Serum lipid levels during oral contraceptive and glucocorticoid administration¹

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SYNOPSIS The effects of oral contraceptives on fasting serum lipid levels have been studied longitudinally in two groups of women. One hundred and twenty-eight subjects (group A) were tested before and during therapy; 52 subjects (group B) were tested initially during therapy and again after this had been discontinued. In both groups oral contraceptive therapy was associated with significantly raised mean serum triglyceride and cholesterol levels. No relation was found between the magnitude of change of serum triglyceride levels and the nature of oestrogen-progestogen combination, age, parity, degree of obesity, family history of diabetes, or duration of therapy. A significant elevation of the mean fasting serum triglyceride level was also found in a group of 19 women receiving low-dose glucocorticoid therapy, though the percentage increase (16%) was less than that in the women receiving oral contraceptives (49%).

Studies carried out in the decade 1950-59 showed that oestrogen therapy was associated with a reduction in serum cholesterol and low density lipoprotein levels in postmenopausal women and men, many of whom had ischaemic heart disease (Russ, Eder, and Barr, 1951; Oliver and Boyd, 1956a and b; Furman, Howard, Norcia, and Keaty, 1958). As a result of these studies trials of oestrogen therapy in survivors of myocardial infarction were instituted (Oliver and Boyd, 1961; Stamler, Katz, Pick, Lewis, Page, Dick, Kaplan, Berkson, and Century, 1960). Progesterone administration was found to have no significant effects on serum lipid levels (Oliver and Boyd, 1956a and b), though a synthetic progestogen (17 α ethinyltestosterone) lowered serum cholesterol levels in 12 hypercholesterolaemic men with ischaemic heart disease (Oliver and Boyd, 1956a).

The demonstration of raised fasting serum cholesterol, triglyceride and low density and very low density lipoprotein levels in certain women receiving oral contraceptive therapy was an unexpected finding (Aurell, Cramér, and Rybo, 1966; Wynn, Doar, and Mills, 1966; Wynn, Doar, Mills, and Stokes, 1969; Wynn and Doar, 1969b; Gershberg, Hulse, and Javier, 1968).

The mechanism and clinical significance of these changes is unknown. We have suggested

that the relative impairment of oral glucose tolerance commonly found during oral contraceptive therapy is 'steroid diabetes' (Wynn *et al*, 1966, 1969) caused by elevation of both the free and protein-bound plasma cortisol levels (Burke, 1969). It is possible that the altered serum lipid levels during oral contraceptive therapy may also result from the same mechanism. There have, however, been no detailed studies of the effects of glucocorticoid therapy on serum lipid levels.

The present study of women tested while receiving and not receiving oral contraceptive therapy was carried out to evaluate further the changes in serum lipid and lipoprotein levels during oral contraceptive therapy.

Subjects

One hundred and eighty women were studied while taking and not taking oral contraceptive therapy. One hundred and twenty-eight subjects (group A) were tested before and during therapy, and 52 subjects (group B) were tested initially during therapy and again after this had been discontinued. No subject was taking any drug known to affect lipid metabolism (excepting oral contraceptives). Details of the two groups are shown in Table I and of the oral contraceptives used in

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	Group A	Group B
No. of women	128	52
Mean age (years)	26 (range 17-46)	32 (range 21-49)
Mean body weight as percentage of ideal body weight	103 (range 74-175)	113 (range 77-196)
Obese women (%)	13	38
Mean parity	1.0 (range 0-5)	2.0 (range 0-7)
Positive family history of diabetes (%)	48	48
Mean time (months) on (group A) and off (group B) oral contraceptive therapy	6.0 (range 3-48)	4.4 (range 2-19)
Mean duration of oral contraceptive therapy in group B subjects (months)		25.4 (range 2-72)

Table I Details of group A and B subjects

	Group A	Group B
Ovulen	42	21
'Step-up' ¹	11	—
Anovlar/Gynovlar	19	12
Norinyl	24	2
Lyndiol	13	7
Volidan	7	4
Orthonovin	5	4
Sequential preparations	7	1
Enavid/Enavid E	—	1

