

# Modulation of Pulsatile Gonadotropin Secretion by Testosterone in Man\*

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**ABSTRACT.** In experimental animals, primary testicular deficiency leads to increased LH pulse frequency. Pulsatile FSH secretion has not been well characterized in any species. To determine the effect of testosterone (T) on the pattern of pulsatile gonadotropin secretion in man, we performed frequent blood-sampling studies in six normal men and six men with primary hypogonadism. All primary hypogonadal men were studied 6–8 weeks after stopping T replacement therapy. Five of the six hypogonadal men were restudied 6–8 weeks after treatment with T enanthate (200 mg, im, every 2 weeks; sampling in this group was 2 weeks after their last T injection). Blood sampling was done at 10-min intervals for 12 h in all subjects, and the pattern of episodic LH and FSH secretion was determined.

Normal men had a serum T level of  $6.3 \pm 0.3$  ng/ml (mean  $\pm$  SEM), a LH level of  $34 \pm 3$  ng/ml, and a LH pulse pattern characterized by low frequency ( $7.6 \pm 0.7$  pulses/12 h) and low amplitude ( $16 \pm 1$  ng/ml). Compared to normal men, primary hypogonadal men had a significantly lower T level ( $2.9 \pm 0.4$

ng/ml) and significantly higher LH pulse frequency ( $13.0 \pm 1.3$  pulses/12 h), amplitude ( $51 \pm 7$  ng/ml), and mean level ( $222 \pm 26$  ng/ml). Reinstitution of T replacement therapy in hypogonadal men resulted in a significant increase in the T level ( $4.7 \pm 0.5$  ng/ml) and significant decreases in LH pulse frequency ( $7.2 \pm 1.6$  pulses/12 h) and amplitude ( $41 \pm 5$  ng/ml) as well as mean LH level ( $75 \pm 15$  ng/ml). FSH levels fluctuated in a distinctly pulsatile pattern in all three groups. Differences in pulsatile FSH secretion between primary hypogonadal men before and during T therapy and normal men paralleled those in pulsatile LH secretion in both frequency and amplitude.

These results demonstrate that in man 1) diminished T negative feedback results in high frequency (circchoral), high amplitude LH and FSH pulses; 2) T replacement decreased LH and FSH pulse frequency and amplitude as well as mean levels; and 3) the decreased LH and FSH pulse frequency with T treatment implies that T or a metabolite of T acts on the central nervous system to slow the hypothalamic LHRH pulse generator. (*J Clin Endocrinol Metab* 58: 609, 1984)

**I**N SEVERAL mammalian species, including man, the testis exerts negative feedback regulation of pituitary gonadotropin secretion. This negative feedback control of LH and FSH secretion is mediated primarily by the testicular steroid hormones testosterone (T) and estradiol (E<sub>2</sub>) (1, 2). Testicular steroids inhibit LH and FSH release, probably both by decreasing hypothalamic LHRH secretion and by decreasing pituitary responsiveness to LHRH (1). However, the precise mechanisms underlying steroid-mediated negative feedback are not clearly established in any species, including man.

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It is well established that LH is released in an episodic or pulsatile fashion from the pituitary gland (3–5). Furthermore, the pulsatile secretion of LH is a result of episodic hypothalamic discharge of LHRH into the hypothalamo-hypophyseal portal blood and subsequent stimulation of the pituitary (6–8). Therefore, the frequency of LH pulses in blood is a direct reflection of the frequency of episodic release of LHRH from the hypothalamus (7, 8), and characterization of pulsatile LH secretion provides insight into the central nervous system control of gonadotropin secretion. LH secretory dynamics have been well characterized in castrate rats (9), sheep (10), and monkeys (11). With castration and loss of testicular negative feedback in these animals, LH pulse frequency increases. T replacement results in a dose-dependent decrease in LH pulse frequency toward the normal level (9–11). These results imply that T exerts a profound slowing effect on the neural mechanisms involved in the timing of episodic LHRH secretion. In the present work, we investigated whether T also modulates the frequency of hypothalamic LHRH pulse generation in man. In men who were androgen deficient due

to primary hypogonadism, we characterized the effect of T replacement therapy on the patterns of pulsatile LH and FSH secretion and compared them to gonadotropin patterns in normal men.

