Further studies of the effects of an anovulatory drug on lipid metabolism in the rat

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ABSTRACT The effect of various levels of the oral contraceptive drug, Enovid E, on serum and liver lipid levels of adult female rats has been investigated. Doses ranging from 0.052 to 1.04 mg/day have been employed in rats fed control or cholesterol-containing diets. It has been confirmed that after administration of even low, physiological doses of the drug, esterified cholesterol in serum and adrenals decreases rapidly while at the same time it accumulates in the liver; cholesteryl oleate is increased while the relative amount of cholesteryl arachidonate is reduced. Serum phospholipids also are decreased; the α/β lipoprotein ratio is significantly reduced due to the decrease of α-lipoproteins. Most of these changes also occur in cholesterol-fed rats. The observed effects are not related to a decreased food intake.

SUPPLEMENTARY KEY WORDS oral contraceptive drug · serum lipids · liver lipids · α-lipoprotein:β-lipoprotein ratio · cholesterol metabolism · cholesteryl arachidonate

PREVIOUS REPORTS from our laboratory concerning the effects of contraceptive drugs on lipid metabolism involved the use of large, nonphysiological doses administered to adult female rats fed diets ad lib. Under these conditions, EE given at a level of 1.04 mg (1.0 mg of norethynodrel and 0.04 mg of mestranol) for 4 days resulted in decreased plasma and adrenal cholesterol levels, increased liver cholesterol concentration, and decreased polyunsaturated fatty acids in the cholesteryl esters of plasma and adrenals. Although the treated animals excreted less fecal cholesterol, cholesterol biosynthesis was stimulated in adrenal and ovary but depressed in liver (1, 2).

Abbreviations: EE, Enovid E; HDL, high density lipoproteins.

Recently, several reports relating oral contraceptive therapy to lipid metabolism have appeared in the literature; hypertriglyceridemia, as well as hypercholesterolemia, has been observed in human patients (3–5). It has been suggested (4, 6) that a reduced lipoprotein lipase activity may be a factor contributing to the increased plasma lipid. The activity of this enzyme was found to be influenced in menopausal women by the administration of estrogens (7). Similarly, the lipemia of pregnancy might be due to a diminished uptake of triglyceride fatty acids by the adipose tissue (8). Sachs, Wolfman, and Herzig (9) observed an increase in pre-β-lipoproteins during administration of oral contraceptives and postulated an interference in the formation of β-lipoproteins. Whether these effects would be seen in rats is open to further clarification. In rats, Schweppe and Jungmann (10) observed that whereas low doses of estradiol stimulated in vitro cholesterol esterification in the liver, higher doses antagonized this esterification process.

In the investigation to be reported here, further experiments have been carried out on the effects of EE on lipid metabolism in rats. The dose level of the oral contraceptive was decreased to the amount required for control of fertility. Pair-feeding experiments were also undertaken to eliminate the possibility that the results obtained after hormone administration were due to partial anorexia.

MATERIALS AND METHODS

Female rats of our inbred strain (5–6 months old, avg wt 225 g) were kept on a stock diet of Purina pellets and were administered oral doses of 0.052, 0.52, or 1.04

mg of EE dissolved in sesame seed oil for 4 days before they were killed. In some instances, the rats were put for 4 wk on a high cholesterol diet (1% cholesterol, 0.25% bile salts, and 15% corn oil) prior to being treated with the contraceptive drug. At the end of the experimental period the rats were anesthetized with Nembutal, blood was withdrawn from the heart, and the organs were removed for subsequent analyses.

