthetic progestin, such as norgestrel, and not the oestrogen component of combined-type oral contraceptives which is responsible for the increase in CSI of bile associated with the use of these preparations [16]. However, further work is required to establish whether it is the oestrogen or progestin in Eugynon-30 which is responsible for the early change in CSI of bile of the subjects reported here.

In summary, treatment of 8 healthy young women with a low oestrogen–progestin oral contraceptive daily for only 7 days caused an increase in the CSI of bile collected by duodenal intubation. All previous work on the effects of oral contraceptives has been carried out on non-homogeneous groups of subjects who have been taking various types of oral contraceptives for many months. The work reported here has an important bearing on the relationship between oral contraceptive use and an increased tendency to develop cholesterol gallstones, since these stones probably take many years to develop. On the basis of the present findings it is apparent that the production of abnormal bile with an increased risk of gallstone formation begins almost immediately upon starting the use of mixed-type oral contraceptives, and this may explain why some women are unable to tolerate this type of preparation.

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REFERENCES

1. Anderson A., James O. F. W., MacDonald H. S., Snowball S. and Taylor W.: The effect of ethynyl oestradiol on biliary lipid composition in young men. *Eur. J. clin. Invest.* **10** (1980) 77–80.
2. Taylor W.: The bile acid composition of rabbit and cat gallbladder bile. *J. steroid Biochem.* **8** (1977) 1077–1084.
3. Hirst B. H., Lund P. K., Reed J. D., Sanders D. J., Shaw B. and Taylor W.: Effects of a combined oestrogen-progestin preparation on gastric acid and pepsin secretion, serum gastrin concentration and secretion of bile acids, phospholipids, and cholesterol in the cat. *Br. J. Pharmac.* **65** (1979) 87–95.
4. Thomas P. J. and Hofman A. F.: A simple calculation of the lithogenic index of bile: expressing biliary lipid composition on rectangular coordinates. *Gastroenterology* **65** (1973) 698–700.
5. Hegardt F. G. and Dam H.: The solubility of cholesterol in aqueous solutions of bile salts and lecithin. *Z. Ernaehrungswiss.* **10** (1971) 223–233.

6. Holzbach R. T., Marsh M., Olszewski M. and Holan K.: Cholesterol solubility in bile: evidence that supersaturated bile is frequent in healthy man. *J. clin. Invest.* **52** (1973) 1467–1479.
7. Carey M. and Small D. M.: The physical chemistry of cholesterol solubility in bile: Relationship to gallstone formation and dissolution in man. *J. clin. Invest.* **61** (1978) 998–1026.
8. Smals A. G. H., Kloppenborg P. W. C. and Benraad T. J.: The effect of oestrogen administration on plasma testosterone, FSH and LH in patients with Klinefelter's Syndrome and normal man. *Acta endocr., Copenh.* **77** (1974) 765–783.
9. Bennion L. J., Ginsberg R. L., Garnick M. B. and Bennett P. H.: Effects of oral contraceptives on the gallbladder bile of normal women. *New Engl. J. Med.* **294** (1976) 189–192.
10. Low-Beer T. S., Wicks A. C. B., Heaton K. W., Durrington P. and Yeates J.: Variation in bile composition and serum lipids during the menstrual cycle. In *Bile Acid Metabolism in Health and Disease* (Edited by G. Paumgartner and A. Stiehl). MTP Press, Lancaster (1977) pp. 183–190.
11. Bennion L. J., Ginsberg R. L., Garnick M. B. and Bennett P. H.: Effects of the normal menstrual cycle on human gallbladder bile. *New Engl. J. Med.* **294** (1976) 1187.
12. Whiting M. J., Down R. H. L. and Watts J. McK.: Precision and accuracy in the measurement of the cholesterol saturation index of duodenal bile. *Gastroenterology* **80** (1981) 533–538.
13. Kern F., Everson G. T., Demark B., McKinley C., Showalter R., Erfling W., Braverman D. Z., Sczepanik-van-Leeuwen P. and Klein P.: Biliary lipids, bile acids, and gallbladder function in the human female. Effects of pregnancy and the ovulatory cycle. *J. clin. Invest.* **68** (1981) 1229–1242.
14. Yamamada M. and Miyaji H.: Binding of sex hormones by male rat liver microsomes. *J. steroid Biochem.* **16** (1982) 437–446.
15. Maton P. N., Reuben A. and Dowling R. H.: Relationship between hepatic cholesterol synthesis and biliary cholesterol secretion in man: hepatic cholesterol synthesis is not a major regulator of biliary lipid secretion. *Clin. Sci.* **62** (1982) 515–519.
16. Down R. H. L., Whiting M. J., Watts J. McK. and Jones W.: Effect of synthetic oestrogens and progestagens in oral contraceptives on bile lipid composition. *Gut* **24** (1983) 253–259.