

Pulsatile gonadotrophin secretion in women with polycystic ovary syndrome after gonadotrophin-releasing hormone agonist treatment

Anthony P.Cheung^{1,3}, John K.H.Lu² and R.Jeffrey Chang¹

¹Department of Obstetrics and Gynecology, University of California, California, and ²Department of Obstetrics and Gynecology, University of California, Los Angeles, California, USA

³To whom correspondence should be addressed at: Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of British Columbia, Vancouver Hospital and Health Sciences Centre, 805 West 12th Avenue, Vancouver, BC, Canada V5Z 1M9

In polycystic ovary syndrome (PCOS), increased luteinizing hormone (LH) pulse frequency has been attributed to either the hypothalamic gonadotrophin-releasing hormone (GnRH) pulse generator or ovarian oestrogen feedback. To address this issue, a detailed examination of pulsatile LH secretion was undertaken during recovery from GnRH agonist (GnRHa) suppression. Each of six women with PCOS and six normal ovulatory women received daily GnRHa treatment for 14 weeks. Frequent blood samples were collected and assayed for gonadotrophins, androgens and oestrogens before, during and up to 4 weeks after treatment. Women with PCOS had higher basal LH pulse frequency and amplitude and increased serum concentrations of LH, androstenedione, testosterone and oestrone than controls. After 3 months of GnRHa treatment, all these parameters were suppressed with no differences observed between the two groups. One week after cessation of GnRHa, LH pulse frequency promptly returned to pre-treatment range with no between-group differences noted, whereas LH pulse amplitude, serum gonadotrophins and ovarian steroids remained maximally suppressed and equivalent in the two groups. Subsequent LH pulse frequency remained constant while LH pulse amplitude and circulating concentrations gradually increased in parallel with a return of serum oestrogen to pre-treatment values. Despite comparable resumption of LH secretion in the two groups, corresponding androgen concentrations in women with PCOS were greater than those of normal ovulatory women. Thus, the immediate restoration of LH pulse frequency after discontinuing GnRHa treatment is largely independent of ovarian oestrogen production and reflects primacy of the GnRH pulse generator in determining basal LH pulse frequency. Equivalent LH pulse frequency rates in the two groups during the recovery period do not suggest an intrinsic hypothalamic-pituitary hyperactivity in PCOS.
Key words: GnRH agonist/LH pulse frequency/PCOS

Introduction

Polycystic ovary syndrome (PCOS) is a clinical condition characterized by menstrual irregularity, hirsutism and infertility. One of the predominant endocrinologic features of PCOS is abnormal gonadotrophin secretion (Yen *et al.*, 1970b; Rebar *et al.*, 1976; Zumoff *et al.*, 1983; Apter *et al.*, 1994). In particular, the pattern of luteinizing hormone (LH) release has been associated with increased pulse amplitude and pulse frequency relative to that observed in the follicular phase of the normal menstrual cycle (Burger *et al.*, 1985; Waldstreicher *et al.*, 1988). The latter, however, has not been uniformly reported by all investigators (Kazer *et al.*, 1987; Venturoli *et al.*, 1988). While gonadotrophin-releasing hormone (GnRH) pulsatile release cannot be assessed *in vivo* from the hypothalamic portal circulation in humans, it is generally recognized that peripheral LH pulses correlate directly with those of hypothalamic gonadotrophin-releasing hormone (Clarke and Cummins, 1982; Levine and Ramirez, 1982). Further support for increased GnRH pulse frequency is the recent observation of a simultaneous rise in α -subunit pulsatile secretion in addition to LH (Berga *et al.*, 1993). Whether this increased pulse frequency reflects intrinsic hyperactivity of the hypothalamic GnRH pulse generator or a feedback effect secondary to altered concentrations of circulating hormones is unknown.

Previously, we and others have demonstrated that in patients with PCOS, resumption of gonadotrophin secretion following long-term GnRH agonist (GnRHa) suppression is marked by an initial rise of serum follicle-stimulating hormone (FSH) and a subsequent gradual increase of LH which appeared to be commensurate with the return of ovarian oestrogen secretion (Cologero *et al.*, 1987; deZiegler *et al.*, 1989). This sequence of gonadotrophin recovery was similar to that observed in women with hypothalamic amenorrhoea receiving pulsatile GnRH (Marshall and Kelch, 1979). The current study was conducted to determine whether in PCOS the resumption of gonadotrophin secretion, in particular LH pulsatile release, was primarily a result of endogenous GnRH activity or an effect of ovarian steroid feedback. An understanding of the underlying mechanism(s) involved might provide an insight into the role of the GnRH pulse generator in the aetiology of this disorder.

Materials and methods

Subjects

Six women with PCOS with a clinical history of chronic anovulation and evidence of hirsutism were recruited for study. The mean modified Ferriman–Gallwey score \pm SD was 18.5 ± 2.74 (range 15–21) after excluding the scores for forearms and legs. Six normal ovulatory

Table I. Luteinizing hormone (LH) radioimmunoassay precision at six control dose concentrations

LH (IU/l)	4.5	17.8	22.5	26.3	28.1	70.6
Intra-assay CV (%)	9.9	4.1	4.1	6.2	6.9	7.1
Interassay CV (%)	17.8	12.4	19.9	16.1	17.6	19.2

women were recruited as controls. Each subject with PCOS had polycystic ovaries on ultrasound examination based on previously described criteria (Adams *et al.*, 1986; Ardaens *et al.*, 1991). The mean age \pm SEM for women with PCOS and normal ovulatory women were respectively 25.67 ± 2.40 years and 29.83 ± 2.15 years ($P = 0.226$). The corresponding values for height were respectively 1.65 ± 0.03 and 1.68 ± 0.03 m ($P = 0.415$). The mean body mass index of women with PCOS was significantly higher than that of controls (33.06 ± 2.68 versus 23.49 ± 1.24 , $P = 0.009$). Among women with PCOS, four were 40% overweight and one was 20% overweight, based on a 1983 Metropolitan Life Insurance Company Table (Burton *et al.*, 1985). In contrast, only one normal ovulatory woman was 20% overweight.

