# Aromatase inhibition causes increased amplitude, but not frequency, of hypothalamic-pituitary output in normal women

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**Objective:** To better understand the site and mode of action of aromatase inhibitors.
**Design:** Prospective study.
**Setting:** Academic research environment.
**Patient(s):** Five eumenorrheic (without polycystic ovary syndrome), early follicular phase women with a normal body mass index (mean: 20.47 ± 0.68 kg/m$^2$), and 12 normal weight, midreproductive aged, early follicular phase women with a normal body mass index (mean: 20.8 ± 1.7 kg/m$^2$) as historical controls.
**Intervention(s):** 2.5 mg letrozole daily for 7 days, with daily urine collection (first morning void), thrice weekly blood sampling, and 4 hours of blood sampling every 10 minutes.
**Main Outcome Measure(s):** Serum luteinizing hormone (LH) measured by a well-characterized immunofluorometric assay with LH pulse characteristics compared between treated and control groups using *t* tests.
**Result(s):** Mean LH and LH pulse amplitude more than doubled in the women who had taken letrozole compared with the controls, but the LH pulse frequency did not differ between the women taking letrozole and the controls.
**Conclusion(s):** These results indicate that the release of negative feedback inhibition of estradiol on the hypothalamic-pituitary axis in normal women by aromatase inhibitors creates an amplitude-related increase in endogenous hypothalamic-pituitary drive. The finding that the mean LH and LH pulse amplitude, but not the frequency, increased after letrozole suggests a possible pituitary site of action. (Fertil Steril® 2011;95:2063–6. ©2011 by American Society for Reproductive Medicine.)

**Key Words:** Aromatase inhibitor, hypothalamic-pituitary-gonadal axis, letrozole, luteinizing hormone, ovary, pituitary

Clomiphene citrate, a mixed estrogen receptor agonist, has been a first-line agent for ovulation induction for over 45 years (1). Its mechanism of action involves opening the negative feedback loop of estradiol on the hypothalamic-pituitary axis, resulting in a greater than 50% increase in endogenous follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (2). The increased gonadotropin stimulation, in turn, leads to ovulatory rates of 60% to 85% and cumulative pregnancy rates of 30% to 40% over three to six cycles (1, 2). Recent studies have indicated a potential role for aromatase inhibitors in ovulation induction (3). Similar to clomiphene, aromatase inhibitors are taken orally for a relatively short period, are relatively inexpensive, and have few side effects (3, 4).

The overall efficacy of letrozole appears comparable with or better than clomiphene and tamoxifen. It appears to be associated with reduced multiple follicular development (1, 3, 5, 6), and it has a significantly reduced rate of multiple gestation compared with clomiphene citrate (5). Despite the increased use and clinical potential of letrozole for ovulation induction, its precise mechanism of action remains unconfirmed. Because it is not a mixed agonist, the response of the hypothalamic-pituitary axis to letrozole cannot be assumed to be identical to that of clomiphene.

We examined whether letrozole's mechanism of action is primarily driven by a hypothalamic site of action, which would favor an increase in the *frequency* of gonadotropin-releasing hormone (GnRH)-LH pulses, or a pituitary site of action, which would favor an increase in LH pulse *amplitude* without a change in pulse frequency. We examined the pulsatile LH response of normally cycling adult women to the administration of an aromatase inhibitor to localize its site of action.

## MATERIALS AND METHODS

### Participants

This protocol was approved by the Albert Einstein College of Medicine Clinical Research Center Protocol Review Committee, and Committee on

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**TABLE 1**

**Anthropometric characteristics by study group.**

| Characteristic | Letrozole (n = 5) | Control (n = 12)[a] | *P* value |
|---|---|---|---|
| Age (y) | 30.8 ± 5.9 | 24.9 ± 4.8 | .09 |
| BMI (kg/m$^2$) | 21.0 ± 1.0 | 20.8 ± 1.7 | .81 |
| Menstrual cycle length (d) | 29.2 ± 1.2 | N/A | — |

*Note:* BMI = body mass index; N/A = not available.
[a] Historical data from Berga et al. (7).

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**TABLE 2**

**Luteinizing hormone (LH) measurements in letrozole-treated women and controls.**

| Characteristic | Letrozole (n = 5) | Control (n = 12)[a] | *P* value |
|---|---|---|---|
| LH pulse frequency/4 hours | 2.0 ± 1.4 | 2.4 ± 1.7 | .55 |
| LH pulse amplitude | 5.1 ± 2.8 | 1.6 ± 0.7 | <.01 |
| Mean LH, IU/L | 9.4 ± 3.5 | 3.4 ± 0.7 | <.01 |

[a] Historical data from Berga et al. (7).

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Clinical Investigations. All participants provided their informed consent before participation. Five women who met the following inclusion criteria were recruited between July 2009 through May 2010: aged 18 to 40 years; body mass index (BMI) 18 to 25 kg/m$^2$; no history of chronic disease affecting hormone production, metabolism, or clearance; normal thyroid stimulating hormone (TSH) at screening; baseline hemoglobin ≥11 g/dL; and regular menstrual cycles every 25 to 35 days. Exclusion criteria included use of medications known to alter or interact with reproductive hormones (e.g., thiazolidinediones or metformin) or excessive exercise (>4 hours per week). Participants underwent a transvaginal ultrasound examination at screening to exclude women with ovarian pathology.

