

## DOES TESTOSTERONE AFFECT THE NORMAL MENSTRUAL CYCLE?

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### SUMMARY

In order to throw further light on the role of androgens in the aetiology of the polycystic ovary syndrome (PCO) we have examined the effect of artificially increasing serum testosterone levels on menstrual function in a group of ovulating women. Six women were studied who had either severe premenstrual syndrome or loss of libido for which they were treated with 100 mg testosterone by s.c. implantation. All had regular menstrual cycles. For 1 month before implantation serum LH, FSH, oestradiol ( $E_2$ ), progesterone and testosterone were measured three times per week. All women showed normal cyclical variation of LH, FSH,  $E_2$  and progesterone. Following implantation, three times weekly blood samples were taken during the first and third cycles. No patient had any disturbance of menstrual pattern. All continued to show cyclical changes of LH, FSH,  $E_2$  and progesterone. Serum  $E_2$  and progesterone were lower but not significantly so in the luteal phase of the treated cycles. This was despite a mean serum testosterone which rose from 1.3 to 7.1 nmol/l at the end of the third week following implantation and to 4.1 nmol/l at the end of the third month. Sex hormone binding globulin levels fell as expected by 18.5% during the first cycle. The lack of significant effect of a markedly elevated serum testosterone level on cyclical hormone changes is indirect evidence that in PCO the primary cause of the menstrual disturbance is not excessive production of ovarian or adrenal testosterone.

The aetiology of the polycystic ovary syndrome (PCO) is unknown. Affected women show a variable combination of clinical features including hirsutism, obesity, oligomenorrhoea and infertility (Stein & Leventhal, 1935). Most have modestly elevated serum levels of testosterone, androstenedione and other androgens (Bardin & Lipsett, 1967; Kirschner *et al.*, 1973; Givens *et al.*, 1975), increased testosterone blood production rates (Bardin & Lipsett, 1967) elevated free testosterone and reduced sex hormone binding globulin (SHBG) levels. In women with oligomenorrhoea modestly elevated serum levels of LH are seen without the normal cyclical variation (Rebar *et al.*, 1976; Baird *et al.*,

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1977). Characterization of the underlying defect is difficult. For example there may be a vicious cycle of events whereby increased androgen production, either from ovaries or adrenals, leads to increased peripheral oestrogen formation, and so to abnormal feedback at pituitary/hypothalamic level and elevated acyclical serum LH. These elevated LH levels in turn may produce chronic hyperstimulation of the ovarian theca cells with consequent overproduction of androgens. The primary abnormality is unknown (Yen, 1980). We have attempted to study one aspect of this problem in an indirect way by examining the effect of an artificially elevated serum testosterone level on cyclical hormone changes in a group of women with normally regular menstrual cycles.

### PATIENTS AND METHODS

Six women were studied with approval from the Salford Health Authority Ethical Committee. Five had severe symptoms of premenstrual syndrome and had failed to respond to other forms of therapy. One complained of loss of libido and in the past had responded well to a single injection of mixed testosterone esters (Sustanon, Organon Laboratories Ltd, Cambridge, England). All had regular menstrual cycles (mean length 28 d, range 27–29), and none was hirsute. The mean age was 28 years (range 22–44). All subjects attended three times a week for a blood sample throughout a single basal menstrual cycle. Blood (10 ml) was taken by venepuncture between 0900 and 1400 h. A 100 mg fused pellet of T (Organon Laboratories Ltd, Cambridge, England) was then implanted in the lower abdominal wall under local anaesthetic. This was performed as close as possible to the first day of menstruation. The series of thrice-weekly blood samples was then repeated in the first and third cycles following implantation. After standing the sample at room temperature for 1 h to allow for clot retraction, serum was separated and stored at  $-20^{\circ}\text{C}$  until the time of the assay.