Study design

Informed consent was obtained from all subjects. The study was approved by the Human Subjects Review Committee of the University of California, Davis. Each of six women with PCOS and six normal ovulatory women received the GnRH α [(imBzl)GnRH α ⁶, Pro⁹-NET]-GnRH, daily for 14 weeks at a dose of $2 \mu\text{g}/\text{kg}$ subcutaneously. Each subject underwent 10 min blood sampling for a 10 h period before GnRH α treatment, at 12 weeks during treatment and at 1, 2 and 4 weeks after discontinuing treatment. Pre-treatment blood sampling was performed on cycle day 2 in normal controls. Women with PCOS had not been on hormone treatment for at least 3 months prior to the study. One subject with PCOS withdrew from the study 12 weeks into the treatment for social reasons. GnRH α administration was extended from 12 to 14 weeks to provide 2 extra weeks so that frequent blood sampling from study subjects could be safely resumed 1, 2 and 4 weeks after discontinuing treatment.

Hormone assay

Serum concentrations of LH, FSH, androstenedione, testosterone and oestrone were measured by conventional radioimmunoassay as previously reported (Yen *et al.*, 1970a; Yen *et al.*, 1972; DeVane *et al.*, 1975; Anderson *et al.*, 1976). Serum oestradiol was measured by an extraction-method kit from Pentax, Santa Monica, CA, USA. Similarly, progesterone and dehydroepiandrosterone sulphate were determined by radioimmunoassay kits from Diagnostic Products Corporation, Los Angeles, CA, USA. Steroid hormones were assayed in serum pooled from blood samples at baseline, 5 h and 10 h during each of the 10 h sampling periods. Circulating LH concentrations were measured in duplicate at all times. The samples were divided into seven LH assays and in each assay, quadruplicate samples from six established LH serum pools were included as controls and the coefficients of variation (Table I) were computed using the method described by Rodbard (1974). The corresponding mean dose concentrations and standard deviations of the six serum pools were also calculated to generate the Baxter parameters (Baxter, 1980) as required by the Munro Program (Taylor, 1987) for the 'pulsar' algorithm (see below). In a typical assay run, the complete LH data set for the whole study of one subject with PCOS and one control were included.

Table II. Basal hormone profile of women with polycystic ovary syndrome (PCOS) and normal ovulatory women

Basal hormone profile	PCOS	Normal	<i>P</i> value
LH (IU/l)	24.48 ± 4.56	8.52 ± 1.59	0.0025
FSH (IU/l)	11.31 ± 1.68	13.12 ± 1.96	0.403
LH:FSH ratio	2.16 ± 0.25	0.71 ± 0.17	0.001
LH pulse amplitude (IU/l)	14.41 ± 2.58	4.03 ± 0.58	0.0009
LH pulse frequency (peaks/10 h)	8.20 ± 0.58	6.50 ± 0.34	0.036
Androstenedione (nmol/l)	8.14 ± 0.87	3.46 ± 0.59	0.001
Testosterone (nmol/l)	1.77 ± 0.24	0.90 ± 0.14	0.008
Oestradiol (pmol/l)	290 ± 26	220 ± 18	0.05
Oestrone (fmol/l)	481 ± 70	263 ± 26	0.015
Progesterone (nmol/l)	2.58 ± 0.16	2.96 ± 0.76	0.441
Dehydroepiandrosterone sulphate ($\mu\text{mol}/\text{l}$)	76.26 ± 7.06	60.25 ± 6.24	0.124

To convert the above hormones to conventional units, divide the appropriate values by the following factors: luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (mIU/ml), 1; androstenedione (ng/ml), 3.492; testosterone (ng/ml), 3.467; oestradiol (pg/ml), 3.671; oestrone (pg/ml), 3.7; progesterone (ng/ml), 3.18; and dehydroepiandrosterone sulphate ($\mu\text{g}/\text{ml}$), 0.02714.

Pulse detection

LH peaks were objectively identified from the hormone series using the 'cluster analysis' algorithm (Veldhuis and Johnson, 1986). To determine the precision of LH measurements for the programme, the three variance models of linear, quadratic and power function were compared using at least 720 (360 duplicate) samples from each of the seven LH radioimmunoassays. The power function $B_1x^{B_2}$ uniformly gave the least fitted variance and was the model selected for within-assay errors. In the analysis, the following parameters were chosen: cluster size for peak = 1, nadir = 2; *t* statistics for both up and down strokes = 2 and peak size = 1. The pulsar algorithm (Merriam and Wachter, 1982) was also used to assess whether study results were affected by the choice of a particular pulse detection programme. It was set to a type 1 error rate of 5% by entering the appropriate set of *G* values as per algorithm.