## Materials and Methods

### Subjects

**Primary hypogonadal men.** Six men, aged 21–40 yr, with androgen deficiency due to primary testicular failure, were recruited and volunteered to participate in this study. Five of these men had Klinefelter's syndrome. The remaining subject had primary hypogonadism associated with a seminoma, which was previously treated with unilateral orchidectomy and periaortic irradiation. All hypogonadal men had low basal serum T levels and elevated LH and FSH levels before treatment. They were otherwise healthy and were not receiving any medication other than T replacement therapy (mean duration, 4 yr; range, 1–10 yr).

All six primary hypogonadal men were studied 6–8 weeks after discontinuing T replacement therapy. Five of the six hypogonadal men were restudied 6–8 weeks after restarting T replacement therapy with T enanthate (Delatestryl, E. R. Squibb and Sons, Princeton, NJ; 200 mg, im, every 2 weeks). In these subjects, blood sampling (see protocol) was performed 2 weeks after their last injection of T enanthate.

**Normal men.** Six normal men, aged 29–44 yr, were recruited and volunteered to participate in this study. These subjects had normal basal serum T, LH, and FSH levels, at least 6 normal seminal fluid analyses obtained over a 3-month period (*i.e.* sperm density >20 million/ml, sperm motility >50%, and >60% oval forms), and normal LH and FSH responsiveness to a 4-h continuous iv infusion of 50 µg LHRH. In addition, these men had a normal medical history and physical examination, complete blood count, 12-panel chemistry battery, and urinalysis. They were not receiving any medication.

**Experimental protocol.** The study protocol was reviewed and approved by the Human Subjects Review Committee of the University of Washington. Informed consent was obtained from each subject after a thorough explanation of the purpose and design of the study. All subjects were admitted to the Clinical Research Center of the University of Washington Hospital or to the Special Studies Unit of the V.A. Medical Center. Beginning at 0800–0900 h, blood sampling was performed through an indwelling 18-gauge polyethylene cannula placed in an arm vein. Blood samples were obtained at 10-min intervals for 12 h in all subjects. The patency of the iv line was maintained with a continuous infusion of heparinized normal saline. Before taking each 4-ml blood sample, the iv line was cleared by removing the first 6 ml of blood via a three-way stopcock. After obtaining a blood sample for hormone determinations, the 6 ml of blood mixed with heparinized saline were reinfused, and the line was flushed with heparinized saline until clear. Blood was allowed to clot at room temperature, and serum was separated, frozen, and stored at –20 C until assayed.

Serum LH and FSH levels were measured in duplicate by

RIA in all blood samples, and serum T levels were measured by RIA on the initial blood sample.

### Hormone assays

**LH and FSH RIA.** The RIA for serum LH (12) used a reference standard (LER 907) and first antibody (antihuman LH batch 2) supplied by the National Pituitary Agency. The tracer was purified hCG radioiodinated with <sup>125</sup>I using chloramine-T (13). The limit of detectability of this assay was 6 ng/ml, and the intra- and interassay coefficients of variation were 5.5% and 8.4%, respectively.

The RIA for serum FSH (12, 14) used reagents distributed by the National Pituitary Agency. The reference standard was LER 907, the first antibody was antihuman FSH (batch 5), and the tracer was HS-1, radioiodinated with <sup>125</sup>I using chloramine-T (13). The limit of FSH detectability was 25 ng/ml. The intraassay variability was 7.3%, and the interassay variability was 9.7%. Lack of significant cross-reactivity of LH in the FSH RIA was demonstrated by pulsatile administration of highly purified human LH (LER 1549) to hypogonadotropic men at a dosage that resulted in high amplitude (~200 ng/ml) LH pulses. At this LH dosage, FSH levels, determined by RIA, remained undetectable, and no FSH pulses were produced.

Assay results for both LH and FSH RIA were calculated using the computer program of Burger *et al.* (15).

