The effect of oral contraceptives on cortisol metabolism

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SYNOPSIS The administration of oral contraceptives which contain oestrogen increases non-protein-bound plasma cortisol levels at 9 am as well as protein-bound and total cortisol levels. These increases are dependent on the dose of oestrogen; they are not usually seen with progestogen-only or 'low-dose' oestrogen (0.05 mg mestranol or less) preparations. 'Standard' oral contraceptives (0.1 mg mestranol or equivalent) produce some elevation of unbound cortisol levels at 9 am (from a normal mean of 0.66 $\mu\text{g}/100\text{ ml}$ to 1.02 on the 'pill') but this elevation is less than that associated with high-dose oestrogen treatment of, for example, prostate cancer (mean 1.8 $\mu\text{g}/100\text{ ml}$). Since unbound cortisol levels in plasma are controlled by a hypothalamic feedback mechanism, it appears that oral contraceptives have some effect on this mechanism. Possible long-term effects of oral contraceptives on hypothalamo-pituitary function require examination.

However, the plasma unbound cortisol to which the tissues are exposed at 9 am does not measure the overall exposure of tissues to cortisol throughout the 24 hours. Neither does measurement of cortisol production rate or urinary metabolite excretion accurately reflect the exposure of tissues to cortisol during oestrogen treatment, because of the complex effects of oestrogen on hepatic metabolism of steroids, steroid-protein binding, and the increased size of the extracellular cortisol pool.

The overall exposure of tissues to unbound cortisol is measured better by urinary free cortisol excretion. Urinary free cortisol excretion is a measure of the integrated area under the diurnal curve of plasma unbound cortisol, ie, of the 24-hour exposure of tissues to unbound cortisol. Urinary free cortisol excretion is normal in women taking low-dose oestrogen or progestogen-only contraceptives, and is only trivially increased by the standard 'pill'. Thus increased exposure of tissues to unbound cortisol is likely to be only a minor factor in the metabolic responses to oral contraceptives. In contrast, urinary free cortisol excretion (mean normal 38 $\mu\text{g}/24\text{ hours}$) is increased by high-dose oestrogen administration for prostatic cancer (mean 110 $\mu\text{g}/24\text{ hours}$); this is because the diurnal rhythm of unbound cortisol is impaired.

It is thus unwise to ascribe effects of oral contraceptives to increased exposure of tissues to cortisol, except in the liver where it is possible that the increased concentration of protein-bound cortisol they cause may exert metabolic effects. The preparations which cause least change in cortisol metabolism are the low-dose oestrogen or progestogen-only contraceptives.

Much has been published concerning the effect of administering various steroids upon cortisol metabolism; in the case of oral contraceptives these effects relate largely to the oestrogen component, for administration of progestogens alone has not been shown to have any consistent effect on cortisol metabolism. The literature relating to the effects of oestrogen on cortisol metabolism is complex. The purpose of this paper is to show how a simple approach suggests that the effect of oral contraceptives in increasing general tissue exposure to cortisol is slight, and is related to the daily oestrogen dose. However, it must be conceded that standard oral contraceptives cause gross disturbances of cortisol metabolism which raise doubt about the wisdom of taking them for long periods.

It has long been known that oestrogens increase total plasma cortisol levels but do not increase urinary excretion of cortisol metabolites (Robertson, Stiefel, and Laidlaw, 1959; Peterson, Nokes, Chen, and Black, 1960). The conflict between this observation and the fact that oestrogen-treated subjects do not clinically resemble patients with Cushing's syndrome having similar plasma cortisol levels was clarified by the demonstration of oestrogen-induced increases in cortisol binding to plasma proteins (Sandberg and Slaunwhite, 1959; Sandberg, Slaunwhite, and Carter, 1960). It is quite certain that administering oestrogen causes an increase in plasma levels of corticosteroid-binding globulin (called CBG) (Daughaday, Adler, Mariz, and Rasinski, 1962; De Moore, Steeno, Brosens, and Hendriks, 1966; Doe, Fernandez, and Seal, 1964). The magnitude of this increase is dependent on the dose of oestrogen given (Doe, Mellinger, Swaim, and Seal, 1967a). The increase in corticosteroid-binding globulin concentration is not apparently due to its increased catabolism (Sandberg, Woodruff, Rosenthal, Nienhouse, and Slaunwhite, 1964); it is probably due to increased hepatic synthesis of corticosteroid-binding globulin, in parallel with the rises in other specific serum proteins induced by oestrogen (Musa, Doe, and Seal, 1967). The raised level of corticosteroid-binding globulin falls to normal within two to three weeks after stopping the oestrogen. Increased levels are not produced by progesterone or prevented by androgens (Sandberg *et al*, 1960).