Statistical analysis

The design of the study allowed simultaneous comparison of within- and between-group changes during and after GnRH α treatment using two-factor analysis of variance (Winer, 1971), with repeated measures on one factor (i.e. the five selected periods of observation). Within-group comparisons among the five selected periods were further characterized using one-factor analysis of variance with repeated measures. A-priori comparison, if appropriate, was made as well as post-hoc pairwise comparison using the Scheffé *F* test or LSD (least significant difference) test. Between-group comparison at each corresponding period was also analysed by two-sample, unpaired Student's *t*-test. Since normality of data was not assumed, between-group comparison was re-analysed using the non-parametric, Wilcoxon rank sum test, which made no difference to the findings.

Results

Basal hormone profile

In women with PCOS, the mean (\pm SEM) basal concentration of LH, 24.48 ± 4.56 IU/l, was substantially higher than that observed in the control group, 8.52 ± 1.59 IU/l (Table II, Figure 1). In contrast, mean concentrations of FSH were similar in both groups. The pattern of LH release in PCOS revealed significant increases in pulse amplitude and pulse frequency

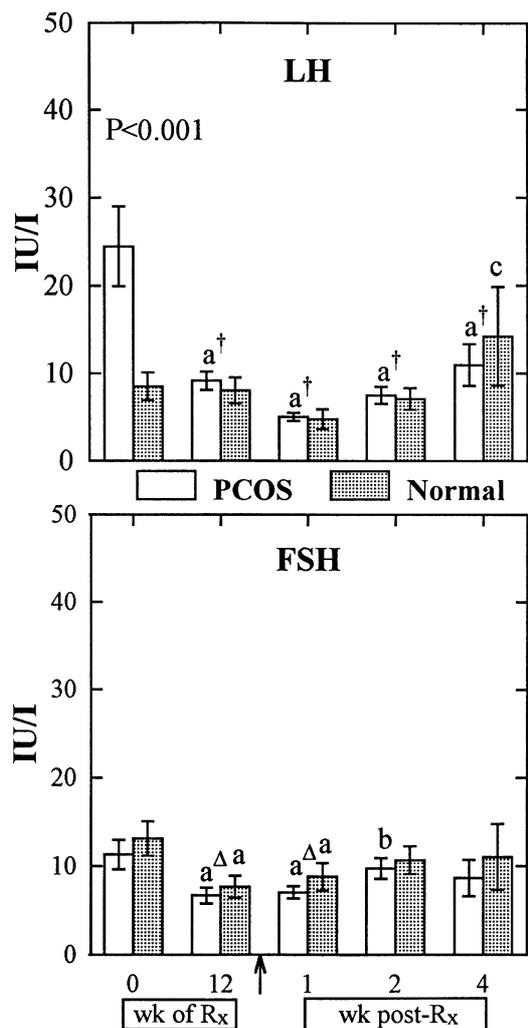


Figure 1. Mean serum concentrations \pm SEM of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) for women with polycystic ovary syndrome (PCOS) and normal ovulatory women before gonadotrophin-releasing hormone agonist (GnRHa) treatment (0 wk of R_x), at 12 weeks of treatment (12 wk of R_x) and at 1, 2 and 4 weeks after discontinuing treatment (1, 2 and 4 wk post- R_x). The duration of GnRHa treatment was 14 weeks (arrow). A significant between-group difference at a particular time point is illustrated by a P value above the relevant pair of error bars. Significant within-group differences between time points are represented by a, b, c and d (relative to 0 wk of R_x , 12 wk of R_x , 1 wk post- R_x and 2 wk post- R_x respectively) above the relevant error bars. † and Δ indicate differences at $P < 0.001$ and $P < 0.01$ respectively while letters without superscripts indicate $P < 0.05$.

compared to those of normal ovulatory women. The mean pulse amplitude in women with PCOS was 14.41 ± 2.58 IU/l, which was substantially greater than in the control group, 4.03 ± 0.58 IU/l. Measurements of amplitude varied widely in individual patients as values ranged from 9.20 IU/l to 21.82 IU/l. In contrast, the magnitude of LH pulses among normal ovulatory women was relatively consistent (Figure 2). The mean LH pulse frequency in women with PCOS, 8.20 ± 0.58 peaks/10 h, was greater than that found in normal ovulatory women, 6.50 ± 0.34 peaks/10 h. While this difference was statistically significant, there was some overlap of individual values between the two groups.

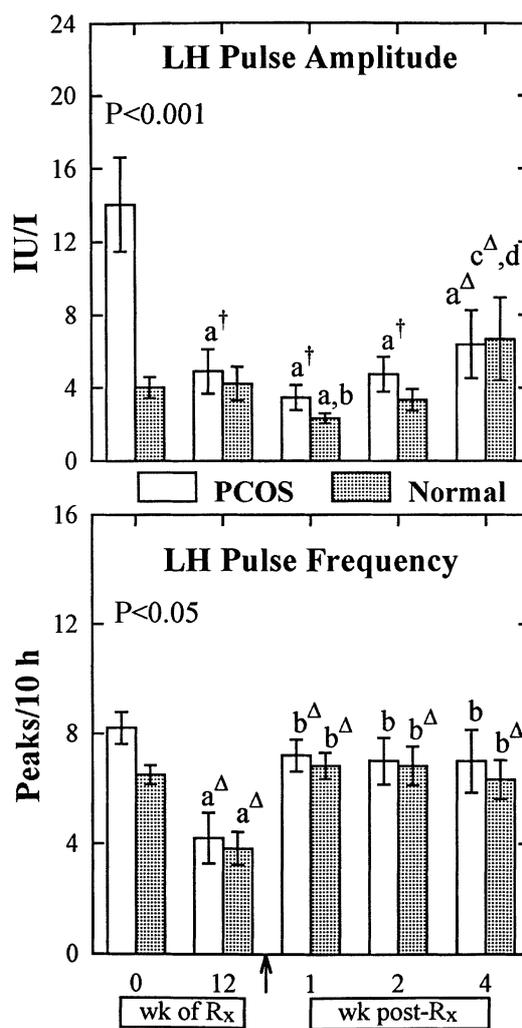


Figure 2. Mean values \pm SEM of luteinizing hormone (LH) pulse amplitude and LH pulse frequency for women with polycystic ovary syndrome (PCOS) and normal ovulatory women derived from the 'cluster' algorithm. The duration of GnRHa treatment was 14 weeks (arrow).