Table II Nature of oestrogen-progestogen therapy in groups A and B

¹'Step-up' (0.1 mg mestranol + 0.1 mg ethynodiol acetate for 16 days, 0.1 mg mestranol + 0.5 mg ethynodiol acetate for seven days)

Table II. A further group of 19 women receiving glucocorticoid therapy (prednisolone, mean daily dose 11 mg, range 5-25 mg) was also tested. Indications for glucocorticoid therapy in these subjects were uveitis (12), asthma (4), pemphigus (1), systemic lupus erythematosus (1), and polyarteritis nodosa (1). The clinical condition was well controlled in all 19 subjects at the time of testing.

Methods

Samples of venous blood were taken after an overnight fast of at least 12 hours, care being taken to standardize the effects of posture and hydrostatic pressure on serum lipid levels (Stoker, Wynn, and Robertson, 1966). Serum cholesterol and triglyceride levels were measured by semi-automated fluorimetric techniques (Cramp and Robertson, 1968; Robertson and Cramp, 1969). Oral glucose tolerance tests were also carried out by methods previously described (Wynn and Doar, 1966) in the majority of subjects studied. Each subject's degree of obesity was assessed by expressing the body weight as a percentage of

ideal body weight (Documenta Geigy, 1956). Subjects exceeding 120% of their ideal body weight were considered to be obese.

Standard statistical techniques were used, including Student's *t* test for paired data, analysis of variance, the product moment correlation coefficient, and Snedecor's 'F' test. Serum lipid levels of subjects receiving glucocorticoid therapy were compared with those of a control group of women of similar mean age and degree of obesity using Student's *t* test for unpaired data. Logarithmic conversion of serum triglyceride levels was carried out before analysis because of positively skewed distributions.

Results

Mean serum triglyceride and cholesterol levels in group A and B subjects while taking and not taking oral contraceptive therapy are shown in Table III, and the changes in serum triglyceride levels in individual subjects in Figures 1 and 2.

SERUM TRIGLYCERIDE LEVELS

The mean fasting serum triglyceride level in 128 group A subjects (67.8 ± 22.6 mg/100 ml) increased during therapy (101.2 ± 30.0 mg/100 ml, $P < 0.001$); the mean level in 52 group B subjects during therapy (126.9 ± 42.6 mg/100 ml) fell after therapy was discontinued (86.5 ± 28.1 mg/100 ml, $P < 0.001$). The increase in serum triglyceride levels during oral contraceptive therapy was an almost invariable finding in that 95% of group A subjects and 88% of group B subjects were affected. We consider the upper limit of normal serum triglyceride in women of this age group to be 131 mg/100 ml (Wynn and Doar, 1966). Control values for group A subjects were all below this level with two exceptions, and during therapy abnormally raised levels occurred in 16 subjects (13%). Twenty-three of the 52 (44%) group B subjects had abnormally raised serum triglyceride levels during therapy, and of these all but five returned to the normal range after therapy was stopped. The variance of serum triglyceride levels was significantly increased ($P < 0.01$) in both groups during therapy, suggesting that some subjects were affected more than others. The correlation between the control serum triglyceride and the change in serum

		Therapy (mean $\pm$ SD)			
		Number of Subjects	Off (mg/100 ml)	On (mg/100 ml)	P
Serum triglyceride	Group A	128	67.8 ± 22.6	101.2 ± 30.0	<0.001
	Group B	52	86.5 ± 28.1	126.9 ± 42.6	<0.001
Serum cholesterol	Group A	128	180.2 ± 35.0	187.0 ± 29.8	<0.05
	Group B	52	198.8 ± 28.2	207.7 ± 40.3	<0.02

Table III Mean serum triglyceride levels in group A and B subjects off and during oral contraceptive therapy

Fig. 1 Serum triglyceride levels in 128 group A subjects before and during oral contraceptive therapy. A 45° line is shown.