Progesterone, oestradiol ( $\text{E}_2$ ), LH, and FSH were measured on all samples, and testosterone at weekly intervals throughout each study cycle. Progesterone was measured by a direct radioimmunoassay as described by Ratcliffe *et al.* (1982) using danazol to displace progesterone from high affinity binding proteins.  $\text{E}_2$  was measured using a Steranti oestradiol direct kit (Steranti Research Ltd). SHBG levels were measured by the method of Hammond & Lähteenmäki (1983). LH, FSH and PRL were measured by specific radioimmunoassay (RIA). Following ether extraction of serum, androstenedione was measured by radioimmunoassay using antiserum HP/5/665-1A from Guildhay Antisera, Guildford:  $^{125}\text{I}$ -LH and  $^{125}\text{I}$ -FSH were prepared by K. Ferguson in the Chelsea Hospital for Women, London. Rabbit anti-LH and anti-FSH (codes F87/2 and M93/2) were obtained as gifts from Professor W. Butt. The LH standard was NIBSC 68/40 and the FSH standard NIBSC 78/459. Testosterone was assayed following extraction with diethyl ether, by RIA using an antiserum raised in a rabbit to testosterone-3-0-(carboxymethyl-oxime)-conjugate (Bioanalysis, Cardiff), with  $^{125}\text{I}$ -testosterone-3-0-(carboxymethyl-oxime)histamine (Amersham International, Aylesbury, Bucks, England) as tracer. When analysing the data, the sample closest to the presumptive LH peak (24 h before progesterone started to rise) was identified and that day designated day 0 although it is acknowledged that the LH value on that day will not necessarily be the highest achieved in that cycle. Because it was not possible to ensure that subjects attended on identical sample days, for the purposes of analysis samples were grouped as follows: days  $-10$  to  $-8$ ; days  $-7$  to  $-6$ ; days  $-5$  to  $-4$ ; days  $-3$  to  $-2$ ; day  $-1$ , day 0, day  $+1$ ;

days + 2 to + 3; days + 4 to + 5; days + 6 to + 7; and days + 8 to + 10. Because data was long-normally distributed analysis was performed using log transformed data. Comparison between pre- and post-treatment values was performed using *t*-tests on matched paired data. A comparison was also made between these women and the cyclical hormone data obtained from 30 ovulatory cycles (Petsos *et al.*, 1985) using *t*-tests on unpaired data. Levels of testosterone, SHBG and PRL were measured on four samples each at weekly intervals in the control and first and third treatment cycles.

## RESULTS

Geometric mean  $\pm$  SD serum testosterone levels are shown before and after treatment in Fig. 1. Mean serum testosterone levels rose from 1.3 to 7.1 nmol/l at the end of the third week following implantation, falling to 4.1 nmol/l at the end of the third month (the upper limit of the normal female range is 2.0 nmol/l). Geometric mean serum and androstenedione levels showed a small but significant increase from basal levels of 8.0 nmol/l (SD, 5.8–11.1) to 10.1 (8.1–12.5) nmol/l at the end of the first treatment cycle ( $P < 0.05$ ). By the end of the 3rd post-treatment cycle the geometric mean level had fallen again to 8.5 (5.9–12.2) nmol/l.

Pre- and post-treatment LH and FSH levels are shown in Figs 2a and b respectively. There was no difference between pre- and either of the post-treatment cycles and similarly the differences between these values and those previously found in 30 ovulatory cycles was not significant. Serum  $E_2$  is shown in Fig. 2c. Again there were no significant differences between pre- and post-treatment cycles. Both before and after treatment the values in the luteal phase of the cycle were rather lower than those previously found by Petsos *et al.* (1985), but not significantly so. Follicular phase serum progesterone levels were for the most part undetectable. Luteal phase serum progesterone levels are shown in Fig. 2d. Again no statistically significant differences were found between pre- and post-treatment cycles or between these values and those previously obtained in ovulatory cycles. SHBG levels were measured before and 3 weeks and 3 months after implantation; levels fell significantly from a mean of 54.2 to 44.2 nmol/l at 3 weeks ( $P < 0.05$ ); levels at 3 months were 47.6 nmol/l (NS). Prolactin levels did not change significantly (control cycle  $249 \pm 60$  (SD) mU/l, first treatment cycle  $215 \pm 47$  mU/l, third treatment cycle  $230 \pm 64$  mU/l).

