Augmented frequency and mass of LH discharged per burst are accompanied by marked disorderliness of LH secretion in adolescents with polycystic ovary syndrome

Maria Cecilia García-Rudaz, María Gabriela Ropelato, Maria Eugenia Escobar, Johannes D Veldhuis and Marta Barontini

Abstract

The aim of this study was to quantify pulsatile LH secretion, burst frequency and mass, LH half-life, and the approximate entropy (ApEn) or (dis-)orderliness of LH release in adolescents with polycystic ovary syndrome (PCOS), combining a high-precision immunofluorimetric LH assay with deconvolution techniques.

We sampled LH concentration profiles every 20 min overnight in 12 girls with PCOS (mean ± S.E.M. age 16.4 ± 0.57 years, body mass index (BMI) 24.4 ± 1.6 kg/m²) and 11 eumenorrheic early-follicular-phase controls (mean ± S.E.M. age 16.5 ± 0.47 years, BMI 22.2 ± 1.0 kg/m²). Fasting serum levels of androstenedione, testosterone, 17-hydroxyprogesterone (17-OHP), estrone, estradiol, FSH and sex hormone-binding globulin (SHBG) were determined. Compared with euandrogenic girls, PCOS adolescents had significantly (P < 0.005) elevated serum LH/FSH ratios, 17-OHP, androstenedione, estrone and testosterone levels, decreased SHBG, and similar estradiol. PCOS subjects exhibited a 3-fold higher mean serum LH concentration with almost no overlap with controls (8.8 ± 1.2 and 2.8 ± 0.3 IU/l respectively, P < 0.001). We initially used a conventional serum hormone concentration peak analysis method (Cluster) to evaluate the characteristics of pulsatile LH release. Cluster analysis disclosed a significant increase in serum LH concentration maximal peak height, a higher LH peak frequency and a higher mean serum LH concentration in interpulse nadirs in the PCOS group. Deconvolution analysis of mechanisms underlying the foregoing showed a higher pulsatile production rate of endogenous LH in the PCOS group (41 ± 4.2 IU/l per day in the PCOS group vs 18 ± 2.3 IU/l per day in the controls, P < 0.01). The mean estimated half-life of endogenous LH disappearance was also significantly higher in patients with PCOS than in controls (110 ± 8.5 and 77 ± 3.7 min respectively, P < 0.01). To quantify the orderliness of LH release, we used ApEn. PCOS patients had remarkably increased disorderliness (higher ApEn) of LH release (1.09 ± 0.04 vs 0.77 ± 0.08 in controls, P = 0.002). Mean serum LH concentration, mass of LH secreted per burst, and LH production rate in PCOS, but not in normal adolescents, correlated positively with androstenedione (P < 0.02, 0.02 and 0.05 respectively). The same parameters also correlated positively with 17-OHP (P < 0.05, 0.02 and 0.05 respectively). Stepwise regression analysis unmasked a negative influence of BMI in PCOS on both mass of LH secreted per burst (r = −0.77, P < 0.005) and LH production rate (r = −0.70, P < 0.01). We conclude that PCOS adolescents secrete LH molecules with amplified frequency and burst mass and with markedly disrupted orderliness. A rise in basal (non-pulsatile) LH release, more basic LH isoforms, and/or a prolongation or asymmetry of the LH secretory burst could account for the apparently prolonged LH half-life. Determining whether disorderliness of the amplified pituitary LH release process is an intrinsic abnormality in PCOS, or reflects androgen excess, may help to clarify the pathophysiology of this oligo-ovulatory syndrome in young women.
time (usually 6–12 h) confirmed a marked increase in mean serum LH concentration related to augmented pulse amplitude in PCOS women compared with the early or mid-follicular phase of the menstrual cycle in normal women (3, 5–6). Later studies in which more intensive blood sampling was implemented and the duration of sampling was extended to 24 h demonstrated an increase in LH pulse frequency as well as pulse amplitude in many patients with PCOS (7–9). The increase in LH pulse frequency provided evidence for accelerated hypothalamic gonadotropin-releasing hormone (GnRH) pulse generator output in this metabolic syndrome. Two recent independent studies (10, 11) have confirmed an attenuation of LH pulse amplitude and pituitary GnRH response (but not of LH pulse frequency) in association with obesity in PCOS women.