Increased CBG-bound cortisol in the plasma of oestrogen-treated subjects has been demonstrated directly (Doe, Zinneman, Flink, and Ulstrom, 1960; Mills, Schedl, Chen, and Bartter, 1960). The importance of this is that cortisol is biologically not available to most tissues as long as it remains bound to corticosteroid-binding globulin (Slaunwhite, Lockie, Back, and Sandberg, 1962; Blecher, 1966; Matsui and Plager, 1966). For this reason much interest has centred round non-protein-bound

cortisol levels in oestrogen-treated subjects. Early reports (Doe *et al*, 1960; Mills *et al*, 1960; Plager, Schmidt, and Staubitz, 1964) gave contradictory results for unbound cortisol levels in oestrogen-treated subjects, due largely to difficulties with methodology. Later work with better methods has resolved the discrepancies, the consensus of opinion now being that oestrogen administration increases unbound biologically active cortisol levels in plasma (Murray, 1967; Doe, Dickinson, Swaim, Zinneman, and Seal, 1967b; Doe, Dickinson, Zinneman, and Seal, 1969; O'Connell and Welsh, 1969) and this is true of oral contraceptives (Burke, 1969a). When the oestrogen is given cyclically, there is no significant change in unbound cortisol levels during the cycle (O'Connell and Welsh, 1969).

These findings concern measurement at one or more points in time only. The average effect of oestrogens on cortisol metabolism over 24 hours might be estimated by the rate of production of cortisol; but in fact this is a poor reflection of the tissue exposure to cortisol during oestrogen treatment. The reason for this is that the metabolic clearance rate of cortisol is decreased and the plasma half-life prolonged by oestrogen treatment (Peterson, 1959; Robertson *et al*, 1959), so that although the miscible pool of cortisol is larger the flux through it is less (Layne *et al*, 1962). Two factors contribute: the increased protein-bound pool in plasma and extracellular fluid (Sandberg, Rosenthal, Schneider, and Slaunwhite, 1966) and the slowed metabolic degradation. Steroid dynamics under these conditions are hard to interpret, and cortisol production rates do not reflect exposure of tissues to cortisol during oestrogen administration. It may be noted, moreover, that little of this work refers explicitly to oral contraceptives.

I have adopted a simpler approach to these complexities, namely, to estimate the 'tissue exposure' to free cortisol averaged over 24 hours. As an example, we may consider the curve of plasma cortisol plotted against time in a normal subject shown in Figure 1. The area under the lower curve of plasma unbound cortisol is a measure of the 'tissue exposure' (concentration $\times$ time) to unbound cortisol for 24 hours. This area would be tedious to measure, were it not for the fact that unbound cortisol is ultrafiltered by the kidney (Beisel, Cos, Horton, Chao, and Forsham, 1964). Thus the area under the curve can be estimated by measuring urinary free cortisol excretion, the kidney performing an integration of the curve of plasma unbound cortisol with respect to time.

I have therefore estimated urinary free cortisol excretion and plasma unbound cortisol levels as a measure of exposure of tissues to cortisol in subjects taking various doses of oestrogen, including oral contraceptives.

Fig. 1 The diurnal variation of unbound plasma cortisol (—) and total plasma cortisol (---) in a normal subject.

Fig. 2 Steady-state gel filtration of 25 ml of human serum on a 30×1.4 cm column of Sephadex G-50 at 37°C . Serum total cortisol concentration $21 \mu\text{g}/100 \text{ ml}$, serum protein concentration equal to height of protein plateau shown. There are two plateaux of cortisol concentration, the first equal to the total plasma cortisol and the second equal to the unbound plasma cortisol.