None of the women reported any alteration in cycle length. Mean cycle length was 29 d with a range of 28–30 d both before treatment and in the two observed post-treatment cycles. From the timing of the LH peak there did not appear to be any alteration in lengths of follicular and luteal phase, although as mentioned previously it was not possible to time

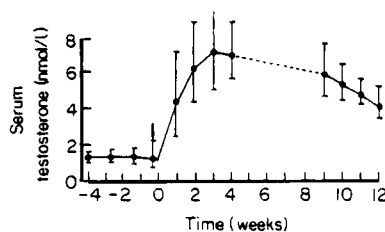


Fig. 1. Geometric mean  $\pm$  SD serum testosterone before and after subcutaneous implantation of 100 mg testosterone (arrow).

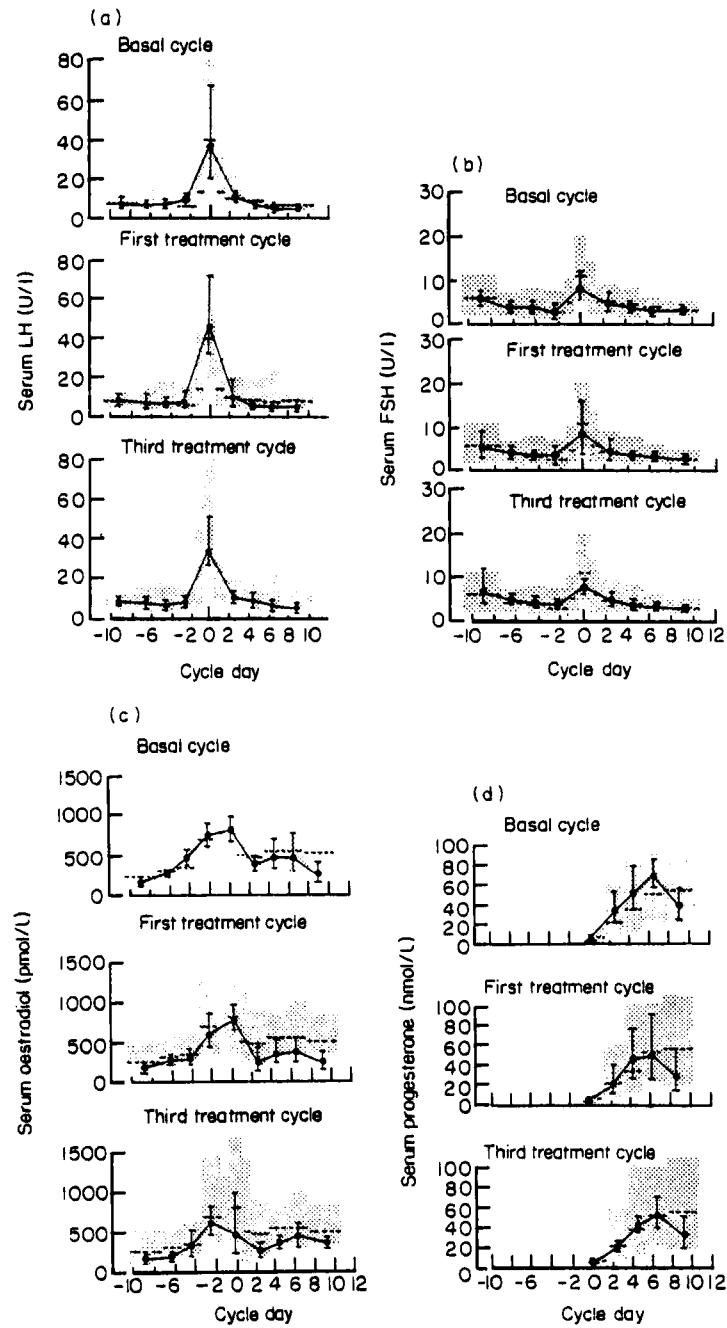


Fig. 2. Geometric mean  $\pm$  SD (a) serum LH, (b) serum FSH, (c) serum oestradiol and (d) serum progesterone throughout a basal cycle and during first and third cycle post implantation with 100 mg testosterone. The shaded areas show geometric mean  $\pm 2$  SD from 30 ovulatory cycles (Petsos *et al.*, 1985). No significant differences were observed between the three cycles.

this peak precisely. Incidentally, none reported hirsutism or any side-effects attributable to androgen excess and all reported some relief of their underlying symptoms. The use of testosterone implants for intractable premenstrual tension is currently the subject of a double-blind controlled trial.