**T RIA.** The RIA for serum T (16) used reagents supplied by the WHO Matched Reagent Programme (17). The antiserum was raised in a rabbit against T-BSA. Anti-T antiserum exhibited cross-reactivity of 14% with 5 $\alpha$ -dihydrotestosterone (DHT), 6% with 5 $\alpha$ -androstane-3 $\beta$ -diol, and less than 2% with other steroids tested. The T assay was preceded by ether extraction, and separation of bound from free hormone was accomplished using dextran-coated charcoal. The assay sensitivity was 0.1 ng/ml, and the intra- and interassay variabilities were 5.1% and 9.8%, respectively.

### LH and FSH pulse analyses

LH and FSH pulses were analyzed using a computerized method in which duplicate determinations of LH and FSH on sequential blood samples collected from each subject were first analyzed with an analysis of variance. This analysis was used to determine the relative contribution of measurement variability (as indicated by the coefficient of variation of assay duplicates) to the overall pattern of hormone fluctuations in the experimental data. Before pulse analyses, a moving average of each two successive data points was performed to smooth the baseline. A pulse was defined as an increment in hormone level from nadir to peak greater than 2 times the coefficient of variation of assay duplicates. The number of LH and FSH pulses per 12 h and pulse amplitudes (absolute increment in LH or FSH level from nadir to peak per pulse) were then determined by computer, and interpulse intervals (time between peaks of successive LH or FSH pulses) were calculated.

### Statistical analysis

Mean LH, FSH, and T levels and mean LH and FSH pulse frequency, interpulse interval, and pulse amplitude were deter-

mined for each normal subject and each primary hypogonadal subject both before and during T therapy. These data were then compared using Student's paired or unpaired *t* tests as appropriate. Correlation of FSH pulses with LH pulses was performed using cross-correlation analysis. All variances were expressed as the SEM.

## Results

### Serum LH, FSH, and T levels

Normal men had a mean serum T level of  $6.3 \pm 0.3$  ( $\pm$  SEM) ng/ml, a LH level of  $34 \pm 3$  ng/ml, and a FSH level of  $121 \pm 18$  ng/ml. Six to 8 weeks after discontinuation of T replacement therapy, primary hypogonadal men had significantly lower serum T levels ( $2.9 \pm 0.4$  ng/ml;  $P < 0.001$ ) and higher serum LH ( $222 \pm 26$  ng/ml;  $P < 0.001$ ) and FSH ( $1361 \pm 214$  ng/ml;  $P < 0.001$ ) levels than normal men. Treatment of these men with T enanthate (200 mg, im, every 2 weeks for 6–8 weeks) resulted in a significant increase in T to a mean of  $4.7 \pm 0.5$  ng/ml ( $P < 0.001$  compared to the level before T) and suppression of LH and FSH levels to  $75 \pm 15$  and  $409 \pm 49$  ng/ml, respectively (both measured 2 weeks after the last T injection;  $P < 0.001$  compared to the levels off T). LH and FSH levels were suppressed to a comparable degree (66% and 69%, respectively), but remained elevated compared to those in normal men.

### LH pulse patterns (Figs. 1 and 2)

Examples of the LH pulse patterns demonstrated by primary hypogonadal men before and during T therapy and by normal men are depicted in Fig. 1.

Normal men had a mean LH pulse frequency of  $7.6 \pm 0.7$  pulses/12 h (Fig. 2A, right), an interpulse interval of  $86 \pm 8$  min (not shown), and a LH pulse amplitude of  $16 \pm 1$  ng/ml (Fig. 2B, right). Compared to normal men, primary hypogonadal men before T replacement therapy had a significantly greater LH pulse frequency ( $13.0 \pm 1.3$  pulses/12 h; Fig. 2A, left), a shorter interpulse interval ( $55 \pm 2$  min;  $P < 0.01$ ; not shown), and a greater LH pulse amplitude ( $51 \pm 7$  ng/ml; Fig. 2B, left). T replacement therapy in these hypogonadal men resulted in a significant decrease in LH pulse frequency ( $7.2 \pm 1.6$  pulses/12 h; Fig. 2A, middle), a lengthening of the interpulse interval ( $122 \pm 38$  min;  $P < 0.01$ ; not shown), and a decrease in LH pulse amplitude ( $41 \pm 5$  ng/ml; Fig. 2B, middle) compared to values in these men off T treatment.