Cholesterol determinations, thin-layer separations of lipid fractions, and gas–liquid chromatography of fatty acids were performed on various tissues as described previously (1). In this investigation, a Varian Aerograph instrument, Series 200, was used with temperature programming (4°C increase/min from 150°C to 200°C; hydrogen flame detector; the column was packed with 14% Hi-Eff-2BP on Gas-Chrom P, 80–100 mesh). Triglyceride determinations were done using the method of Kaplan and Lee (11); phospholipids were determined by the method of Bartlett (12). Lipoproteins were separated on hydrocellulose gel strips in barbitone acetate buffer with albumin, using a current of 2.5 ma/strip for 45 min. Staining was performed overnight with a saturated solution of Sudan black B in 60% ethanol. Sections of duplicate strips were eluted with acetone and the absorbance of the solution was read at 580 nm.

β-Lipoprotein precipitation from the serum was performed according to the method of Burstein (13).

RESULTS

Experiment I. Pair-feeding Using a Dose Level of 0.52 mg EE

It has been previously observed that rats given doses of 1.04 mg of EE consumed less food than control animals, and lost weight. The possibility that some of the effects of the drug on lipid metabolism could be ascribed to anorexia rather than to the primary hormonal effect required clarification. For this purpose three groups of seven rats each were treated as follows: one group was dosed for 4 days with 0.52 mg EE in sesame oil, two other groups were given sesame oil alone. One of the latter groups of animals was allowed to eat Purina pellets ad lib., while the other group was pair-fed to the drug-treated group. In addition to measuring food consumption and weight gain, feces were collected, dried, and analyzed for cholesterol and total lipid content.

After 4 days of treatment and an overnight fast, rats were killed and serum and liver lipid concentrations were determined. The results are presented in Tables 1–4. As has been reported previously, both serum cholesterol and phospholipid levels were markedly decreased as a result of EE administration; in addition, the cholesterol:phospholipid ratio changed from 0.46 for the control rats to 0.33 for the treated animals. These effects were not secondary to a decreased food consumption. α-Lipoproteins, ordinarily quite abundant in the rat, were very significantly decreased in treated rats; this was verified by the decrease in α/β ratios in these animals (Table 1).

When determinations of cholesterol and phospholipids were made on serum from which β-lipoproteins had been precipitated by heparin and $MnCl_2$, it became obvious that the decrease in cholesterol was primarily in the α-lipoprotein fraction; approximately 90% of this cholesterol disappeared.

There was a significant increase in the weight of the liver of the treated animals (Table 2). Comparisons with pair-fed and ad lib. control groups confirm the fact that these effects are due to the drug and not to changes in food consumption.

Food consumption was markedly reduced, from 8 g per day to 0.5 g per day, as the drug administration progressed; as a result, less feces were excreted by the treated animals (Table 3). Table 4 shows the cholesterol content of feces during EE administration. The cholesterol content of feces was unaffected by diet or treatment with the drug. However, when the average daily excretion of cholesterol was calculated from the total amount of fecal material excreted over the 4-day period, there were noticeable differences between the groups. The treated group excreted less than the ad lib. control ($P < 0.005$) and also less than the pair-fed control ($P < 0.05$).

TABLE 1 EFFECT OF ENOVID E ON CHOLESTEROL AND PHOSPHOLIPIDS IN SERUM AND α-LIPOPROTEINS

Treatment	Cholesterol			Phospholipids				
	Total (C)	αLP (A)	A/C	Total (P)	LP (a)	a/P	C/P	αLP/βLP†
	mg/100 ml		%	*mg/100 ml*		%		
0.52 mg EE	10.8 ± 1.3^{ab}*	3.4 ± 0.4^{cd}	31.6	32.1 ± 6.2^{ef}	29.2 ± 3.4^{gh}	91.0	0.33	25/65
Control, pair-fed	41.6 ± 3.0^{a}	31.0 ± 2.1^{c}	74.5	99.2 ± 10.6^{e}	89.5 ± 16.2^{g}	90.0	0.41	
Control, ad lib.	46.3 ± 9.9^{b}	33.1 ± 2.7^{d}	71.5	100.4 ± 15.2^{f}	74.9 ± 18.9^{h}	75.0	0.46	176/63

Numbers with the *same* letter in superscript are significantly different at $P < 0.001$.

* Mean value ± SD, seven rats per group.

