

Gonadotropin-releasing hormone-induced changes in testosterone secretion in normal women*

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This study investigated the pattern of testosterone (T) secretion in spontaneous (n = 14) and gonadotropin-releasing hormone (GnRH)-treated (n = 6) menstrual cycles in normal women. In spontaneous cycles, T was found to increase progressively over the follicular phase ($P \leq 0.001$), with the peak T value occurring on cycle day 0 (luteinizing hormone [LH] surge). The mean (\pm standard error of the mean [SEM]) T values on cycle day -14 and cycle day 0 were 35 ± 4 and 51 ± 4 ng/dl, respectively. GnRH was administered intravenously to six women at 1.3 to 1.7 μ g per dose every 30 minutes in a study that assessed the ovarian effects of a rapid gonadotropin pulse frequency. In three of the women, the T levels followed a normal follicular phase pattern, whereas in the remaining three GnRH-treated women, there were marked increases in T with peak levels of 97, 123, and 81 ng/dl on day 0. The GnRH-treated subgroup with increased T levels had significantly increased follicular levels of LH, follicle-stimulating hormone (FSH), LH-bio and number of preovulatory ovarian follicles. This study demonstrated that increased levels of LH, FSH, and LH/FSH are capable of acutely increasing the secretion of ovarian androgens. *Fertil Steril* 48:423, 1987

In normal reproductive age women, the ovaries and adrenal glands are known to secrete similar quantities of testosterone (T).¹ Normal women maintain serum T concentrations within a rather narrow range (e.g., 30 to 60 ng/dl) throughout the menstrual cycle. There is a lack of consensus in the literature regarding whether serum T levels demonstrate a secretion pattern over the menstrual cycle in normal women.²⁻⁶

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Luteinizing hormone (LH) is a primary regulator of ovarian T secretion. The ovarian stroma, in general, and the thecal layer, in particular, respond to LH stimulation with androgen secretion. In polycystic ovarian disease (PCOD), an increased LH/follicle-stimulating hormone (FSH) ratio, primarily reflecting increased LH secretion, promotes increased T secretion from the ovaries.⁷

A study was recently completed that investigated the effects of a physiologic dose of gonadotropin-releasing hormone (GnRH) administered to normal women at a supraphysiologic frequency in the follicular phase of normal menstrual cycles.⁸ This supraphysiologic pulse frequency was used to imitate the rapid gonadotropin secretion pattern found in women with luteal phase deficiency. Changes in gonadotropin levels, ovarian hormone production, follicular development, and corpus luteum function were monitored. Prominent changes in serum T were noted to occur in some of the GnRH-treated women. This report investigates

these changes in T secretion in an attempt to further elucidate the influence of the brain (pituitary) on gonadal (ovarian) function.

MATERIALS AND METHODS

Subjects

Fourteen normal women between ages 23 and 35 years participated as volunteers in this study. These women were within $\pm 10\%$ of ideal body weight (Metropolitan Life Tables, 1980) and had regular menstrual cycles, normal basal body temperature (BBT) charts, and normal serum levels of T (<60 ng/dl), prolactin (PRL) and luteal progesterone (P) (>12 ng/ml) preceding the study. They were taking no medications and had not received any hormone therapy for the previous 12 months.

Protocol

BBT charts were kept and daily (A.M.) venous blood samples were obtained throughout one complete menstrual cycle (cycle 1). These blood samples were analyzed for LH, FSH, estradiol (E_2), and T by radioimmunoassay (RIA) and for LH by bioassay (LH-bio). The RIA for the T concentration was performed on blood samples throughout both cycles. During the study, as each subject approached midcycle, daily morning pelvic sonograms of the ovaries were performed until ovulation was observed.