### FSH pulse patterns

**Correlation of FSH with LH pulses (Fig. 1).** In all groups, fluctuations in FSH levels were distinctly pulsatile, although of lesser magnitude than LH pulses. FSH pulses were significantly correlated in time with LH pulses ( $P$

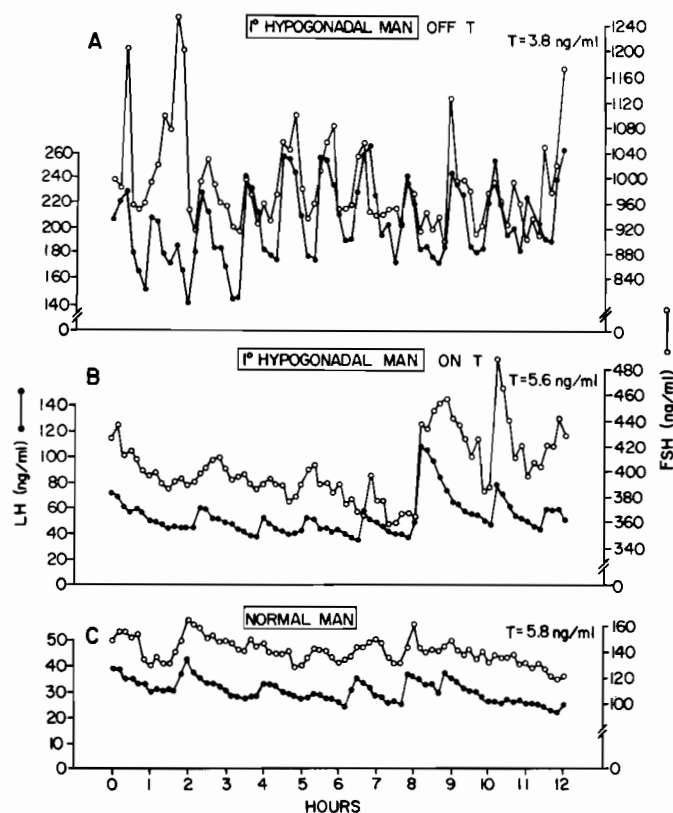


FIG. 1. Examples of serum LH (●) and FSH (○) pulse patterns in a primary (1°) hypogonadal man before T therapy (A) and during T replacement (B) and in a normal man (C). Note the concordance of FSH and LH pulses in each example and the T levels at the beginning of sampling for each subject. Also note the differences in scale.

$< 0.001$ ) in all subjects. Examples of the concordance of FSH pulses with LH pulses in primary hypogonadal men before and during T treatment and in normal men are shown in Fig. 1. FSH pulse peaks were coincident or within 20 min of LH pulse peaks in 39 of the 46 total LH pulses in normal men (85%), in 67 of 78 total LH pulses in primary hypogonadal men before T therapy (86%), and in 31 of 36 total LH pulses in primary hypogonadal men during T treatment (86%).

**FSH pulse parameters (Fig. 2).** The differences in FSH pulse frequency and amplitude in each subject group were similar to those in LH pulse frequency and amplitude. In normal men, the FSH pulse frequency was  $7.7 \pm 0.6$  pulses/12 h (Fig. 2A, right), the interpulse interval was  $84 \pm 4$  min (not shown), and the FSH pulse amplitude was  $17 \pm 1$  ng/ml (Fig. 2B, right). Compared to normal men, primary hypogonadal men before T replacement had a significantly greater FSH pulse frequency ( $13.5 \pm 1.7$  pulses/12 h; Fig. 2A, left), a shorter interpulse interval ( $50 \pm 4$  min;  $P < 0.001$ ; not shown), and a greater FSH pulse amplitude ( $122 \pm 15$  ng/ml; Fig. 2B, left). T therapy in these hypogonadal men resulted in a significant decrease in FSH pulse frequency ( $7.8 \pm 1.1$  pulses/