† Ratio of α-lipoprotein to β-lipoprotein as determined by the average absorbance of eluted selections of hydrocellulose gel strips following electrophoresis.

TABLE 2 EFFECT OF ENOVID E ON WEIGHT AND CHOLESTEROL CONTENT OF LIVER

Treatment	Liver		Cholesterol in Liver		
	Weight	% Body Wt	Total		% Ester
	g		*mg/g*	*mg/liver*	
0.52 mg EE	5.83 ± 0.46^{ad}*	2.78	3.85 ± 0.22^{bc}	22.44	43.8
Control, pair-fed	4.91 ± 0.28^{a}	2.30	2.42 ± 0.14^{b}	11.88	14.0
Control, ad lib.	4.85 ± 0.27^{d}	2.26	2.38 ± 0.08^{c}	11.54	13.5

Numbers with the *same* letter in superscript are significantly different; superscripts a–c indicate significant difference at $P < 0.001$, and d, at $P < 0.005$.

* Mean value ± SD, seven rats per group.

TABLE 3 EFFECT OF ENOVID E ON FOOD CONSUMPTION AND EXCRETION OF FECES

Treatment	Day 1		Day 2		Day 3		Day 4*	
	Food Ingested	Feces Excreted	Food Ingested	Feces Excreted	Food Ingested	Feces Excreted	Food Ingested	Feces Excreted
	g	*g*†	*g*	*g*†	*g*	*g*†	*g*	*g*†
0.52 mg EE	8 ± 1‡	1.33 ± 0.80	6 ± 1	1.02 ± 0.41^{a}§	4 ± 1	0.79 ± 0.26^{bc}	0.5 ± 0.8	0.90 ± 0.31^{d}
Control, pair-fed	9 ± 2	1.73 ± 0.38	6 ± 1	1.66 ± 0.43	4 ± 1	1.45 ± 0.45^{b}	1.0 ± 1.0	0.74 ± 0.26
Control, ad lib.	11 ± 2	1.25 ± 0.39	10 ± 2	2.02 ± 0.72^{a}	12 ± 2	2.00 ± 0.58^{c}	2.0 ± 0.7	1.79 ± 0.47^{d}

* Food was removed from cages 16 hr before the rats were killed.

† Dry weight.

‡ Mean value ± SD, seven rats per group.

§ Superscripts a and b indicate significant difference at $P < 0.01$, and c and d, at $P < 0.005$. Numbers with *same* letter in superscript are significantly different.

TABLE 4 EFFECT OF ENOVID E ON EXCRETION OF CHOLESTEROL IN THE FECES

Treatment	Cholesterol Excretion				Average Daily Excretion, Day 1–4	Fecal Cholesterol, Day 1–4
	Day 1	Day 2	Day 3	Day 4		
	mg/rat/day				*mg/rat*	*mg/g*
0.52 mg EE	4.1 ± 2.4*	4.2 ± 1.1	3.0 ± 1.1	2.7 ± 2.0	3.5 ± 0.4^{ab}†	3.3
Control, pair-fed	6.3 ± 2.0	4.9 ± 1.3	4.5 ± 2.3	2.8 ± 0.3	4.6 ± 0.7^{a}	3.4
Control, ad lib.	4.0 ± 1.3	6.1 ± 2.2	5.8 ± 1.6	3.5 ± 1.6	5.1 ± 0.5^{b}	3.0

* Mean value ± SD, seven rats per group.

† Numbers with superscripts a and b are significantly different at $P < 0.05$ and $P < 0.005$, respectively.

Experiment II. Comparisons of Results Using Two Dose Levels of EE

In this experiment two levels of EE were used: a dose level equal to the minimum which is effective in preventing conception (14, 15) and a dose which was 10 times higher.

