

If chapatti flour were to be enriched with vitamin D, as has been found to be feasible in Glasgow,²⁷ Goans (whose intake of flour is low) would receive least benefit, but they appear to have satisfactory intakes already. Most chapatti flour was consumed by Sikhs and Hindus, whose intakes of vitamin D and serum 25-OHD₃ levels were low.

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Changed levels of endogenous sex steroids in women on oral contraceptives

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Summary

Serum and urinary levels of unconjugated testosterone, dihydrotestosterone, and oestradiol were measured by specific radioimmunoassays in 10 healthy women in the early follicular phase of their menstrual cycle and in nine healthy women taking oral contraceptives. The contraceptive group had testosterone levels 1.3 times higher and dihydrotestosterone levels two times higher than those in the controls. Serum oestradiol levels in the contraceptive group were much lower than those in the controls and similar to levels in postmenopausal women.

The contraceptive group had about twice the urinary excretion of unconjugated (free) testosterone and dihydrotestosterone of the controls, but their excretion of unconjugated oestradiol was 2.7 times lower. The great increase in serum and urinary androgen concentrations, as well as the suppression of oestradiol, may be related to the antiovarian effect of oral contraceptives.

Introduction

The way that oral contraceptives act is still unknown, but they are thought to either inhibit gonadotrophin release or directly affect the ovaries and the genital tract. Oral contraceptives lower serum and urinary gonadotrophin levels,¹ but there is conflicting evidence on whether they reduce oestradiol levels.²⁻⁵ Raised serum testosterone concentrations have been found in women taking oral contraceptives,^{3 6 7} but there are no data on 5 α -dihydrotestosterone, an androgen that is more than twice as potent as testosterone in some bioassays.⁸

Oral contraceptives are often given as long-term replacement therapy in young women with hypogonadism resulting from operations, such as ⁹⁰Y or ¹⁹⁸Au pituitary implantation for treatment of pituitary tumours.⁹

In a small group of healthy women we assessed the effects of oral contraceptives on serum levels of the main sex hormones and measured the simultaneous urinary excretion of the unconjugated hormone.

Subjects and methods

Nineteen healthy members of the hospital staff, aged 18-34, volunteered for this study. Ten women who were not taking any medication acted as controls; they were studied in the first 10 days of their menstrual cycle. The other nine had all been taking oral contraceptives for at least two months and were studied at various days from their last menstrual bleeding. Five women were taking Ovran (ethinyloestradiol 50 μ g and D-norgestrel 250 μ g), two were taking Eugynon 50 (ethinyloestradiol 50 μ g and DL-norgestrel 500 μ g), and two were taking Gynovlar 21 (ethinyloestradiol 50 μ g and norethisterone acetate 3 mg).

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Specimens—The women collected all urine passed between 8 am and 8 pm on one day, keeping the urine refrigerated during the collection. They then made a second collection of urine passed between 8 pm and 8 am the next morning. No preservatives were added to the urine samples, which were frozen immediately on reception and kept at -20°C until assayed. Blood was withdrawn between 3 and 4 pm on the day of the collection and at 9-10 am the next morning. Serum was separated from the blood clot within three hours of sampling and stored at -20°C until assayed.

Assays—Serum and urinary testosterone and dihydrotestosterone concentrations were measured by radioimmunoassay¹⁰ after a separation by thin-layer chromatography. The antiserum used was specific for testosterone (100%) and dihydrotestosterone (66%), other compounds cross-reacting 3% (5 α -androstane-3 β , 17 β -diol) or less. Thin-layer chromatography was carried out on precoated silica gel plates (Merck, 5554) in a cyclohexane:ethyl acetate (3:2) system. Serum and urinary oestradiol was measured by radioimmunoassay using a highly specific oestradiol-antiserum raised in rabbits against oestradiol-6-carboxymethyl oxime-bovine serum albumin. Oestrone, oestriol, and ethinyloestradiol each cross-reacted less than 0.4% with oestradiol in the assay. For urinary oestradiol assays, a thin-layer chromatography step was included (as above but in a benzene:diethyl ether (1:1) system). The step was unnecessary for serum measurements as results were identical with or without it.

Urinary creatinine was measured by the Jaffe reaction as adapted for the AutoAnalyzer (Technicon).

To minimise methodological variance each individual hormone in serum or urine from both groups was measured in the same assay batch.

Results

SERUM LEVELS

Mean serum levels of unconjugated testosterone, dihydrotestosterone, and oestradiol in the two groups of women are shown in the table. The contraceptive group had higher mean values of both testosterone and dihydrotestosterone than the controls, and the difference was highly significant for dihydrotestosterone ($P < 0.01$). Serum oestradiol values were significantly lower in the contraceptive group ($P < 0.001$), being similar to values in postmenopausal women. Fig 1 shows the individual serum dihydrotestosterone and oestradiol values and includes values in postmenopausal women and normal men for comparison.