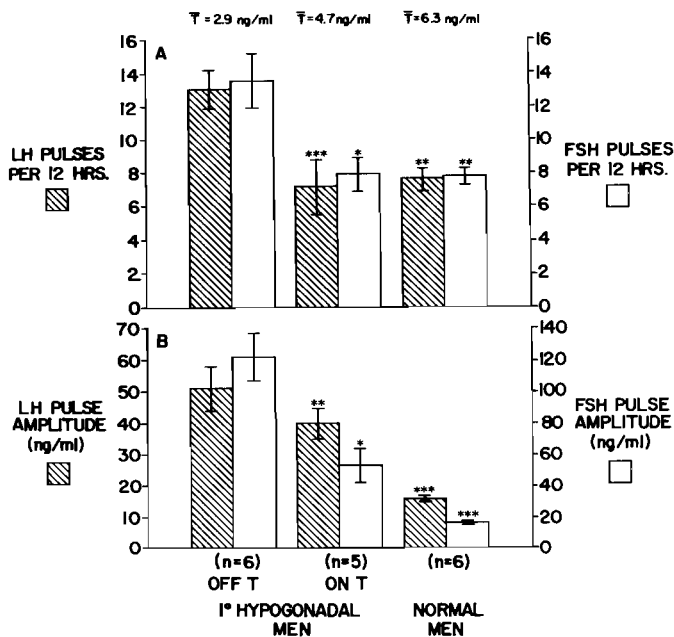


FIG. 2. Mean LH and FSH pulse frequency (pulses per 12 h; A) and LH and FSH pulse amplitude (B) in primary (1°) hypogonadal men before T therapy (*left*) and during T replacement (*middle*) compared to those in normal men (*right*). All values are the mean  $\pm$  SEM. Note the mean T levels for each group above the respective bars. \*\*\*,  $P < 0.001$ ; \*\*,  $P < 0.01$ ; \*,  $P < 0.05$  (compared to hypogonadal men before T therapy).

12 h; Fig. 2A, *middle*), a lengthening of the interpulse interval ( $89 \pm 14$  min;  $P < 0.05$ ; not shown), and a reduction in FSH pulse amplitude ( $53 \pm 11$  ng/ml; Fig. 2B, *middle*) compared to values in these men off T treatment.

## Discussion

These findings demonstrate that diminished T negative feedback in androgen-deficient, primary hypogonadal men resulted in high frequency (circchoral), high amplitude LH pulses. Both LH pulse frequency and amplitude were significantly greater in these hypogonadal men than in normal men. A similar increase in LH pulse frequency with the loss of negative feedback from the testes was reported after castration in the rat (9), red deer (18), ram (10), and monkey (11). In man, several previous studies have documented the presence of pulsatile LH secretion in primary hypogonadal men (10, 19, 20), but in none of these studies was the pattern of episodic LH secretion adequately characterized in a sufficient number of hypogonadal men compared to normal men.

Winters *et al.* (21) initially reported that the frequency of pulsatile LH release in men with primary gonadal failure was similar to that in normal men. These data differ from our results. A likely explanation for the

discrepant results is the difference in blood sampling intervals in the two studies. Winters and coworkers sampled blood every 20 min, while we sampled every 10 min. The need for more frequent sampling to adequately characterize rapid endocrine rhythms has been emphasized by several investigators (22, 23). Using the principles of time series analysis, Yates (23) suggested that in order to detect periodic signals, sampling must be performed at 6 times the expected highest frequency. Therefore, by using a 20-min sampling interval, it is possible that Winters *et al.* (21) may not have been able to detect a significantly increased LH pulse frequency in primary hypogonadal men compared to normal men.

Recently, Winters and Troen (24) reexamined the LH pulse patterns in men with primary testicular failure using a 10-min blood sampling interval and confirmed our findings of a more rapid LH pulse frequency in these men than in normal men. As in our studies, they also found an increased LH pulse amplitude in these men (21, 24).