Six of the 14 women also participated in the second part of the study. Patient information characterizing these women are presented in Table 1. In this part of the study, treatment with GnRH (Factrel, Ayerst Laboratories, New York, NY) was initiated on cycle day 2, 3, or 4 (early follicular) of the subsequent menstrual cycle (cycle 2). GnRH in a

heparinized solution was administered intravenously via an intermittent infusion pump (Auto-syringe, Model A6H, Hookset, NH) at a dose of 25 ng/kg given every 30 minutes. The GnRH dose varied from 1.3 to 1.7 $\mu\text{g/pulse}$ and from 64 to 82 $\mu\text{g/day}$, depending upon the subject's weight. GnRH treatment was continued at the same dose and frequency throughout the follicular phase and was terminated when two or more of the multiple follicles that developed were determined to have undergone ovulation by sonographic criteria. GnRH was administered between 9 and 13 days in the six volunteers. Monitoring of hormone changes during this second cycle was performed in the same manner as the first cycle.

Hormone Assays

The RIA for T and E_2 was performed as described previously,⁹ using reagents provided by the World Health Organization (WHO) Matched Reagent Programme.¹⁰ The sensitivities of the T and E_2 assays were 10 ng/dl and 12 pg/ml, respectively; intra-assay and interassay coefficients of variation were 5.1 and 9.8%, respectively, for the T assay and 8.2 and 8.8%, respectively, for the E_2 assay. In addition, measurement of T and E_2 levels in pooled samples provided monthly by the WHO External Quality Control Program were consistently close to mean values obtained in other laboratories.

Serum samples were analyzed for LH and FSH by double-antibody RIA.^{11,12} Standard National Institutes of Health reagents were used, including the LER-907 reference preparation. The sensitivity of the LH assay was 6 ng/ml; intra-assay and interassay variabilities were 5.5 and 8.4%, respectively. The sensitivity of the FSH assay was 25 ng/ml; intra-assay and interassay variabilities were 7.3 and 9.7%, respectively.

Table 1 Subjects Administered GnRH

| Subject | Age | Gravidity | % IBW ^a | Baseline hormone levels ^b | | |
|---------|-----|-----------|--------------------|--------------------------------------|-------|-------|
| | | | | LH | FSH | T |
| | yrs | | | ng/ml | ng/ml | ng/dl |
| A | 31 | 1 | 104 | 24.9 | 168.7 | 44 |
| B | 33 | 2 | 92 | 45.7 | 145.8 | 33 |
| C | 29 | 0 | 101 | 20.3 | 150.9 | 32 |
| D | 31 | 0 | 90 | 29.7 | 204.3 | 30 |
| E | 23 | 0 | 100 | 40.9 | 186.5 | 37 |
| F | 35 | 2 | 94 | 40.8 | 276.1 | 39 |

^a IBW, ideal body weight; Metropolitan Life Tables, 1980.

^b Values obtained the day prior to initiation of GnRH treatment.

Bioactive serum LH levels were measured as previously described.¹³ The mean intra-assay and interassay coefficients of variation were 8 and 17%, respectively.

Data Analysis and Statistics

The daily hormone data were arranged relative to the day of the peak LH value (surge), which was labeled day 0. The periovulatory phase of the cycle was defined as days -2 to +2, inclusive. Changes in serum T concentrations in blood samples obtained during cycle 1 (n = 14) over the menstrual cycle (days -10 to +10) were compared by two-way analysis of variance (ANOVA). Two-way ANOVA with repeated measures was used to compare T concentrations between control (cycle 1) and treated (cycle 2) cycles in the six women who participated in both parts of the study. Other comparisons of data between the subgroups of subjects with and without changes in T concentrations were made by the one-tailed Student's *t*-test.

RESULTS

Mean serum T concentrations in 14 normal menstrual cycles are illustrated in Figure 1. The mean \pm standard error of the mean (SEM) for serum T over these menstrual cycles was 41.9 ± 2.2 ng/dl, with a range of 14 to 82 ng/dl. A two-way ANOVA indicated there was a significant ($P \leq 0.001$) change in T over the menstrual cycle, with the peak T value occurring on day 0.