In PCOS, mean baseline concentrations of circulating androgens, androstenedione and testosterone were significantly greater than those found in normal controls (Table II). Circulating concentrations of oestrone were increased compared to control subjects whereas differences in serum oestradiol failed to achieve statistical significance between the two groups. Reversal of the normal oestrone:oestradiol ratio in our patients was consistent with that of previously reported studies (DeVane *et al.*, 1975; Rebar *et al.*, 1976; Chang *et al.*, 1982). Mean serum concentrations of progesterone and dehydroepiandrosterone sulphate did not differ between women with PCOS and normal ovulatory women.

Effects of GnRHa

Mean serum FSH concentrations in both groups decreased significantly in response to GnRHa, whereas serum LH concentrations were significantly reduced in PCOS only (Figure 1). While a suppressive effect of GnRHa on LH concentrations in normal ovulatory women was not observed, values were

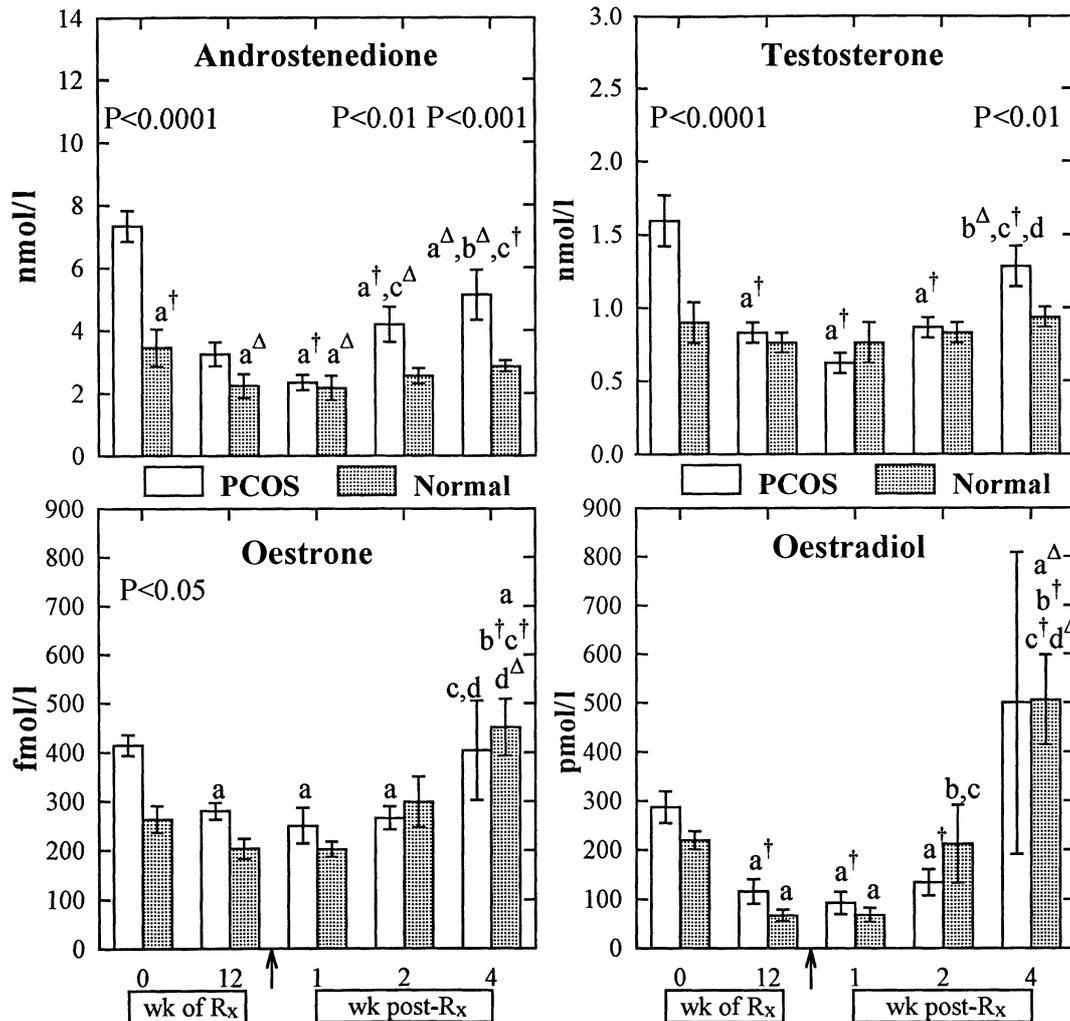


Figure 3. Mean serum concentrations \pm SEM of androstenedione, testosterone, oestrone and oestradiol for women with polycystic ovary syndrome (PCOS) and normal ovulatory women. The duration of GnRH α treatment was 14 weeks (arrow).