Fig. 2 Serum triglyceride levels in 52 group B subjects during therapy and after therapy was discontinued. A 45° line is shown.

triglyceride level in group A subjects after starting therapy ($r = -0.18$, $P < 0.05$) was just significant, but probably due to the phenomenon of regression about the mean.

No significant correlation was found between the changes in serum triglyceride or cholesterol in group A and B subjects and age, parity, or degree of obesity. Analysis of variance showed the mean change of serum triglyceride levels in group A subjects to be similar with various oestrogen-progestogen combinations. The mean changes in serum triglyceride and cholesterol levels while taking and not taking oral contraceptive therapy were similar in group A and B subjects with and without a family history of diabetes mellitus.

While there was no significant correlation between the change of serum triglyceride level and duration of therapy in group A subjects the majority of these had received therapy for a similar period, that is, four to eight months. To investigate this aspect further serum lipid levels were analysed in a group of 169 subjects who had received therapy for periods ranging up to 159 months (Table IV). Analysis of variance showed no significant effect of duration of therapy on serum triglyceride or cholesterol levels.

Studies of diurnal serum triglyceride levels in four subjects off and on oral contraceptive therapy have shown that serum triglyceride levels remain raised during therapy for the whole 24-hour period. An example is shown in Figure 3.

SERUM CHOLESTEROL LEVELS

The mean serum cholesterol level in 128 group A subjects (187.0 ± 29.8 mg/100 ml) was slightly but significantly higher than their mean control level (180.2 ± 35.0 , $P < 0.05$). Similarly, the mean serum cholesterol level in 52 group B subjects during therapy (207.7 ± 40.3) fell slightly after therapy was discontinued (198.8 ± 28.2 , $P < 0.02$), (Table III).

SERUM LIPID LEVELS IN SUBJECTS RECEIVING GLUCOCORTICOID THERAPY

Mean fasting serum cholesterol and triglyceride levels in groups of control and glucocorticoid-treated women are shown in Table V. Slight increases in both mean serum triglyceride and

	Duration of Therapy (months)					
	0-9	10-29	30-49	50-69	70-89	90-159
No. of subjects	46	49	33	22	12	7
Mean age (years)	27	27	31	35	38	41
Mean degree of obesity (% 'ideal body weight')	105	107	106	109	101	107
Mean serum triglyceride $\pm$ SD (mg/100 ml)	103.3 $\pm$ 34.0	110.1 $\pm$ 44.7	112.8 $\pm$ 35.8	121.3 $\pm$ 41.3	100.3 $\pm$ 29.2	107.1 $\pm$ 19.7
Mean serum cholesterol $\pm$ SD (mg/100 ml)	196.6 $\pm$ 29.3	189.6 $\pm$ 32.5	193.4 $\pm$ 37.5	212.6 $\pm$ 38.1	199.9 $\pm$ 42.4	202.3 $\pm$ 30.2

Table IV Effect of duration of oral contraceptive therapy on serum lipid levels

Fig. 3 Diurnal serum triglyceride levels in a 36-year-old woman on and off oral contraceptive therapy.

	Control Subjects	Glucocorticoid-treated Subjects	P
No. of subjects	53	19	—
Mean age (years)	31 (range 25-45)	29 (range 13-45)	—
Mean degree of obesity (% 'ideal body weight')	105 (range 71-175)	108 (range 87-162)	—
Mean serum triglyceride $\pm$ SD (mg/100 ml)	74.6 $\pm$ 26.1	86.9 $\pm$ 26.9	<0.05
Mean serum cholesterol $\pm$ SD (mg/100 ml)	187.4 $\pm$ 38.3	202.6 $\pm$ 42.8	NS

Table V Serum lipid levels in control subjects and subjects receiving glucocorticoid therapy

cholesterol levels were observed in the glucocorticoid-treated subjects but only the former achieved statistical significance.