T replacement therapy in primary hypogonadal men resulted in a significant decrease toward normal in LH pulse frequency and amplitude as well as mean levels. Winters *et al.* (25) found that infusion of T in normal men at its physiological production rate (7.5 mg/day) for 4 days decreased LH pulse frequency without affecting pulse amplitude. However, T infusion in primary hypogonadal men at twice its production rate (15 mg/day) for 4 days failed to decrease either LH pulse frequency or amplitude (23). These investigators concluded that there was resistance to gonadotropin-suppressive effects of androgen in men with primary hypogonadism. These data differ from our results and those in normal men (2, 25). As discussed above, inadequate characterization of LH pulse patterns in their hypogonadal men as a result of a longer blood sampling interval may explain these differing results. Differences in the duration of T treatment, T preparation, and dosage regimen used may have also contributed to the different results.

The frequency of LH pulses is thought to be a direct reflection of the frequency of pulsatile LHRH secretion from the hypothalamus. Two recent reports demonstrated a very close temporal relationship between pulsatile LHRH release into hypothalamo-hypophyseal portal blood and pulsatile LH secretion into peripheral blood (7, 8). Therefore, in the present study, modulation of LH pulse frequency by T suggests that T or a metabolite of T acts on the hypothalamic neural oscillator controlling the frequency of pulsatile LHRH secretion. Although unlikely, another possibility to explain the suppression of LH pulse frequency by T is that T induced a block in transmission of some LHRH pulses at the pituitary level.

The amplitude of LH pulses in blood is a reflection of both the amplitude of LHRH pulses released by the

hypothalamus and the pituitary responsiveness to LHRH. Therefore, suppression of LH pulse amplitude induced by T may represent modulation at a hypothalamic or pituitary site or both. T is metabolized to DHT and  $E_2$  in peripheral tissues, including the hypothalamus and pituitary (26–29). Both DHT and  $E_2$  modulate LH and FSH secretion (2, 25, 30). In normal men, DHT, like T, markedly reduces LH pulse frequency without affecting amplitude (25), while  $E_2$  decreases LH pulse amplitude without affecting frequency (25). The extent to which these steroid metabolites of T may have contributed to the modulation of pulsatile LH secretion by T in our studies cannot be determined.

Episodic FSH secretion was evident in all subject groups and showed a strong temporal correlation with pulsatile LH secretion. Concordance of FSH with LH pulses was demonstrated by previous investigators (4, 30–32). However, characterization of FSH pulse characteristics was not previously undertaken. The changes in pulsatile FSH secretion paralleled those in pulsatile LH secretion in each of our study groups. These results are consistent with the existence of a single hypothalamic releasing factor, LHRH, controlling both LH and FSH secretion (33) and further support the suggestion that T acts on the brain to slow the frequency of the hypothalamic LHRH pulse generator.

The existence of discrete FSH pulses may at first seem untenable, considering the long half-life of FSH (~3 h) in blood (34–36). However, the disappearance of FSH secreted into blood follows a multiexponential decay, and estimation of half-life will vary according to the time at which it is calculated. After injection of FSH into the blood, there is a very rapid rise and fall in FSH due to equilibration into its volume of distribution (35, 36). Therefore, if FSH were secreted by the pituitary in frequent discrete amounts, a rapid FSH pulse pattern in peripheral blood could be produced.

The administration of 200 mg T enanthate by im injection every 2 weeks is a commonly used replacement regimen for men with hypogonadism (1). T levels between injections range from slightly above the normal range just after an injection to the low end of the normal range just before the next injection (37, 38). The effect of LH secretion of this large fluctuation in T levels between T enanthate injections is not known. Blood sampling in this study was performed 2 weeks after the last injection of T enanthate, when blood T levels were at their nadir. This time was chosen because of the relatively smaller changes in T levels that occur toward the end of the injection interval (37, 38). Whether the results would have been different if T levels were maintained at a more constant level is not known.

In conclusion, we have demonstrated that in man, diminished T negative feedback results in high frequency

(circhoral), high amplitude LH and FSH pulses. T replacement decreases LH and FSH pulse frequency and amplitude as well as mean levels. The decrease in LH and FSH pulse frequency with T therapy implies that T or a metabolite of T (such as DHT or  $E_2$ ) acts on the central nervous system to slow the hypothalamic LHRH pulse generator. It remains to be determined whether T administered in more constant, physiological dosages can fully normalize mean levels of LH and FSH as well as their pulse patterns.

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