similar to the reduced concentrations of women with PCOS after 12 weeks of GnRH α treatment. Uniformly, there were significant decreases in LH pulse amplitude and frequency in women with PCOS and normal ovulatory women following 3 months of GnRH α administration (Figure 2). In PCOS, LH pulses were characterized by a mean magnitude of 4.91 ± 1.22 IU/l and a mean frequency of 4.20 ± 0.92 peaks/10 h, both of which were similar to corresponding values measured in the control group. Mean circulating concentrations of androstenedione and oestradiol in both groups were significantly decreased by GnRH α (Figure 3). In addition, significant reduction of serum testosterone and oestrone were noted in women with PCOS. Significant decrements in mean serum testosterone in normal ovulatory women were not observed, a pattern which persisted throughout the study. Serum progesterone concentrations in both groups were decreased in response to GnRH α , whereas serum dehydroepiandrosterone sulphate concentrations remained unchanged (Figure 4). Following 3 months of GnRH α suppression, there were no differences in gonadotrophin and steroid profiles between women with PCOS and the control group. As mentioned previously, GnRH α treatment was extended from 12 to 14 weeks to provide 2 extra weeks

so that frequent blood sampling from study subjects could be safely resumed 1, 2 and 4 weeks after discontinuing GnRH α treatment. Accordingly, no blood samples were collected when GnRH α suppression was expected to be maximal at 14 weeks. However, that circulating concentrations of LH and ovarian steroids and LH amplitude reached a nadir 1 week after withdrawal of treatment would reflect the effects of these 2 additional weeks of GnRH α suppression (see below).

Recovery from GnRH α

One week following discontinuation of GnRH α , serum concentrations of LH and FSH in both groups remained suppressed (Figure 1) compared to values at 12 weeks (i.e. 2 weeks before GnRH α was suspended). In contrast, the pattern of pulsatile LH secretion in PCOS revealed an immediate restoration of mean pulse frequency similar to that found during pre-treatment assessment (Figure 2). These LH pulses, however, were of small magnitude and were lower than the diminished amplitude observed at 12 weeks (Figure 2). The control group also exhibited a return of pre-treatment LH pulse frequency which, interestingly, was not significantly different from the corresponding rate of pulsatile LH release in PCOS. Mean LH pulse

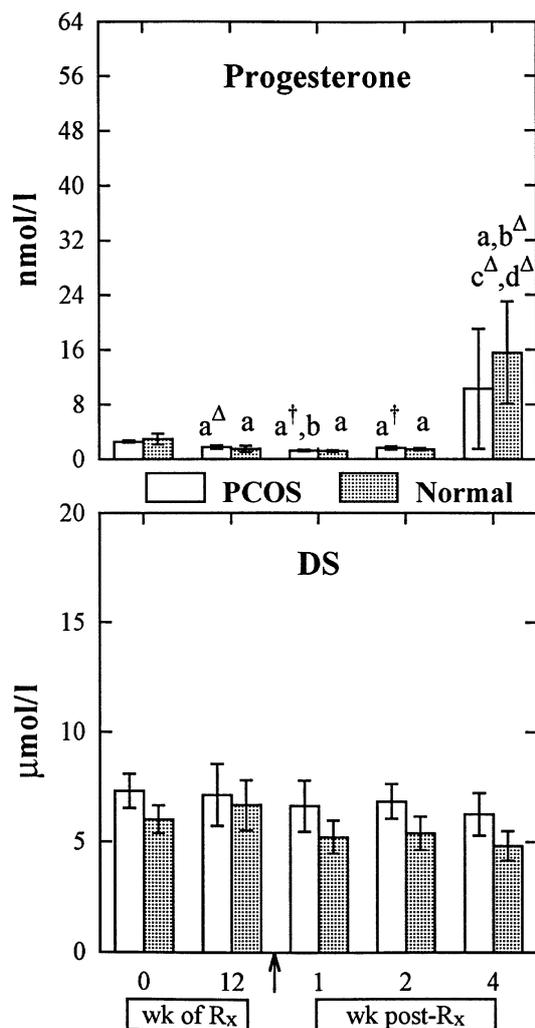


Figure 4. Mean serum concentrations \pm SEM of progesterone and dehydroepiandrosterone sulphate for women with polycystic ovary syndrome (PCOS) and normal ovulatory women. The duration of GnRHa treatment was 14 weeks (arrow).

amplitudes were similar in the two groups. Representative LH pulsatile patterns of a woman with PCOS and a normal ovulatory woman are illustrated in Figure 5. During this early recovery phase, mean steroid hormone concentrations in both groups were maximally suppressed (Figure 3), a pattern similar to that observed for LH pulse amplitude and mean circulating LH concentrations (Figures 1, 2). The lack of ovarian steroid secretion in PCOS immediately following cessation of long-term GnRHa treatment was consistent with our previous published results (deZiegler *et al.*, 1989).