### Measurements

Participants took 2.5 mg of letrozole daily for 7 days starting on menstrual cycle days 1 to 4. On day 6 of letrozole administration, women underwent an 8-hour session in which blood was sampled every 10 minutes, with an intravenous bolus of 75 ng/kg of GnRH given at 4 hours. The GnRH-stimulation portion of the experimental design was a part of a larger, ongoing study. Each participant therefore had a 4-hour window in which we assessed the LH pulse dynamics before the exogenous GnRH was administered. Participant characteristics analyzed included age, body mass index (BMI), LH pulse frequency, LH pulse amplitude, and mean serum LH.

The LH pulsatility results were compared with noncontemporaneous, previously published data from 12 women who had LH pulsatility assessments by means of blood sampling every 15 minutes in an identical LH assay. Prior examination of the role of sampling interval on LH pulse detection indicates that 10- and 15-minute sampling intervals for LH yield essentially identical results (7). The control women were aged 20 to 33 years, had a normal BMI, were ovulatory (determined by luteal phase progesterone), had no systemic disease, and had no history of excessive exercise (7). For both groups, serum LH was measured using a solid-phase, two-site specific immunofluorometric assay (DELFIA; Perkin Elmer, Turku, Finland) (8). The interassay and intra-assay coefficients of variation for serum LH were 5.5% and 2.3%, respectively. Serum estradiol and testosterone were also measured using DELFIA reagents. The assay limit of detection for testosterone was 3.28 nmol/L (11 ng/dL), and the intra-assay coefficient of variation was 12.9% at the level of the lowest standard.

### Statistical Analysis

In each group, LH pulse frequency, amplitude, and mean were the outcomes of interest. The LH pulse frequency was determined by two methods (9, 10), both of which yielded similar results. The data are shown using the modified Santen and Bardin method, which defines LH pulses as a 20% increment over the preceding nadir. Amplitude was calculated as the peak value of the LH pulse minus the preceding nadir. The mean LH was calculated as the average level across the first 4 hours of sampling before the administration of exogenous GnRH. In the controls, the LH pulse frequency was determined by dividing the mean pulse frequency for the 12-hour study by 3. The group means (± standard deviation [SD]) were compared using Student's *t*-test with a two-tailed alpha of 0.05. Analyses were performed using STATA 9.2 (StataCorp LP, College Station, TX).

## RESULTS

The participant characteristics are shown in Table 1. The mean age of the women in the letrozole group (30.8 ± 5.9 years) did not statistically significantly differ from the controls (24.9 ± 4.8 years; *P*=.07). The mean BMI of the letrozole group (21.0 ± 1.0) was nearly identical to the controls (20.8 ± 1.7; *P*=.82). Menstrual cycle length in days of the letrozole group was within normal limits.

### LH Pulsatility Parameters

As shown in Table 2 and in Figures 1 and 2, letrozole-treated women had 2.0 ± 1.4 LH pulses per 4 hours compared with 2.4 ± 1.7 per 4 hours (*P*=.55) in controls. The mean LH pulse amplitude for the letrozole-treated group was more than twice that of controls: 5.1 ± 2.8 IU/L versus 1.6 ± 0.7 IU/L (*P*<.01). Mean LH for the letrozole-treated women was also substantially increased compared to controls: 9.4 ± 3.5 IU/L versus 3.4 ± 0.7 IU/L (*P*<.01). The GnRH bolus did not change the LH pulse frequency (before: 2.0 ± 0.5, versus after: 1.8 ± 0.4; *P*=.74), while the mean LH after stimulation increased by approximately 50% (data not shown).

### Sex Steroids

The serum estradiol levels decreased, as expected, to the assay limit of detection during letrozole administration and remained there for the course of the cycle. Testosterone levels increased transiently during letrozole administration from a mean pre-letrozole minimum of 0.86 ± 0.27 nmol/L to a maximum of 1.64 ± 0.33 nmol/L (*P*=.02) in the four women who had available serum for testosterone measurement.

## DISCUSSION

Aromatase constitutes the rate-limiting step in the conversion of C19 steroids (testosterone and androstenedione) into C18 steroids (estradiol and estrone). Nonsteroidal aromatase inhibitors target the active site of the aromatase cytochrome P450 and bind in a competitive manner to prevent estrogen synthesis (11). Aromatase messenger RNA (mRNA) has been localized in the pituitary gland as well as several areas of the hypothalamus in zebrafish (12) and rainbow trout (13). Rats exhibit moderate levels of aromatase within the periventricular preoptic nucleus and medial preoptic nucleus, with several other areas of the hypothalamus demonstrating low but detectable aromatase activity (14). Nonhuman primates demonstrate aromatase mRNA in both the hypothalamus and pituitary gland.