Discussion

The present observation of significantly raised fasting serum triglyceride and cholesterol levels during oral contraceptive therapy confirms and extends our previous findings (Wynn *et al*, 1966, 1969; Wynn and Doar, 1969b). We have also noted increased low density and very low density lipoprotein levels during therapy and a decreased rate of flotation of the modal component of the Sf 0.12 lipoproteins (Wynn *et al*, 1969). Since the contribution of chylomicra to fasting serum triglyceride levels was similar in control subjects and women receiving oral contraceptive therapy (Wynn *et al*, 1969), the elevation of serum triglyceride levels during oral contraceptive therapy is probably due to the raised serum low and very low density lipoprotein levels. The small increase in serum cholesterol during therapy is surprising but may be due to the reduction in high density

lipoprotein cholesterol noted by Aurell *et al* (1966). Mean serum cholesterol and triglyceride levels were higher in group B than group A subjects, both while taking and not taking oral contraceptive therapy. The group B subjects, however, were on average older, more parous and more obese than group A subjects. A notable finding was that the raised serum lipid and lipoprotein levels returned towards normal in group B subjects after therapy was discontinued. The increase in serum triglyceride during therapy affected 95% and 88% of group A and B women respectively. If the day-to-day variation is taken into account it is likely that all subjects were affected. In this and previous studies (Wynn *et al*, 1966, 1969; Wynn and Doar, 1969b) we have found no relation between the change in serum lipid levels during therapy and the subject's age or degree of obesity. Similar changes were also noted with various oestrogen-progestogen combinations (Wynn *et al*, 1969). Although the changes in serum lipid levels qualitatively resemble those occurring during pregnancy, there are important differences. In particular, the elevation of serum cholesterol is much more marked during the latter (Svanborg and Vikrot, 1965). Gershberg *et al* (1968) found a weak but significant correlation ($r = 0.29$, $P < 0.05$) between the serum triglyceride level and duration of oral contraceptive therapy in 49 women treated with Enovid for three to 51 months. In the present study, however, we found no significant correlation between the change in serum triglyceride and duration of therapy in a longitudinal study of 128 group A subjects who had received therapy for three to 48 months; analysis of variance of serum triglyceride and cholesterol data also showed no effect of duration of therapy in a further group of 169 women treated for periods ranging from two to 159 months. There is evidence to suggest that the increase in serum triglyceride during oral contraceptive therapy occurs rapidly. Hazzard, Spiger, and Bagdade (1969) found a 47% mean increase in serum triglyceride after two weeks' administration of ethinyl oestradiol, 0.05 mg, and medroxyprogesterone acetate, 10 mg, a figure similar to that observed in our present longitudinal study (49%) in which the mean duration of therapy was six months. In individual studies we have also observed a rapid rise in serum triglyceride (Fig. 4) during therapy. There is a large body of literature relating to the effects of natural and synthetic steroids on serum lipid and lipoprotein levels in man. In general, oestrogens decrease total serum cholesterol and serum low density lipoprotein levels and raise serum high density lipoprotein levels (Russ *et al*, 1951; Oliver and Boyd, 1956a and b; Furman *et al*, 1958) and triglyceride levels (Robinson and Le Beau, 1965; Gershberg *et al*, 1968; Wynn and Doar, 1969b). Androgens, some of which bear a chemical similarity to

Fig. 4 Effect of oestrogen administration on serum lipid levels in a 59-year-old woman with maturity-onset diabetes mellitus (ideal body weight 58.4 kg).

progestogens, usually increase serum low density and decrease serum high density lipoprotein levels; serum total cholesterol levels, however, are little affected (Russ *et al*, 1951; Robinson, Le Beau, and Cohen, 1964; Furman *et al*, 1958). Progesterone administration has no significant effect on serum lipid levels (Svanborg and Vikrot, 1966). Serum cholesterol levels, however, fell in 12 hypercholesterolaemic men receiving a synthetic progestogen, 17 α ethinyltestosterone (Oliver and Boyd, 1956a). Brody, Kerstell, Nilsson, and Svanborg (1968) noted serum lipid levels to be unchanged during treatment with megestrol acetate but serum cholesterol was reduced during norethisterone acetate therapy.