Mean serum levels of dihydrotestosterone and oestradiol correlated well in the normal group (fig 2; $r = 0.74$; $P < 0.02$) but poorly in the contraceptive group ($r = 0.21$). Linear correlations between serum testosterone and dihydrotestosterone and between serum testosterone and oestradiol were not significant at the 5% level in either group.

There were no differences between morning and afternoon values of the hormones in either group.

Mean (\pm SD) serum and urinary levels of unconjugated testosterone, dihydrotestosterone, and oestradiol in 10 healthy women in early follicular phase of menstrual cycle and nine women on oral contraceptives. Ranges are given in parentheses

	Testosterone	Dihydrotestosterone	Oestradiol
<i>Serum levels (nmol/l)</i>			
Control group	0.722 \pm 0.184 (0.451-1.041)	0.313 \pm 0.145 (0.082-0.616)	0.126 \pm 0.054 (0.044-0.217)
Contraceptive group	0.972 \pm 0.382 (0.382-1.596)	0.623 \pm 0.243 (0.372-1.118)	0.038 \pm 0.012 (0.022-0.051)
t Test	NS	$P < 0.01$	$P < 0.001$
<i>Urinary excretion (nmol/24 h)</i>			
Control group	320 \pm 264 (115-902)	134 \pm 81.2 (36.5-282)	88.1 \pm 91.8 (20.9-274.52)
Contraceptive group	694 \pm 579 (108-1947)	259 \pm 146 (68.1-488.5)	25 \pm 21.3 (5.5-73.8)
t Test	NS	$P < 0.05$	$P < 0.05$

NS = Not significant at 5% level.

Conversion: SI to traditional units—Serum testosterone: 1 nmol/l \approx 288 pg/ml. Urinary testosterone: 1 nmol/24 h \approx 0.288 g/24 h. Serum dihydrotestosterone: 1 nmol/l \approx 290 pg/ml. Urinary dihydrotestosterone: 1 nmol/24 h \approx 0.29 g/24 h. Serum oestradiol: 1 nmol/l \approx 272 pg/ml. Urinary oestradiol: 1 nmol/24 h \approx 0.27 g/24 h.

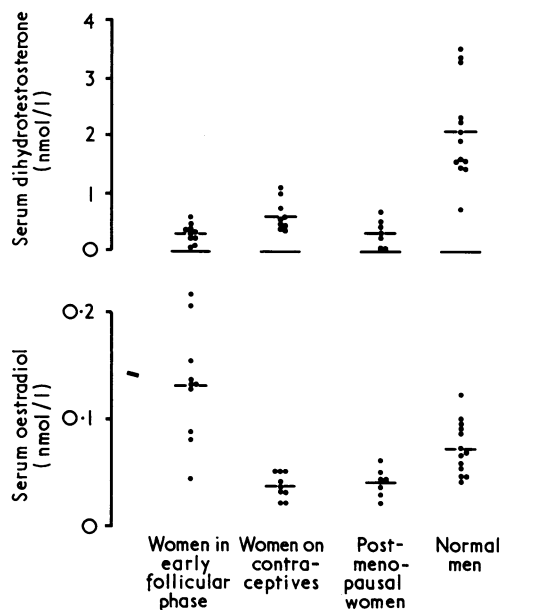


FIG 1—Serum levels (means of day and night values) of oestradiol and dihydrotestosterone in control and contraceptive groups. Levels in postmenopausal women and normal men are shown for comparison.

Conversion: SI to traditional units: Oestradiol: 1 nmol/l \approx 272 pg/ml. Dihydrotestosterone: 1 nmol/l \approx 290 pg/ml.

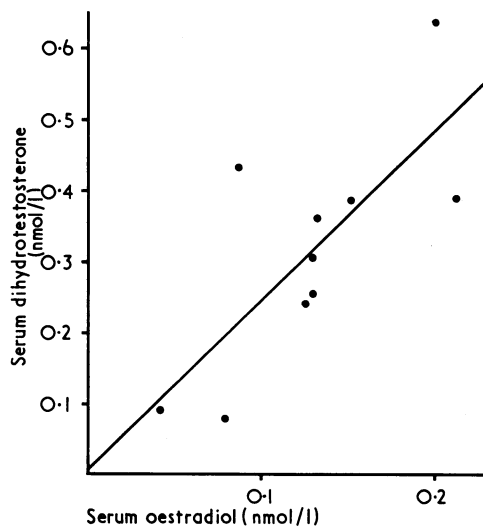


FIG 2—Relation between serum levels (means of day and night values) of dihydrotestosterone and oestradiol in the early follicular phase in 10 normal women: $y = 2.20x + 0.02$; $r = 0.74$; $P < 0.02$.

URINARY LEVELS

The 24-hour urinary excretion of the unconjugated hormones is shown in the table. The contraceptive group excreted more testosterone and dihydrotestosterone than the controls, and, again, the difference was significant for dihydrotestosterone. Testosterone and dihydrotestosterone values in the night collections tended to be lower in both groups, but the values overlapped and the averages of the night and day collections were not significantly different.