Methods

Free cortisol excretion was measured by competitive protein-binding analysis of dichloromethane extracts of urine (Beardwell, Burke, and Cope, 1968), the method being based on that of Murphy (1968). Total plasma cortisol was measured in the

same way. This assay is relatively specific for cortisol and corticosterone; progesterone and 11-desoxycortisol interfere but this is unlikely to be significant in the samples tested. The oral contraceptives encountered in this study caused no significant interference in the assay (Table I). Values for plasma cortisol by this method were

Name of 'Pill'	Constituents	Apparent Cortisol Content of Whole Tablet (μg)	Effect of Constituent Steroids as Fraction of Same Weight of Cortisol
Metrolen-M	Ethinodiol diacetate 1 mg	0	0
Ovulen	Mestranol 0.1 mg	0	0
Lyndiol 2.5	Lynestrenol 2.5 mg	0	0
	Mestranol 0.075 mg		
Metrolen	Ethinodiol diacetate 2 mg	0.48	0.0002
	Mestranol 0.1 mg		
C-Quens 21	Chlormadinone acetate 1.5 mg	0.36	0.0002
	Mestranol 0.1 mg		
Gynovlar 21	Norethisterone acetate 3 mg	2.40	0.0008
Controvlar	Ethinylloestradiol 0.05 mg		
Anovlar 21	Norethisterone acetate 4 mg	2.80	0.0007
	Ethinylloestradiol 0.05 mg		
Minovlar	Norethisterone acetate 1 mg	1.44	0.0014
	Ethinylloestradiol 0.05 mg		
Enavid E	Norethynodrel 2.5 mg	3.60	0.0014
Feminor	Mestranol 0.1 mg		
Previson			
Enavid	Norethynodrel 5 mg	4.44	0.0009
Feminor seq.	Mestranol 0.075 mg		
Norinyl-1	Norethisterone 1 mg	2.40	0.0023
	Mestranol 0.05 mg		

Table I Effect of oral contraceptives in competitive protein-binding cortisol assay

about 70% of the plasma 11-hydroxysteroid concentrations in the same samples determined by Mattingly's method (1962), and were within 10% of true cortisol concentrations as determined by paper chromatography in 10 samples.

Plasma unbound cortisol was measured by steady-state gel filtration (Burke, 1969b). This method preserves the labile equilibrium between cortisol and its binding proteins, and is carried out on undiluted plasma at body temperature and pH. The principle is that the plasma sample is passed continuously into a Sephadex G-50 column at 37°C until a steady state is reached. The leading edge of the sample gives up cortisol to the column, so that the first protein fractions in the effluent are devoid of cortisol (Fig. 2); this dissociated cortisol is retarded by molecular sieving and overtaken by the fresh incoming sample. When the cortisol concentration in the gel builds up to the unbound cortisol concentration in the incoming sample, no further net dissociation of steroid-protein complexes takes place and the rest of the sample, irrespective of its size, passes through the column at equilibrium. A plateau of cortisol and protein concentrations identical to those in the sample is then seen in the effluent, as shown in Figure 2. When the supply of sample is stopped, the protein and protein-bound cortisol concentrations in the effluent fall to zero and the equilibrium unbound cortisol present in the column is eluted as a second cortisol plateau by displacement with a suitable buffer. Thus the cortisol concentration in the final effluent plateau is equal to the true non-protein-bound cortisol concentration in the original sample of plasma at 37°C. A sample of this plateau is removed and assayed for cortisol as described above; alternatively its cortisol concentration may be derived from the total plasma cortisol concentration if tritiated cortisol is added to the plasma before gel filtration, by determination of the tritium concentrations in the two cortisol plateaux. The physicochemical principles to be satisfied are discussed elsewhere (Burke, 1969b). In practice, 10 ml plasma samples can be used on a 20 by 0.9 cm column, the plasma being gassed with 5% CO₂ before analysis to restore pH. The coefficient of variation of 14 replicate estimates of unbound cortisol on pooled serum was 3.5%.