Certain important conclusions may be drawn from these studies. First, serum triglyceride and very low density lipoprotein levels were not measured by the majority of early investigators. The elevation of serum triglyceride levels during oestrogen therapy has only been appreciated in recent years. Second, there have been no detailed studies of the effects of individual gonadal steroids in healthy premenopausal women. The majority of subjects investigated were hypercholesterolaemic, hypogonadal, or had ischaemic heart disease. The effects of gonadal steroids on serum lipid levels in such subjects may well differ from those caused by such compounds in healthy premenopausal women. It has been observed, for example, that the serum lipid response to oestrogens (Feldman, Wang, and Adlersberg, 1959) and androgens (Howard and Furman, 1962) may differ both qualitatively and quantitatively in subjects with abnormally raised serum lipid levels, compared with the changes observed in subjects with normal serum lipid levels. We have found the elevation of serum cholesterol during methandienone therapy to be more marked in subjects with maturity-onset diabetes mellitus than in non-diabetic subjects (Wynn,

Fig. 5 Effect of methandienone therapy in a 52-year-old woman with maturity-onset diabetes mellitus.

1967). An example is shown in Figure 5. Third, certain steroid combinations appear to act in a synergistic rather than in an additive manner on serum lipid levels (Hood and Cramér, 1959). It is therefore difficult to predict the effect of any given oestrogen-progestogen combination on serum lipid levels. A further difficulty in making such predictions arises from the fact that certain synthetic progestogens are metabolized into oestrogenically active compounds (Pincus, 1965).

The few longitudinal studies of serum lipid and lipoprotein levels during the administration of oestrogen-progestogen combinations have shown conflicting results. Pincus (1965) found no change in serum cholesterol or β -lipoprotein levels in 41 women treated with Enavid for one year. Aurell *et al* (1966), however, noted raised serum triglyceride and cholesterol levels in eight subjects treated with Anovlar for one year, though Brody *et al* (1968) found no significant change in either lipid level in nine women receiving the same drug combination for a similar period. The latter group, however, noted raised serum triglyceride levels during Ovulen therapy. Gershberg *et al* (1968) found serum triglyceride levels to be raised during Enavid therapy but serum cholesterol levels were unchanged.

The mechanism by which serum lipid levels become raised during oral contraceptive therapy is unknown. Theoretically this must result from

an increased rate of entry into or a decreased rate of removal of lipid from the circulation or a diminished volume of distribution. Current techniques do not allow distinction between these possibilities. Indirect evidence has suggested that both increased triglyceride synthesis secondary to raised fasting plasma insulin levels, and decreased triglyceride removal consequent upon diminished post-heparin lipolytic activity may contribute to the increase in serum triglyceride levels (Hazzard *et al*, 1969). The majority of workers, however, have found the fasting plasma insulin to be unchanged during oral contraceptive therapy (Spellacy and Carlson, 1966; Javier, Gershberg, and Hulse, 1968; Wynn and Doar, 1969b) and we previously found no significant correlation between the changes in fasting serum triglyceride and plasma insulin levels in subjects studied before and during therapy (Wynn and Doar, 1969b). It is not clear whether serum lipid levels during therapy are due to a primary effect of the oestrogen and/or progestogen on lipoprotein metabolism or secondary to increased circulating levels of other hormones, such as cortisol, thyroxine, or growth hormone. We have suggested that the raised oral glucose tolerance test plasma glucose and blood pyruvate levels found during therapy are 'steroid diabetes' secondary to oestrogen-induced elevation of the free and protein-bound cortisol levels (Wynn and Doar, 1966; Doar and Wynn, 1970; Slightly raised (Adlersberg, Schaeffer, and Drachman, 1950) or lowered serum cholesterol levels (Oliver and Boyd, 1956b; Moses *et al*, 1962) but unchanged serum triglyceride levels (Moses, Jablonski, Sunder, Greenman, and Danowski, 1962) have been observed during glucocorticoid or ACTH therapy. Our present cross-sectional study showed low-dose glucocorticoid therapy to be associated with a slight increase in mean serum triglyceride and cholesterol levels above those of the control women, though only the former achieved statistical significance. The percentage increase in serum triglyceride during therapy, however, was only 16% in the glucocorticoid group compared with 49% in the oral contraceptive group. While the glucocorticoid data are difficult to interpret without knowledge of the influence of the underlying disease process on serum lipid levels, nevertheless the changes in serum lipid levels do not quantitatively resemble those found during oral contraceptive therapy. Raised circulating thyroid hormone levels during therapy are unlikely to be responsible for the change in serum lipid levels since we found thyrotoxicosis (Doar, Stamp, Wynn, and Audhya, 1969) and the administration of L-triiodothyronine to control subjects (Stamp, Doar, and Wynn, 1969) to be associated with lowered serum cholesterol and unchanged serum triglyceride levels. The effect of raised plasma growth hormone levels on serum lipid levels in acromegaly or during human growth hormone