The women taking contraceptives excreted less unconjugated oestradiol than the controls, and the difference between the means of the two groups was just significant. There was no difference in mean values between night and day collections.

As a check urinary creatinine excretion was measured in night and day collections from six women in each group. The average 24-hour values (\pm SD) were 8.8 ± 1.8 mmol/24 h (0.99 ± 0.20 g/24 h) and 7.5 ± 2.6 mmol/24 h (0.85 ± 0.29 g/24 h) for the control and contraceptive groups respectively.

Discussion

Our findings have shown profound changes in the endogenous sex hormone concentrations in women on oral contraceptives when compared with values in the early follicular phase of normal cycles. All changes in serum levels in the contraceptive group were corroborated by corresponding changes in the urinary excretion of the unconjugated form of each hormone.

Changes in serum dihydrotestosterone levels in the contraceptive group corresponded to the changes in testosterone concentrations. Our control serum dihydrotestosterone values were lower than those that have been reported in the normal follicular phase,^{11,12} probably because all measurements in our controls were made within the first 10 days of the menstrual cycle. Some, though not all,^{13,14} workers have detected small but significant changes in serum dihydrotestosterone levels during the normal menstrual cycle.

Serum oestradiol levels in our contraceptive group were reduced to the postmenopausal range. Although others have found low levels,^{4,5} they have not been this low, and the difference is probably due to differences in methods. None of the other workers used antiserum as specific for oestradiol as the one we used. Furthermore, as serum determinations with our antiserum do not need any prior chromatography step, our assay is more sensitive and can detect quantities as small as 7.5 pmol (2 pg).

The different contraceptives used by our subjects produced no different effects on the sex steroid levels. As ethinyloestradiol (50 µg) was the only ingredient common to all three preparations, it probably induced the hormonal changes, although the role of progestins needs further investigation.

Sex hormone binding globulin (SHBG) concentrations are raised in women taking the pill,^{15,16} and it has been suggested that changes in serum SHBG concentration might influence the peripheral effects of sex hormones.¹⁷ In our contraceptive group the androgen levels increased, while oestradiol levels decreased. It is difficult to reconcile these changes with the idea, on the one hand, that they were caused by increased SHBG levels and, on the other, that changes in gonadotrophins caused the observed sex hormone changes. It is more probable that the oral contraceptives had a direct effect on steroid metabolism. This possibility is supported by the fact that oral contraceptives induce large increases in serum concentrations of the unrelated steroids cortisol and aldosterone.^{18,19} In normal reproductive women over 95% of oestradiol comes from the ovaries,²⁰ whereas dihydrotestosterone comes almost entirely from peripheral conversion of androstenedione and testosterone.²¹ Our results may therefore indicate that oral contraceptives have different effects on sex steroid synthesis in different organs.

The changes in testosterone and dihydrotestosterone levels in our contraceptive group seem to correspond with those of cortisol.¹⁸ Levels of both total and non-protein-bound unconjugated serum cortisol are increased in women on oral contraceptives, and serum non-protein-bound cortisol levels correlate well with the urinary excretion of the unconjugated cortisol.²² In another study we also found a significant correlation between unconjugated testosterone and dihydrotestosterone and urinary creatinine in normal men and women,²³ which suggests that glomerular filtration may be the main renal excretory mechanism for the unconjugated hormones.

The increased urinary excretion of unconjugated androgens by women taking contraceptives suggests that they have an increased level in circulating "tissue-available" androgens. As symptoms of virilism do not occur in women on the pill despite their increased exposure to the two most potent naturally occurring androgens⁸ the contraceptives themselves must block their effects—that is, they must have a peripheral anti-androgenic effect.

The significant positive correlation between the serum dihydrotestosterone and oestradiol levels in the control group suggests the existence of an inherent balance in the body between these two hormones. Such a balance may also be inferred from animal experiments showing a synergistic effect

between the hormones when administered.^{24,25} The absence of this correlation in those taking contraceptives might indicate that oral contraceptives act by destroying that balance.

The raised serum testosterone levels in women taking contraceptives contrast with the reduced levels found in men after ethinyloestradiol administration.^{26,27} This opposite response in the two sexes is probably related to differences in sex hormone metabolism. The conflicting reports on the effects of oral contraceptives on serum testosterone levels in hirsute women^{7,28} might mean that these patients show varying degrees of "male-like" responses.

The antiovarian effects of the oral contraceptives are probably related to the changes they induce in sex hormone levels. Thus low serum oestradiol levels preclude oestradiol's positive feedback action on the pituitary release of gonadotrophins,²⁹ and the increased androgen levels would tend to inhibit the hypothalamus. More significantly, however, the low serum oestradiol levels indicate low oestradiol production and intraovarian oestradiol levels, and the ovary itself is likely to be the primary target of the contraceptive, although there has been no study on the effects of oral contraception on oestradiol production by ovarian tissue.

The dihydrotestosterone:oestradiol ratio in the contraceptive group was over six times higher than that in the normal group. Further study might show whether this ratio could be used as an index of the antiovarian efficiency of contraceptive preparations.

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