Three groups of adult female rats were treated as follows for 4 days: group 1, sesame oil; group 2, 0.052 mg EE (0.050 mg progestin and 0.002 mg estrogen) in sesame oil; and group 3, 0.52 mg EE (0.50 mg progestin and 0.02 mg estrogen in sesame oil). The rats were killed following an overnight fast. It can be seen (Table 5) that even at the low physiological dose (0.052 mg) of EE, there were marked changes in lipid levels; in serum, cholesterol decreased from 47.8 to 15.5 mg per 100 ml and phospholipid decreased from 118.5 to 62.8 mg per 100 ml. The C/P and the α/β lipoprotein ratios also decreased. Higher doses of the drug enhanced most of these changes.

In liver (Table 6), the triglyceride levels were decreased and the cholesterol content was increased following hormonal treatment. No changes were observed in the phospholipids even when the larger dose level, 0.52 mg of EE, was administered.

Table 7 shows the fatty acid composition of the liver cholesteryl esters and the concentration of individual cholesteryl esters. The decrease of arachidonate observed in those animals receiving the larger dose of EE was accompanied by an increase of oleate. However, taking into account the concentration of liver cholesteryl esters (Table 6), the actual amount of cholesteryl arachidonate did not change while other esters increased appreciably.

Experiment III. Effects of Various Levels of EE on Cholesterol-fed Rats

In this experiment adult female rats were fed a high cholesterol diet for 4 wk. The animals were then divided

TABLE 5 EFFECT OF DOSE OF ENOVID E ON SERUM CHOLESTEROL AND PHOSPHOLIPIDS

Treatment	Cholesterol			Phospholipids (P)	C/P	αLP/βLP*
	Total (C)	Free	% Ester			
	mg/100 ml			*mg/100 ml*		
Control	47.8 ± 5.4†[a]	15.9 ± 2.9[b]	66.7	118.5 ± 13.2[c]	0.40	165/64[de]
0.052 mg EE	15.5 ± 3.6[a]	4.8 ± 0.4[b]	69.0	62.8 ± 11.4[c]	0.25	19/56[d]
0.52 mg EE	4.2 ± 1.9[a]	2.0 ± 0.9[b]	52.5	23.6 ± 4.7[c]	0.18	26/69[e]

Numbers with the *same* letter in superscript are significantly different at the following levels: a, b, and c, $P < 0.001$; d, $P < 0.025$; and e, $P < 0.01$.

* See footnote (†) to Table 1.

† Mean value ± SD, six rats per group.

TABLE 6 EFFECT OF DOSE OF ENOVID E ON LIVER LIPIDS

Treatment	Liver		Cholesterol			Phospholipids	Triglycerides
	Weight	% Body Wt	Total		Ester % Ester		
	g		*mg/g*	*mg/organ*	*mg/g*	*mg/g*	*mg/g*
Control	5.80 ± 0.32*	2.46	2.48 ± 0.12[ac]†	14.38	0.31 ± 0.15[d] 12.5	25.8 ± 3.8	17.2 ± 6.8[fg]
0.052 mg EE	6.06 ± 0.66	2.55	2.84 ± 0.33[ab]	17.21	0.59 ± 0.35[e] 20.8	23.8 ± 8.2	8.9 ± 2.9[f]
0.52 mg EE	6.01 ± 0.73	2.73	3.31 ± 0.24[bc]	19.88	1.00 ± 0.23[de] 30.0	24.9 ± 11.8	7.5 ± 0.7[g]

* Mean value ± SD, six rats per group.

† Numbers with the *same* letter in superscript are significantly different at the following levels: a, e, and f, $P < 0.05$; b and g, $P < 0.025$; c, $P < 0.001$; and d, $P < 0.005$.

into three groups, and they were given diets supplemented with sesame oil, 0.52 mg EE in sesame oil, or 1.04 mg EE in sesame oil, for 4 days. The results of the lipid analyses performed on these animals are shown in Tables 8–10.