administration to hypopituitary dwarfs is difficult to interpret because of the impaired thyroid and/or adrenocortical function and altered glucose tolerance commonly found in these situations.

Slight elevation of serum lipid levels in subjects with mild chemical diabetes mellitus is well known, but difficult to interpret since these subjects are often obese. Oral glucose tolerance, assessed as the total area between the oral glucose tolerance test plasma glucose curve and the abscissa, is relatively impaired during oral contraceptive therapy in 80 to 90% of subjects (Wynn and Doar, 1969a) and we have considered the possibility that raised fasting serum triglyceride levels during therapy may be related to this deterioration in glucose tolerance. No significant correlations were found, however, between the change in serum triglyceride level before and during therapy and the control oral glucose tolerance test glucose area or the change in that glucose area after starting therapy (Wynn and Doar, 1969b).

The relation between rates of hepatic synthesis of lipid and protein (lipoprotein apoprotein) moieties of lipoprotein is unknown. Raised circulating plasma non-esterified fatty acid (Nefa) levels, as in diabetic ketosis, are known to stimulate hepatic synthesis and the release of large amounts of very low-density lipoprotein (Havel, 1961). Hepatic very low density apoprotein synthesis is presumably stimulated by increased lipid formation from Nefa. It is conceivable, however, that in certain metabolic situations the converse may occur. Oestrogen administration is associated with raised circulating levels of several carrier proteins, including transcortin, thyroxine-binding globulin, transferrin, and caeruloplasmin (Doe, Mellinger, Swaim, and Seal, 1967). If the rate of hepatic apoprotein synthesis were increased by oestrogen therapy, stimulation of hepatic lipoprotein synthesis and release into the circulation might follow. The rapid rise in serum triglyceride levels during oral contraceptive therapy (Hazzard *et al*, 1969) resembles that of other carrier proteins during oestrogen therapy (Doe *et al*, 1967).

Raised serum lipid and lipoprotein levels are known to be associated with the development of clinical manifestations of atherosclerosis (Kannel, Dawber, Friedman, Glennon, and McNamara, 1964; Brown, Kinch, and Doyle, 1965). While not all subjects receiving oral contraceptive therapy develop serum lipid levels outside the normal range, it is possible that any elevation of any low-density serum lipoprotein level may accelerate the rate of development of atherosclerosis. The safety of long-term oestrogen-progestogen administration must therefore be seriously questioned. A possible link between thrombosis and altered serum lipid levels during oral contraceptive therapy has been provided by Bolton, Hampton, and Mitchell (1968) who found altered platelet behaviour, caused by an-

abnormality of low-density lipoprotein lecithin similar to that observed in patients with arteriosclerosis. It is clear that the effects of any synthetic steroid on serum lipid and lipoprotein levels cannot be confidently predicted, and, further, that these effects may differ in healthy subjects from those who already have metabolic abnormalities such as impaired glucose tolerance or raised serum lipid levels. It will be many years before the answers to these problems are resolved but the numbers of women at risk are large. The incidence of cerebral thrombosis has already been shown to be increased in women taking oral contraceptives (Inman and Vessey, 1968; Vessey and Doll, 1969).

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