Two weeks after withdrawal of GnRHa, mean FSH concentrations returned to pre-treatment values and remained similar in both women with PCOS and normal ovulatory women (Figure 1). In PCOS, mean LH concentrations were still suppressed from pre-treatment values. Mean LH pulse frequency in women with PCOS and normal ovulatory women remained at pre-treatment values and were not different between the two groups (Figure 2). In PCOS, a small increase in LH pulse amplitude was noted which did not represent a significant change within the group nor a significant difference

between the two groups. Correspondingly, changes in mean steroid hormone concentrations in both women with PCOS and normal ovulatory women began to reflect resumption of ovarian steroidogenesis during the second week of recovery (Figure 3). During this period, a modest but significant increment in the mean serum androstenedione concentration was detected in women with PCOS whereas subjects in the control group failed to demonstrate similar evidence of increased ovarian androstenedione production. The resultant increase in circulating androstenedione in PCOS was statistically different from the mean androstenedione value in normal ovulatory women (Figure 3). Unlike androstenedione, serum testosterone levels remained unchanged in both groups. In PCOS, increases of serum oestrone and oestradiol concentrations were not observed compared to values found during the first week of recovery. In the control group, serum oestrone was unaltered whereas oestradiol concentrations increased significantly to achieve pre-treatment values among some controls. Progesterone levels remained suppressed and similar in both groups (Figure 4).

Four weeks following the cessation of GnRHa administration, mean FSH levels in both groups remained in the pre-treatment range (Figure 1). In women with PCOS, there was a small increase in mean serum LH concentrations which did not achieve statistical significance. By comparison, the control group exhibited an increase of circulating LH which was significantly greater than that previously measured during recovery. In both groups, the mean LH pulse frequency was maintained at pre-treatment rates (Figure 2). Examination of mean LH pulse amplitude in normal ovulatory women revealed a significant increase which was greater than the magnitude of LH pulses noted at 1 and 2 weeks after GnRHa withdrawal and which were comparable to baseline pre-treatment values. In PCOS, the mean LH pulse amplitude rose slightly but remained well below that of baseline values established prior to GnRHa administration. The mean LH amplitude between the two groups was not significantly different. In both groups, a clear trend of increasing values of mean LH pulse amplitude, which closely mirrored that of circulating LH, could be demonstrated over the four-week recovery period (Figures 1, 2). During this interval of study in PCOS women, mean serum androstenedione concentrations continued to increase but had not returned to baseline values. In normal ovulatory women, serum androstenedione concentrations remained unaltered, thereby increasing the difference between groups. Concomitantly, PCOS patients exhibited a significant increase in serum testosterone concentrations, which essentially restored baseline values (Figure 3). In contrast, normal ovulatory women failed to demonstrate any change in serum testosterone concentrations, which was a consistent finding throughout the study. Marked increases in serum oestrone and oestradiol concentrations were noted in both patients with PCOS and normal ovulatory women. These changes were reflective of significant ovarian follicular activity as serum progesterone concentrations also exhibited substantial increases at this time. Mean dehydroepiandrosterone sulphate concentrations were not altered throughout the entire course of study (Figure 4).

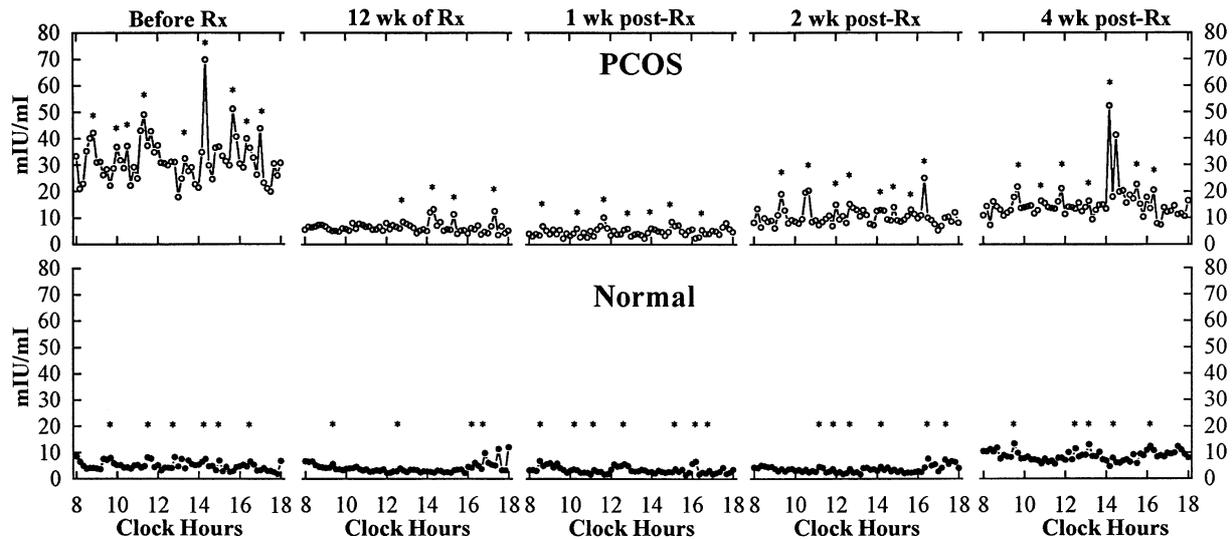


Figure 5. Representative plots of serum luteinizing hormone (LH) concentrations at 10-min intervals for a 10 h duration before treatment (R_x), at 12 weeks of R_x and at 1, 2 and 4 weeks post- R_x in a woman with polycystic ovary syndrome (PCOS) and a normal ovulatory woman. Significant LH peaks detected by the 'cluster' algorithm are marked by asterisks (*).

Cluster versus pulsar algorithm

Mean values of LH pulse amplitude derived from the pulsar algorithm (Figure 6) closely resembled those from the cluster algorithm (Figure 2). Within- and between-group changes in mean LH pulse frequency rates during the course of study were also similar, despite variations from the cluster algorithm in the number of LH pulse peaks detected among individuals. Because the model of least fitted variance for the cluster algorithm was directly derived from the study data set, results using this algorithm were considered more robust and were referred to in the following discussion.