Subjects

Normal subjects were healthy volunteers from hospital staff. The women taking oral contraceptives were otherwise healthy, and had been taking these drugs for at least six months. Twenty-one of these were taking standard oral contraceptives, containing 0.1 mg of mestranol or ethinyloestradiol combined with a variety of progestogens as shown in Table I. Two women were taking chlormadinone acetate, 0.5 mg daily,

alone, and four others were taking oral contraceptives which contained 0.05 mg mestranol; the results in these two subgroups were indistinguishable and they were combined as a 'low-dose oestrogen' group. Seven men with prostatic carcinoma who were being treated with a variety of oestrogens equivalent to a daily dose of between 0.2 and 3.0 mestranol per day were studied; none was clinically ill. Ten cases of Cushing's syndrome were included for comparison.

Results

EFFECT OF OESTROGEN DOSAGE ON TOTAL PLASMA CORTISOL LEVELS AT 9 AM

Figure 3 shows the increases in plasma cortisol in the groups studied. Since it seemed likely that the effects of oral contraceptives upon cortisol metabolism might be related to their oestrogen content, the cortisol levels measured were

Fig. 3 Total plasma cortisol concentrations at 9 am in oestrogen-treated subjects and patients with Cushing's syndrome. The shaded area is the range of values in normal subjects.

Fig. 4 Unbound plasma cortisol concentrations at 9 am in normal and oestrogen-treated subjects and in patients with Cushing's syndrome.

arranged in groups according to oestrogen dosage as shown. It can be seen that low-dose oestrogen or progestogen-only oral contraceptives have no significant effect on total plasma cortisol levels at this time of day.

The administration of standard oral contraceptives is associated with a substantial rise in plasma cortisol (normal female mean 12 $\mu\text{g}/100\text{ ml}$, mean on standard 'pill' 32 $\mu\text{g}/100\text{ ml}$) as is well known, but high-dose oestrogen treatment causes a greater increase (mean 63 $\mu\text{g}/100\text{ ml}$). The values in the patients with Cushing's syndrome are shown for comparison.

EFFECT OF OESTROGEN DOSAGE ON UNBOUND PLASMA CORTISOL LEVELS AT 9 AM
Figure 4 shows the levels of unbound cortisol

found at 9 am in the normal and oestrogen-treated subjects, and in the patients with Cushing's syndrome. The low-dose oestrogen and progestogen-only preparations had no significant effect on unbound cortisol levels. The 'standard' preparations are associated with a significant rise in the mean level of unbound cortisol at this time of day, but most of the values are within the normal range as previously described (Burke, 1969a). High-dose oestrogen treatment is associated with much higher levels of unbound cortisol in some cases, approaching values found in Cushing's syndrome.

The means and standard deviations depicted in Figs. 4 and 5 are derived from the $\log_{10}$ values, unbound cortisol being approximately log-normally distributed; the mean and range of absolute values are shown in Table II.

Group	Total Plasma Cortisol ($\mu\text{g}/100\text{ ml}$)		Unbound Plasma Cortisol ($\mu\text{g}/100\text{ ml}$)		Urinary Free Cortisol Excretion ($\mu\text{g}/24\text{ hr}$)	
	Mean	Range	Mean	Range	Mean	Range
Normal males	10.8	5.5-16.0	0.68	0.1-1.8	38	0-98
Normal females	12.0	4.0-20.4	0.66	0.1-1.4		
'Low-dose pill'	13.9	9.0-17.5	0.45	0.3-0.6	58	19-107
'Standard pill'	32.1	16.7-58.8	1.02	0.5-1.8		
Prostatic cancer on oestrogens	63.0	53.5-76.0	1.84	0.6-2.8	110	45-260
Cushing's syndrome	30.1	15.0-62.5	4.03	1.5-11.2	417	125-1410

Table II Absolute values for plasma cortisol at 9 am and for urinary free cortisol excretion

Fig. 5 Daily urinary free cortisol excretion in normal and oestrogen-treated subjects and patients with Cushing's syndrome.

EFFECT OF OESTROGEN ON DAILY URINARY FREE CORTISOL EXCRETION

Figure 5 shows the daily urinary free cortisol excretion in normal and oestrogen-treated subjects and in Cushing's syndrome. It will be noted that the slight increase in urinary free cortisol excretion seen in subjects on the 'pill' is statistically not significant. Only one value in the women taking standard oral contraceptives was outside the normal range. High-dose oestrogen treatment appears to raise urinary free cortisol excretion, but not consistently to levels seen in Cushing's syndrome.