There was a marked decrease in serum total cholesterol levels as well as in serum triglyceride levels (Table 8). Both doses of the drug exerted rather similar effects, although a significant decrease in triglyceride values was not achieved until the larger dose was administered. The amount of cholesteryl esters in the serum decreased, with arachidonate being lowered more than the other cholesteryl esters. The ovarian cholesterol content did not change when expressed as per gram of tissue (Table 9). However, the size of the ovary decreased as a result of EE administration and therefore total cholesterol was depressed when expressed as the cholesterol content of the whole organ. Adrenal cholesterol (per gram of tissue or per gland) was decreased by EE administration.

TABLE 7 EFFECT OF DOSE OF ENOVID E ON FATTY ACID COMPOSITION AND CONCENTRATION OF CHOLESTERYL ESTERS IN THE LIVER

	Control	Treatment	
		0.052 mg EE	0.52 mg EE
16:0*	29.1 ± 4.3† (9)‡	27.5 ± 3.7 (16)	24.8 ± 2.5 (25)
16:1	5.8 ± 1.5 (2)	6.5 ± 1.4 (4)	7.4 ± 2.9 (7)
18:0	9.5 ± 0.7 (3)	9.8 ± 1.6 (6)	7.3 ± 1.6 (7)
18:1	16.4 ± 2.1[ab]§ (5)	23.4 ± 2.6[a] (14)	29.4 ± 3.5[b] (29)
18:2	10.9 ± 1.7 (3)	11.4 ± 1.7 (7)	14.2 ± 1.4 (14)
20:4	15.1 ± 1.7[ed] (5)	8.3 ± 0.9[e] (5)	6.3 ± 1.8[d] (6)

* No. of carbon atoms : no. of double bonds.

† % of total cholesteryl ester fatty acids, mean ± SD, six rats per group.

‡ Values in parentheses are concentrations (mg/g of liver) of individual cholesteryl esters.

§ Numbers with the *same* letter in superscript are significantly different at $P < 0.001$.

The effect of the drug on liver lipids (Table 10) of cholesterol-fed rats differed from what was observed in rats fed stock diets. Since a large amount of cholesterol accumulated in the liver as a result of cholesterol feeding, the increase in hepatic cholesterol resulting from drug administration for 4 days was masked and was not significant. However, the triglyceride concentration was markedly decreased (from 25.14 to 9.30 mg/g).

DISCUSSION

The results of the first experiment have confirmed our previous findings that lipid metabolism of female rats is significantly affected by the administration of an oral contraceptive drug. In these experiments, a much lower dose than the one used previously also resulted in reduced serum cholesterol and phospholipid levels and increased cholesteryl esters in the liver. It has been shown that these effects were not secondary to a decreased food consumption. Cholesterol excretion was decreased in

TABLE 8 EFFECT OF DOSE OF ENOVID E ON SERUM LIPIDS IN CHOLESTEROL-FED RATS

Treatment	Cholesterol		Cholesteryl			Triglycerides
	Total	Esterified	Oleate	Linoleate	Arachidonate	
	mg/100 ml			*mg/100 ml*		*mg/100 ml*
Control	125.1 ± 25.0*[ab]	93.1 ± 7.1[cd]	19	22	29	40.1 ± 18.3[e]
0.52 mg EE	57.4 ± 16.8[a]	33.7 ± 7.7[c]	9	9	7	20.9 ± 10.9
1.04 mg EE	59.4 ± 20.3[b]	39.1 ± 6.1[d]	10	10	5	14.7 ± 11.6[e]

Numbers with the *same* letter in superscript are significantly different; superscripts a–d indicate significant difference at $P < 0.001$, and e, at $P < 0.025$.
* Mean value ± SD, six rats per group.