## DISCUSSION

Despite the fact that PCO was described as long ago as 1935 (Stein & Leventhal, 1935) the underlying causes remain uncertain. It is well known that there is increased production of androgens by the ovaries in PCO (Laatikainen *et al.*, 1980; Kasuga, 1980) and that tissue from affected ovaries shows poor aromatase activity (Axelrod & Goldzieher 1961; Short & London, 1961). However, Erikson *et al.* (1979) demonstrated that cultured granulosa cells from polycystic ovaries would produce predominantly oestrogens rather than androgens provided that they were cultured in a medium rich in FSH. In fact when cultured in such a medium they behaved in a similar manner to those from normal ovaries. This suggested that rather than there being a primary ovarian abnormality, polycystic ovaries are behaving appropriately to excess LH stimulation.

Other studies have shown that although there is increased production of adrenal androgens, in a significant proportion of patients with PCO there is unlikely to be a classical enzyme deficiency (Givens *et al.*, 1975; Lachelin *et al.*, 1979; Child *et al.*, 1980). It may be that in PCO, there is a minor modulation of adrenal enzyme activity in response to elevated serum androgen levels. It has been shown that androgens can inhibit 21- and 11-hydroxylase enzymes *in vitro* (Morrow *et al.*, 1967; Sharma *et al.*, 1973; Hornsby 1980, 1982).

Studies which have attempted to define a pituitary abnormality in PCO have shown that there is an exaggerated response of LH to 'standard' doses of GnRH (Patton *et al.*, 1975; Katz & Carr, 1976; Rebar *et al.*, 1976; Baird *et al.*, 1977). However, a similar response can also be obtained in normal women by prior treatment with oestrogens (Yen *et al.*, 1974; Shaw *et al.*, 1975). Thus, in women with PCO it appeared possible that elevated serum androgen levels led to increased peripheral oestrogen formation which in turn leads to abnormal feedback at pituitary/hypothalamic level with consequent elevation of serum LH. Indeed it has been demonstrated that while serum E<sub>2</sub> levels are normal in PCO, oestrone levels are elevated (De Vane *et al.*, 1975; Baird *et al.*, 1977; Kandeel *et al.*, 1978; Lobo *et al.*, 1981).

The present study appears to be the first in which the effect of a marked elevation of testosterone on the female cycle has been examined. In the premenstrual syndrome, serum E<sub>2</sub> and progesterone levels have been claimed to be abnormal during the luteal phase, although the evidence is inconclusive (Bancroft & Bäckström, 1985). In the subjects studied, cyclical fluctuations in E<sub>2</sub>, progesterone and gonadotrophins were initially within the ranges established for normal ovulatory cycles, and were essentially unchanged post-implant. Serum total (and therefore free) testosterone levels rose post-implant (100 mg testosterone) to about twice those seen in PCO for at least 3 months. The rise in androstenedione as modest compared with that of testosterone, and the levels seen at the end of the first treatment cycle were lower than those commonly seen in patients with PCO. It may be argued that the basic abnormality in PCO is the increased extra-glandular production of oestrogen from increased androstenedione produced by both the ovaries and adrenals; nevertheless we suggest these findings are indirect evidence against the

primary abnormality in PCO being over-production of androgens. These findings are contrary to those of Dunaif *et al.* (1984) who found that abnormal gonadotrophin secretion returned to normal when an androgen secreting tumour was removed in a 29-year-old woman. However, the serum testosterone in that patient was about three times higher than the maximum level seen in this study and other hormones such as E<sub>2</sub> were markedly elevated.

Some studies have suggested that there may be abnormal dopaminergic control of gonadotrophin secretion in PCO (Quigley *et al.*, 1981; Luciano *et al.*, 1984). Our findings suggest that further research into the underlying cause in PCO should be concentrated at hypothalamic and pituitary levels.

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