Discussion

The results of this study have demonstrated that in women with PCOS and normal ovulatory women, return of gonadotrophin secretion after cessation of long-term GnRHa treatment was characterized by an early restoration of LH pulse frequency followed by a gradual resumption of pulse amplitude. The suppressive effect of GnRHa on ovarian steroid secretion was substantial as significant decreases in circulating androstenedione, testosterone, oestrone and oestradiol were demonstrated in PCOS. Concentrations of androstenedione and oestradiol were also significantly suppressed in normal ovulatory women. Clinically, the reduction in oestrogen concentrations was accompanied by the appearance of vasomotor instability among all subjects. The observation at 1 week after discontinuing GnRHa provided a critical window to contrast intrinsic GnRH pulsatility, since circulating concentrations of gonadotrophins and ovarian steroids were maximally suppressed and equivalent in both groups. Notably, LH pulse frequency was not significantly different from their respective basal values and was not significantly different between the two groups. The re-establishment of baseline pre-treatment pulse frequency prior to recovery of ovarian steroid production indicates that oestrogen feedback has minimal, if any, influence on the rate of LH

release during this time. LH pulse frequency remained unaltered during the recovery period despite a return of oestrogen to pre-treatment levels. Our findings are contrary to those of previous studies, which have indicated that increased LH pulse frequency in PCOS may be attributed to an association between GnRH and oestrogen secretion. The ability to distinguish the effect of GnRH from that of gonadal steroid feedback on LH pulse frequency has been achieved in earlier reports utilizing GnRH-deficient models (Belchetz *et al.*, 1978; Spratt *et al.*, 1987). In rhesus monkeys with hypothalamic lesions or men with idiopathic hypogonadotropic hypogonadism, progressive increases in the delivery rate of GnRH have been shown to induce increased LH pulse frequencies much like those of PCOS without apparent influence from gonadal steroid production. In the design of our study model, an effect of ovarian steroid feedback was not evident. Our data, therefore, are consistent with animal (Belchetz *et al.*, 1978; Clarke and Cummins, 1982; Levine and Ramirez, 1982) and limited human (Spratt *et al.*, 1987) data that LH pulse frequency is primarily determined by hypothalamic GnRH activity. However, this does not preclude the well-recognized effect of luteal-phase progesterone concentrations in slowing LH pulse frequency (Soules *et al.*, 1984) and reducing basal LH concentrations (Baird *et al.*, 1977). Indeed, the wider range in LH pulse frequency rates observed at 4 weeks after discontinuing GnRHa was consistent with this effect in one patient with PCOS and two control subjects who demonstrated rising progesterone concentrations and apparent ovulation. Taken together, the fact that there was no difference in LH pulse frequency between the two groups during the recovery period (before ovulation and before full evolution of the chronic, aberrant hormone state pathognomonic of women with PCOS) would suggest that heightened LH pulse frequency observed in women with PCOS was not due to a higher firing rate of GnRH/LH pulsatile secretion intrinsic to the hypothalamic-pituitary unit but a consequence of prolonged anovulation and

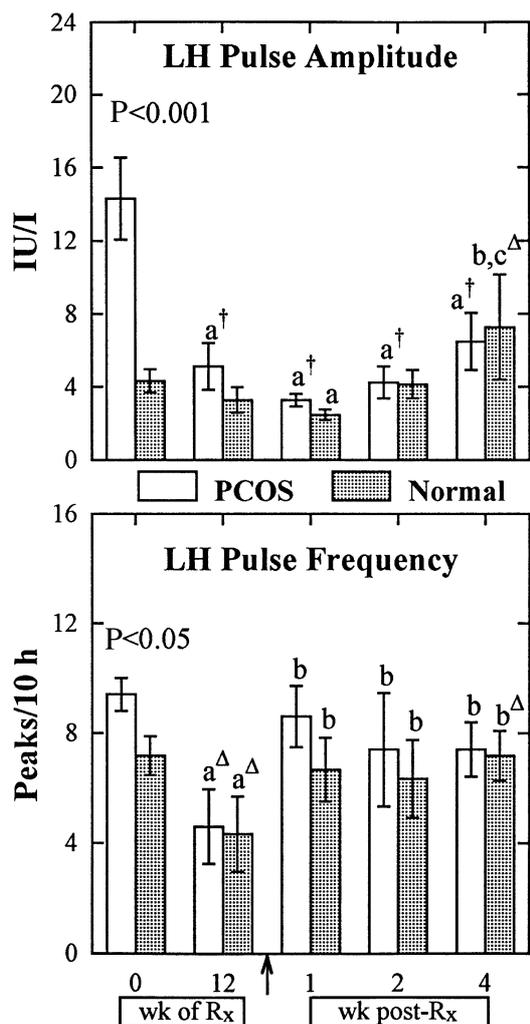


Figure 6. Mean values \pm SEM of luteinizing hormone (LH) pulse amplitude and LH pulse frequency for women with polycystic ovary syndrome (PCOS) and normal ovulatory women derived from the 'pulsar' algorithm. The duration of GnRHa treatment was 14 weeks (arrow).

lack of cyclical exposure to progesterone. In this regard, extending assessment of pulsatile LH secretion beyond 4 weeks after discontinuing GnRHa may provide valuable information, especially among anovulatory women with PCOS.