TABLE 9 EFFECT OF DOSE OF ENOVID E ON ADRENAL AND OVARY CHOLESTEROL IN CHOLESTEROL-FED RATS*

Treatment	Cholesterol in Adrenals		Cholesterol in Ovaries	
	mg/g	*mg/organ*	*mg/g*	*mg/organ*
Control	62.4	2.86	12.8	0.42
0.52 mg EE	37.2	1.94	12.8	0.34
1.04 mg EE	33.2	1.63	12.4	0.33

* Analyses were carried out on pooled organs.

animals given the oral contraceptive drug. Although fecal cholesterol determinations are of limited value in appraising cholesterol balance, the fact that the treated animals fed a cholesterol-free diet excreted less cholesterol or its metabolities, or both, than the untreated animals should be accepted at least as an indication of some change in cholesterol metabolism. We have previously observed more highly significant differences in fecal sterol excretion when higher doses of the drug were administered (2).

Since most of the phospholipids are present in the α-lipoproteins, the effect observed in total serum phospholipids was clearly reflected by decreases in phospholipids of α-lipoprotein. However, the phospholipids were not decreased at the same rate as cholesterol since the resulting cholesterol:phospholipid ratio was depressed. Hill and Dvornik (16) have also shown a fall in the cholesterol:phospholipid ratio in lipoproteins as a result of estradiol-17-β administration to male rats.

In the second experiment reported here, physiological doses of the drug (with antifertility activity) also resulted in deviations from "normal" cholesterol metabolism. The dose levels used here were 1/10 of the amount which had produced responses in our previous experiments.

The effect of contraceptive drugs on serum lipoprotein levels has also been shown in other investigations. According to Furman (17), estrogen administration to humans diminishes the cholesterol content of α-lipoproteins relative to phospholipid and protein content, and increases the concentration of α-lipoproteins in the serum. Horne, Howie, Weir, and Goudie (18) found elevated serum levels of α-macroglobulin in women taking oral contraceptives.

Mendenhall (19) has reported decreases in albumin and increases in immunoglobulin, ceruloplasmin, and α-antitrypsin in women taking oral contraceptives. Similar, more marked changes have been observed in pregnant women at term.

We have previously reported an enhancement of lecithin–cholesterol acyltransferase activity in plasma of rats treated with the contraceptive drug (1). This enzyme has been shown to be associated with HDL (20). As suggested by Rowen and Martin (21), the disruption of HDL structure exposes lipids to the transferase enzyme with resulting enhancement of its activity.

In the first two experiments reported here cholesteryl esters accumulated in the liver (Tables 2 and 6). The fatty acids esterified with cholesterol were primarily palmitic, oleic, and linoleic; the total amount of cholesteryl arachidonate did not change appreciably, although the proportion of cholesteryl arachidonate decreased

TABLE 10 EFFECT OF DOSE OF ENOVID E ON LIVER LIPIDS IN CHOLESTEROL-FED RATS

Treatment	Liver		Total Lipids	Cholesterol			Triglycerides
	Weight	% Body Wt		Total		Free	
	g		*mg/g*	*mg/g*	*mg/organ*	*mg/g*	*mg/g*
Control	8.00 ± 1.03*	3.3	106.5 ± 2.38	19.50 ± 6.03	15.60	3.30 ± 0.79	25.14 ± 5.13[ab]†
0.052 mg EE	7.79 ± 1.02	3.2	100.9 ± 13.8	20.53 ± 3.66	15.99	3.94 ± 0.65	10.85 ± 2.97[a]
1.04 mg EE	7.74 ± 0.78	3.3	85.9 ± 13.4	22.32 ± 2.43	17.27	3.87 ± 0.45	9.30 ± 4.57[b]

* Mean value ± SD, six rats per group.
† Numbers with the *same* letter in superscript are significantly different at $P < 0.001$.

considerably as the other esters increased. It has been suggested by Gidez, Roheim, and Eder (22) that there is a preferential incorporation of cholesteryl esters of polyenoic acids into the HDL. The reduction of serum HDL rich in highly unsaturated esters would result in a reduced amount of unsaturated cholesteryl esters. We have previously reported that, in plasma, there was a decrease in cholesteryl arachidonate and linoleate with corresponding increases of palmitate, palmitoleate, stearate, and oleate when 1.04 mg EE was administered (1).