In the GnRH-treated follicular phase of cycle 2,

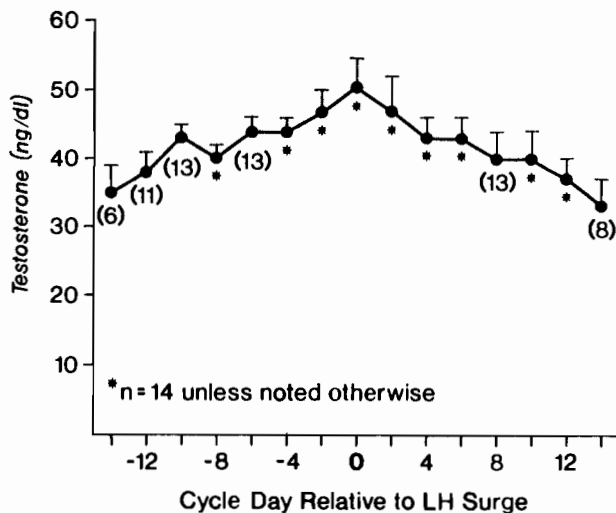


Figure 1 The mean ($\bar{X} \pm$ SEM) serum T concentrations over the menstrual cycle (n = 14).

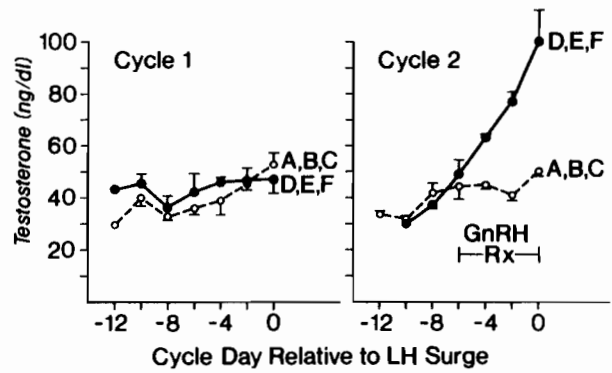


Figure 2 The mean ($\bar{X} \pm$ SEM) serum T levels are indicated in the follicular phases of control and GnRH-treated cycles. Subjects A, B, and C (subgroup I) and subjects D, E, and F (subgroup II) differed significantly ($P \leq 0.01$) from each other in cycle 2 (GnRH Rx).

three subjects were noted to have a day 0 serum T value that exceeded the normal range for that cycle day (21 to 80 ng/dl, 95% confidence interval). These three women (subjects D, E, F) had day 0 serum T levels of 97, 123, and 81 ng/dl, respectively. These peak values followed a progressive increase over the follicular phase from well within the normal range. There was a significant increase ($P \leq 0.001$) in T during the treated follicular phase in these three subjects (D, E, F), whereas T in the remaining three women (A, B, C) did not differ from follicular phase control values (Fig. 2).

In an effort to discern possible causes for the increase in T levels in some of the women in cycle 2, various other hormone parameters during GnRH treatment were examined (Table 2). The response on the common cycle days (-6 to -1) that all subjects received GnRH treatment was analyzed. GnRH led to an increase in LH and FSH in all subjects; however, the mean LH and FSH levels achieved in the three women with elevated T levels were significantly greater than those experienced by the others ($P \leq 0.05$). The mean LH was 196 ± 30 ng/ml (mean \pm SEM) versus 110 ± 13 ng/ml, and the mean FSH was 230 ± 10 ng/ml versus 160 ± 26 ng/ml for the elevated and normal T groups, respectively. This same finding also was true for LH-bio determinations, 0.93 ± 0.09 versus 0.57 ± 0.09 μ g/ml ($P \leq 0.05$). (The LH-bio to immuno ratio decreased in five of the six women during GnRH administration). All subjects experienced elevated LH/FSH ratios compared with normal cycles, but the high T and normal T groups were not significantly different with respect to LH/FSH (Table 2).