It is interesting to note that LH pulsatility was still detectable, albeit at markedly lower frequency and amplitude, during GnRHa treatment despite significant suppression of circulating concentrations of LH and ovarian steroids. This is in contrast to a previous report which showed that pulsatile secretory pattern of both LH and free α -subunit were abolished by a single depot injection of the GnRHa, D-Trp using a non-Gaussian model for time series to detect pulses (Couzinet *et al.*, 1991). Potential factors that might account for the discrepancy include the dose, duration and type of GnRHa treatment, between-subject variability in response to GnRHa suppression and recovery, hormone assay techniques and criteria used to detect LH peaks. The D-His dose of 2 μ g/kg selected in the study was designed to adjust for body weights and was based on a pilot study in which adequate pituitary-ovarian suppression could be demonstrated using comparable

doses for the same treatment duration among women with endometriosis. It remained possible that LH pulses were ablated after 14 weeks of GnRHa treatment but frequent blood sampling during GnRHa suppression was only performed at 12 weeks, for reasons discussed earlier. From our own experience and that reported in the literature (Jaakkola *et al.*, 1990; Cheung and Chang, 1995), it is recognized that LH values measured by the newer monoclonal immunometric assays based on chromofocusing techniques are generally lower than values obtained by conventional radioimmunoassay. We have also observed less intra- and interassay variations. Accordingly, the LH signal-to-noise ratio and the number of LH peaks identified by pulse detection algorithms could differ from that based on radioimmunoassay. Although radioimmunoassay was in fact used in the study, comparison was made specifically within each group and between the two groups using the same LH assay method. It was not the intention of the study to compare LH pulsatility using different assay techniques. With respect to pulse detection algorithm, interpretation of our data was unchanged whether the cluster or pulsar algorithm was used for comparison.

In contrast to LH pulse frequency, detectable increases in pulse amplitude in PCOS were temporally related to progressive increases in serum oestrone and oestradiol concentrations. These results are consistent with the observation that increased exposure of pituitary gonadotrophs to oestrogen is associated with amplified LH responses to administration of GnRH (Jaffe and Keye, 1974; Shaw *et al.*, 1975; Wang *et al.*, 1976; Baird *et al.*, 1977). That LH pulse amplitude in PCOS had not returned to pre-treatment values by the end of recovery might have been due to the limited duration of study following discontinuation of GnRHa. It has been suggested that, in PCOS, increased LH pulse amplitude may be the result of prolonged exposure to chronic oestrogen secretion or a manifestation of self-priming by GnRH or both (Rebar *et al.*, 1976; Hoff *et al.*, 1979). Failure to achieve a sufficient duration of either effect could account for the diminished pulse amplitude in PCOS. These same considerations may also apply to the recovery of circulating LH concentrations in PCOS which remained below baseline levels measured prior to the initiation of GnRHa treatment. By comparison, the group of normal ovulatory women exhibited complete recovery of basal serum LH concentrations as well as LH pulse amplitude at the end of the study.

The pattern of gonadotrophin secretion during recovery from GnRHa suppression also permitted assessment of ovarian stimulation by endogenous LH and FSH. In particular in PCOS, ovarian androgen responses to the resumption of LH secretion revealed significant increases of serum androstenedione and testosterone whereas in the control group, no incremental changes were observed. These findings suggested that androgen-producing tissues of patients with PCOS were more responsive to LH stimulation than those of normal ovulatory women since throughout recovery, basal serum LH concentrations, LH pulse frequency and LH pulse amplitude were similar between the two groups. Our data support the results of in-vitro studies performed on stromal and thecal tissues from women with PCOS in which androstenedione and

testosterone dose-responses to LH stimulation were significantly greater than those of normal ovulatory women (Barbieri *et al.*, 1986; Gilling-Smith *et al.*, 1994). In addition, results of the current study are consistent with those which have demonstrated greater androstenedione and 17α -hydroxyprogesterone responses to GnRHa stimulation in PCOS compared to normal ovulatory women, and a probable over-expression of C-17, 20-lyase and 17α -hydroxylase activities of the enzyme system, cytochrome P-450c17 α , in this disorder (Barnes *et al.*, 1989). Serum oestrone and oestradiol concentrations rose progressively in both groups after the discontinuation of GnRHa to achieve and, in some instances, exceed baseline values. The oestrogen responses were most likely dictated by a gradual resumption of FSH secretion. Indeed, FSH concentrations returned to pre-treatment values two weeks after GnRHa withdrawal, as documented in our earlier report (deZiegler *et al.*, 1989). Additional evidence of FSH stimulation was the demonstration of elevated serum progesterone concentrations and apparent ovulation noted earlier. Because of the more divergent hormone response at 4 weeks after discontinuing GnRHa, the study was also re-analysed after excluding data from this time point. However, this made no difference to the conclusion.

In summary, this study has carefully examined the recovery of pulsatile LH secretion in both women with PCOS and normal ovulatory women following cessation of long-term GnRHa administration. The results demonstrated that the early restoration of LH pulse frequency was due to hypothalamic GnRH pulse generator alone whereas the subsequent return of LH pulse amplitude might represent the combined effects of ovarian oestrogen feedback as well as hypothalamic GnRH activity. These findings may have implications for the establishment of basal LH pulse frequency and amplitude that are important in states of normal or altered reproductive physiology, including PCOS. In addition, heightened LH pulse frequency observed in PCOS is unlikely to be caused by a higher set-point in GnRH ultradian rhythm that is intrinsically-entrained in the hypothalamic-pituitary unit but is possibly the effect of chronic anovulation from a lack of phasic modulation of hormones, particularly progesterone, in the normal menstrual cycle.

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