The fact that cholesteryl arachidonate does not accumulate in the liver may mean that its incorporation into HDL and the subsequent turnover of HDL must be quite rapid. On the other hand, there might be an interference with arachidonate biosynthesis from linoleate; the linoleate content in the liver increases as the dose level of the drug increases. The fact that there are very definite changes in fatty acid pattern of cholesteryl esters both in the serum and in the liver denies the theory of simple redistribution of cholesterol within the body. Obviously, it would seem that cholesteryl arachidonate is being preferentially metabolized.

It has been shown previously that cholesteryl esters act as a reservoir of steroid precursors. Most of the cholesterol in rat adrenal glands is esterified; the fatty acids characteristically contain high percentages of the tetraenoic acids (20:4 and 22:4) (23). Sayers, Sayers, White, and Long (24) showed that administration of ACTH to rats caused a depletion of cholesterol in the adrenal. Recently, Gidez and Feller (25) have shown that a unilateral adrenalectomy causes a selective decrease of cholesteryl arachidonate in the remaining organ. They suggest that selectivity in the depletion of cholesteryl esters is due to differences in their rates of hydrolysis. Apparently, hydrolysis of esters must precede the conversion. In dog adrenals, hydrolysis of esters could be demonstrated as well as synthesis of labeled steroids from cholesterol esters (26).

It has been shown (27) that adrenals are among the most active tissues capable of removing cholesteryl esters from plasma. This process may also be instrumental in supplying cholesterol for further metabolism in these glands (in addition to stimulated biosynthesis) while at the same time being responsible for reduction of cholesterol in plasma.

In an organ overloaded with exogenous cholesterol (experiment III), the effects of the drug were somewhat masked. However, even under these conditions hypolipemic action was observed after drug administration. Here again serum cholesteryl arachidonate was decreased to the greatest extent.

It is possible that the short-term effects of anovulatory drugs may be modified by long-term administration. Studies in which physiological doses of these compounds or their components are given to adult animals over their life span are in order at this time.

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References

1. Aftergood, L., H. J. Hernandez, and R. B. Alfin-Slater. 1968. Effect of large doses of the oral contraceptive Enovid on cholesterol metabolism in the rat. *J. Lipid Res.* **9:** 447–452.
2. Alfin-Slater, R. B., and L. Aftergood. 1968. The effect of a steroid anti-ovulatory agent on cholesterol metabolism in rats. *Proc. West. Hemisphere Nutr. Congr. II.* 253.
3. Zorilla, E., M. Hulse, A. Hernandez, and H. Gershberg. 1968. Severe endogenous hypertriglyceridemia during treatment with estrogen and oral contraceptives. *J. Clin. Endocrinol. Metab.* **28:** 1793–1797.
4. Hazzard, W. R., M. J. Spiger, J. D. Bagdade, and E. L. Bierman. 1969. Studies on the mechanism of increased plasma triglyceride levels induced by oral contraceptives. *N. Engl. J. Med.* **280:** 471–476.
5. Wynn, V., J. W. H. Doar, G. L. Mills, and T. Stokes. 1969. Fasting serum triglycerides, cholesterol and lipoprotein levels during oral contraceptive therapy. *Lancet.* **2:** 756–760.
6. Ham, J. M., and R. Rose. 1969. Platelet adhesiveness and lipoprotein lipase activity in controls and in subjects taking oral contraceptives. *Amer. J. Obstet. Gynecol.* **105:** 628–631.
7. Fabian, E., A. Štork, J. Kobilková, and J. Šponarová. 1967. The activity of the lipoprotein lipase and estrogens. *Enzymol. Biol. Clin.* **8:** 451–455.
8. Otway, S., and D. S. Robinson. 1968. The significance of changes in tissue clearing factor lipase activity in relation to the lipaemia of pregnancy. *Biochem. J.* **106:** 677–682.
9. Sachs, B. A., L. Wolfman, and N. Herzig. 1969. Plasma lipid and lipoprotein alterations during oral contraceptive administration. *Obstet. Gynecol.* **34:** 530–534.
10. Schweppe, J. S., and R. A. Jungmann. 1969. The effect of hormones on hepatic cholesterol ester synthesis in vitro. *Proc. Soc. Exp. Biol. Med.* **131:** 868–870.
11. Kaplan, A., and V. F. Lee. 1965. A micromethod for determination of serum triglycerides. *Proc. Soc. Exp. Biol. Med.* **118:** 296–297.
12. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
13. Burstein, M. 1960. Dosage du cholestérol dans les α et les β lipoprotéines du serum par une methode basée sur la precipitation sélective des β lipoprotéines. *Pathol. Biol.* **8:** 1247–1249.
14. Yang, M. G., V. L. Sanger, and O. Mickelsen. 1969. Feeding norethynodrel and mestranol to immature and adult female rats. *Proc. Soc. Exp. Biol. Med.* **130:** 1146–1149.
15. Banik, U. K., C. Revesz, and F. Herr. 1969. Orally active estrogens and progestins in prevention of pregnancy in rats. *J. Reprod. Fert.* **18:** 509–513.
16. Hill, P., and D. Dvornik. 1969. Agents affecting lipid metabolism. XXXVII. Separation of rat serum lipoproteins with dextran sulfate. *Can. J. Biochem.* **47:** 1043–1048.