Table 2 Patient Data: Follicular Phase of Control and Treated Cycles

| Subjects | GnRH (total daily dose) | Cycle days treated | Mean LH (days -6 to -1) | Mean FSH (days -6 to -1) | Mean LH/FSH (days -6 to -1) | Mean LH-bio (days -6 to -1) | Number of ovarian follicles (≥ 14 mm mean diameter) | Integrated E ₂ (days -6 to 0) | Peak E ₂ | Mean T (days -6 to 0) | Peak T (periovulatory or follicular phase) | cycle day |
|----------------------------|-------------------------|--------------------|---------------------------------|---------------------------------|------------------------------|--------------------------------|---|--|-------------------------------|-----------------------------|--|-----------|
| | | | | | | | | | | | | |
| Cycle one (Control) | | | | | | | | | | | | |
| A | | | 38.3 | 145.7 | 0.26 | 0.36 | 1 | 1345 | 352 | 49 | 58 | 0 |
| B | | | 69.3 | 125.2 | 0.49 | 0.46 | 1 | 1558 | 329 | 44 | 56 | 0 |
| C | | | 42.5 | 175.4 | 0.24 | 0.39 | 1 | 791 | 245 | 37 | 48 | +2 |
| D | | | 69.6 | 185.9 | 0.37 | 0.51 | 1 | 1878 | 405 | 51 | 56 | +2 |
| E | | | 40.8 | 132.5 | 0.30 | 0.22 | 2 | 1832 | 403 | 48 | 55 | 0 |
| F | | | 60.0 | 193.5 | 0.30 | 0.34 | 1 | 2308 | 743 | 37 | 63 | +2 |
| Mean \pm SEM | | | 53.4 \pm 6.0 | 159.7 \pm 11.8 | 0.33 \pm 0.04 | 0.38 \pm 0.04 | 1.2 \pm 0.2 | 1617 \pm 214 | 413 \pm 70 | 44 \pm 2.5 | 56 \pm 2.0 | |
| Cycle two (Rx) | | | | | | | | | | | | |
| Subgroup I | | | | | | | | | | | | |
| A | 82 | | 102.9 | 184.3 | 0.57 | 0.51 | 2 | 2590 | 609 | 45 | 54 | 0 |
| B | 72 | -7 to +1 | 92.7 | 108.4 | 0.86 | 0.46 | 2 | 1991 | 432 | 45 | 49 | 0 |
| C | 82 | -11 to +1 | 135.1 | 187.2 | 0.71 | 0.75 | 1 | 927 | 208 | 46 | 51 | -6 |
| Mean \pm SEM | 78.7 \pm 3.3 | -9 to +1 | 110.2 \pm 12.8 ^a | 160.0 \pm 25.8 | 0.71 \pm 0.08 ^a | 0.57 \pm 0.09 ^b | 1.7 \pm 0.3 | 1836 \pm 486 | 416 \pm 116 | 45 \pm 0.3 | 51 \pm 1.5 | |
| Subgroup II | | | | | | | | | | | | |
| D | 64 | -8 to +3 | 248.8 | 233.8 | 1.07 | 1.06 | 4 | 4243 | 909 | 70 | 97 | 0 |
| E | 82 | -7 to +4 | 146.2 | 211.6 | 0.76 | 0.96 | 5 | 4432 | 1586 | 83 | 123 | 0 |
| F | 82 | -7 to +4 | 193.0 | 245.9 | 0.78 | 0.76 | 3 | 6271 | 1759 | 65 | 81 | 0 |
| Mean \pm SEM | 76.0 \pm 6.0 | | 196.0 \pm 29.7 ^{a,c} | 230.4 \pm 10.0 ^{b,c} | 0.87 \pm 0.10 ^a | 0.93 \pm 0.09 ^{a,c} | 4.0 \pm 0.6 ^{a,d} | 4982 \pm 647 ^{a,d} | 1418 \pm 259 ^{a,d} | 73 \pm 5.4 ^{a,d} | 100 \pm 12.2 ^{a,d} | |

^a $P \leq 0.001$ versus control cycle.