Discussion

EFFECTS OF ORAL CONTRACEPTIVES

These studies suggest that the amount of oestrogen contained in oral contraceptives determines their effects on cortisol metabolism. In this respect, progestogen-only and low-dose oestrogen contraceptives appear relatively innocuous; their effect on total and unbound plasma cortisol and on urinary free cortisol excretion appears to be minimal.

The standard oral contraceptives, containing 0.1 mg of mestranol or equivalent, do increase the concentration of unbound cortisol at 9 am, as well as increasing total plasma cortisol. But they have little effect on daily urinary free cortisol excretion. In normal subjects, little urinary free cortisol is excreted at night (Espiner, 1966; Vagnucci, Hesser, Kozak, Pauk, Lauler, and Thorn, 1965) because plasma unbound cortisol falls to low levels at night. In women taking oral contraceptives also it seems probable that plasma unbound cortisol falls to near normal low levels at night, because of the normal urinary free cortisol excretion.

By these criteria the small doses of oestrogen in standard oral contraceptives (0.1 mg mestranol) used in the present study increased exposure of tissues to unbound cortisol very little, except at 9 am; and low-dose oestrogen (0.05 mg mestranol or less) and progestogen-only oral contraceptives caused no significant change in exposure of tissues to unbound cortisol at all. Thus increased exposure of tissues to unbound cortisol appears to be no more than a minor factor in the metabolic changes produced by oral contraceptives containing 0.1 mg mestranol, and it is probably an insignificant factor with preparations containing less or no oestrogen.

However, the lack of effect of oral contraceptives on tissue exposure to cortisol must be treated with caution in the case of the liver. The hepatic extraction of cortisol from plasma accounts for more than the non-protein-bound fraction (Tait and Burstein, 1964). Keller, Richardson, and Yates (1969) suggested that cortisol bound to CBG may have access to

hepatic cells in the absence of a capillary wall, thereby apparently inducing enzyme changes. If CBG-bound cortisol is at least partly available to the liver, then the increase in such cortisol caused by oral contraceptives may have relevance to the hepatic changes they apparently cause; no precise evidence on this point is available. Low-dose oestrogen or progestogen-only preparations appear to cause minimal increases in concentration of corticosteroid-binding globulin judging by total plasma cortisol levels.

EFFECTS OF HIGH-DOSE OESTROGENS AND PREGNANCY

This minor elevation of unbound cortisol and insignificant change in urinary free cortisol excretion in women on 'standard' oral contraceptives contrasts sharply with the changes seen in pregnancy. In pregnancy the diurnal rhythm of unbound cortisol is grossly disturbed (Burke, 1970; Doe *et al.*, 1969) and in consequence urinary free cortisol excretion increased. In pregnancy tissue exposure to unbound cortisol, judged by plasma unbound and urinary free cortisol measurements, is increased about three-fold. A similar but smaller increase is found in patients treated with high-dose oestrogens for prostatic cancer (Doe *et al.*, 1969); and my own preliminary findings in healthy subjects treated with high-dose oestrogens indicate that the exposure of their tissues to unbound cortisol is raised too, though less so than in pregnancy. These elevations of tissue exposure to cortisol with high-dose oestrogen treatment, and especially pregnancy, are also suggested by similar changes in 'cortisol aminoaciduria' (Zinneman, Seal, and Doe, 1967; Doe, Zinneman, Place, and Seal, 1968). The conclusion may be drawn that high-dose oestrogen treatment does cause increased exposure of tissues to unbound cortisol, but this is less than that found in pregnancy.

HYPOTHALAMIC EFFECTS

The increase in unbound cortisol induced by 'standard' oral contraceptives raises doubts about their long-term use. Unbound cortisol appears to be the fraction of plasma cortisol to which the hypothalamic control mechanism for corticotrophin release responds (Kawai and Yates, 1966). Raised unbound cortisol levels associated with oestrogen treatment thus imply interference with cortisol homeostasis at hypothalamic level. In the case of oral contraceptives, the extent of this interference is a matter of degree, apparently depending on the dose of oestrogen; but the mechanism of its production and its long-term consequences are quite unknown. It would perhaps be surprising if the disturbances of cortisol metabolism associated with taking oral contraceptives other than 'low-

dose' preparations were proved eventually to be completely harmless.

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