17. Furman, R. H. 1969. Gonadal steroid effects on serum lipids. *In* Metabolic Effects of Gonadal Hormones and Contraceptive Steroids. H. A. Salhanick, editor. Plenum Press, N.Y. 247–264.
18. Horne, C. H. W., P. W. Howie, R. J. Weir, and R. B. Goudie. 1970. Effect of combined estrogen-progestogen oral contraceptives on serum-levels of α_2 macroglobulin, transferrin, albumin, and IgG. *Lancet*. **1:** 49–50.
19. Mendenhall, H. W. 1970. Effect of oral contraceptives on serum protein concentrations. *Amer. J. Obstet. Gynecol.* **106:** 750–753.
20. Lossow, W. J., S. N. Shah, and I. L. Chaikoff. 1966. Isolation of cholesterol-esterifying activity in rat serum by ultracentrifugal flotation. *Biochim. Biophys. Acta.* **116:** 172–174.
21. Rowen, R., and J. Martin. 1963. Enhancement of cholesterol esterification in serum by an extract of group A Streptococcus. *Biochim. Biophys. Acta*. **70:** 396–405.
22. Gidez, L. I., P. S. Roheim, and H. A. Eder. 1965. Effect of diet on the cholesterol ester composition of liver and of plasma lipoproteins in the rat. *J. Lipid Res*. **6:** 377–382.
23. Gidez, L. I. 1964. Occurrence of a docosatrienoic acid in the cholesterol esters of adrenals of rats on EFA deficient diet. *Biochem. Biophys. Res. Commun*. **14:** 413–418.
24. Sayers, G., M. A. Sayers, A. White, and C. N. H. Long. 1943. Effect of pituitary adrenotropic hormone on cholesterol content of rat adrenal glands. *Proc. Soc. Exp. Biol. Med*. **52:** 200–202.
25. Gidez, L. I., and E. Feller. 1969. Effect of the stress of unilateral adrenalectomy on the depletion of individual cholesteryl esters in the rat adrenal. *J. Lipid Res.* **10:** 656–659.
26. Dailey, R. E., L. Swell, and C. R. Treadwell. 1963. Hydrolysis and utilization of cholesterol esters for steroid synthesis by canine adrenal homogenates. *Arch. Biochem. Biophys*. **100:** 360–363.
27. Brot, N., W. J. Lossow, and I. L. Chaikoff. 1964. In vitro uptake and hydrolysis, by rat tissues, of cholesterol esters of a very low density, chyle lipoprotein fraction. *J. Lipid Res*. **5:** 63–67.