^b $P \leq 0.05$ versus control cycle.

^c $P \leq 0.05$ versus subgroup I.

^d $P \leq 0.01$ versus subgroup I.

There was also a difference in the ovarian response between the groups (Table 2). The three women with elevated T levels had significantly more medium to large (≥ 14 mm in diameter on day prior to ovulation) preovulatory follicles: four versus two ($P \leq 0.01$). In terms of peak T levels, the women could be properly ranked in ascending order by the number of ovarian follicles that developed (the more follicles the higher the T level). As the number of preovulatory follicles was reflected in significant increases in peak and integrated E₂ values, there were increased ($P \leq 0.01$) levels of E₂ in the three women with elevated T concentrations in cycle 2.

DISCUSSION

There is disagreement in the medical literature regarding whether T levels change throughout the menstrual cycle. Separate studies by Lobotsky et al.² and Judd and Yen³ concluded that there was a subtle, but significant, increase in serum T in the periovulatory interval compared with the other phases of the cycle. Goebelsmann and colleagues reported that the highest serum T levels in eight normal women were found on the day of the LH surge.⁴ However, two other studies, by Valette et al.⁵ and Dupon et al.,⁶ failed to find any discernible

changes in T concentrations over the menstrual cycle. The findings in the present study quite clearly demonstrate an increase in serum T throughout the follicular phase of normal spontaneous cycles with a peak on the day of the LH surge. Perhaps these changes in T were more apparent in this study because a larger number of subjects were involved.

Support for a definite periovulatory increase in serum T in normal cycles can be drawn from other sources. In women, the midcycle production rates of T and androstenedione ($\Delta^4\text{A}$) have been shown to be increased.¹⁴ There is a significant increase in $\Delta^4\text{A}$ concentrations in the ovarian vein draining the ovary that contains the dominant preovulatory follicle compared with concentrations in the contralateral ovarian vein.¹⁵ Approximately 50% of circulating T is derived from the peripheral conversion of $\Delta^4\text{A}$.¹ Based upon the periovulatory production rates for both T and $\Delta^4\text{A}$, and making calculations using the known conversion rates for these hormones, a small but definite increase in circulating T would be expected in the periovulatory phase. Further evidence for a midcycle rise in T is provided by a case report of a modest, but significant, increase in periovulatory T levels in a woman who had received bilateral adrenalectomies.¹⁶

LH is a principal determinant for gonadal T secretion in both men and women. During ovarian follicular development, LH stimulates thecal cells to secrete androgens that are aromatized to estrogen by the intrafollicular granulosa cells under the influence of FSH.¹⁷ The thecal layer under the influence of LH secretes Δ^4 A and lesser amounts of T in increasing quantities as a follicle approaches ovulation. An increased LH/FSH ratio and an exaggerated LH secretory pattern are key features of the pathophysiology of PCOD.^{7,18} All women with PCOD have an increased production rate of T.¹

A subgroup of women (n = 3) in the present study demonstrated acute increases of T during GnRH treatment in cycle 2. These women had higher levels of serum LH and more ovarian follicles. (The increased number of follicles in subgroup D, E, F was presumably secondary to a differential increase in FSH in these women). These findings were compatible with the evidence that: (1) increased T production is associated with the dominant follicle and (2) LH is a key regulatory hormone of T secretion. It is presumed that the increased T secretion was secondary to the combination of a LH increase in the presence of multiple follicles. A condition with some similarities to PCOD was induced in this subset of normal subjects: LH was increased, as was the LH/FSH ratio; multiple ovarian follicles were present; and elevated levels of T were found. However, there were important differences between this acutely induced state of hyperandrogenism and PCOD: the multiple follicles in PCOD are smaller and do not usually proceed to ovulation, and E_2 is relatively low in PCOD while estrone is the dominant estrogen. Nevertheless, it is interesting to speculate whether a chronic state of excess androgen secretion with PCOD features could be induced with more prolonged GnRH treatment administered in a similar fashion.

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