Most previous studies used classical polyclonal RIA methods to evaluate plasma levels of LH in the adult. Studies of pulsatile characteristics of LH secretion in patients with PCOS evaluated with more precise and/or specific immunoassays to measure LH levels are scarce (12). Apter et al. (13) employing a high-precision time-resolved immunofluorimetric assay (IFMA) showed an increase in 24 h LH pulse frequency and amplitude in a group of hyperandrogenic girls. These findings supported earlier predictions of peripubertal onset of this syndrome (14).

The characteristics of LH secretion may provide an insight into the neuroendocrine mechanisms that underlie the pathophysiology of PCOS (15). Therefore we here investigated the pulsatile characteristics of LH secretion in a group of adolescents with PCOS and a group of controls in the early follicular phase of the menstrual cycle using a highly specific LH IFMA, deconvolution analysis to calculate individual LH secretory properties (16), and approximate entropy (ApEn) to assess the orderliness of LH release (17). To our knowledge, no previous studies have combined these contemporary and complementary strategies to appraise the nature of neuroendocrine pathophysiology in adolescents with PCOS.

Patients and methods

Subjects

Twelve adolescents with PCOS (age 13–21 years) and 11 eumenorrheic controls (ages 13–19 years) were studied. All PCOS patients were below 17 years of chronological age except for one who was 20.5 years old. None of the subjects was hypertensive or had evidence of Cushing’s disease or drug-induced hirsutism. Hyperprolactinemia and thyroid disease were ruled out by normal serum measurement of these hormones. Subjects had not been taking any medication including contraceptives pills for at least 3 months before the study. The diagnosis of PCOS was based on the presence of at least three of the following features: clinical signs of hyperandrogenism (hirsutism, evaluated by a Ferriman–Gallwey score of at least 9 (18), and/or acne), perimenarcheal onset of oligomenorrhea or amenorrhea, and elevated serum testosterone and/or androstenedione concentrations. In PCOS patients, late-onset congenital adrenal hyperplasia was excluded by a normal serum 17-hydroxyprogesterone (17-OHP) concentration response 60 min after an adrenocorticotropic hormone stimulation test (19). The protocol of this study was approved by the ethical committee of Ricardo Gutiérrez Children’s Hospital, and written informed consent was obtained from each subject and her parents.

Clinical protocol

Control volunteers with regular menstrual cycles (of similar age, see above) and oligomenorrheic PCOS patients were studied during the early follicular phase (days 2–5) of the menstrual cycle, and amenorrheic PCOS patients were studied on a random day. In no patient with PCOS had recent ovulation occurred, as evidenced by retrospective measurement of serum progesterone levels. Progestin withdrawal bleeding was avoided because administration of progestins may slow LH pulsatility (20). Subjects were admitted to the hospital at 1800 h. Blood samples were withdrawn through an indwelling i.v. catheter every 20 min for 12 h beginning at 1900 h. Subjects refrained from drinking caffeinated beverages during the study and received standard meals at 2100 h. They were allowed to sleep from 2200 to 0600 h. Serum LH concentrations were determined in each sample collected at 20 min intervals and also concentrations of follicle-stimulating hormone (FSH), sex hormone-binding globulin (SHBG) and sex steroid hormones on 0700 h fasting samples. Each individual’s samples were analyzed in one assay in duplicate.

Assays

Serum LH and FSH concentrations were measured by IFMA (Delfia; Pharmacia, Turku, Finland), as described by Ropelato et al. (21). The intra- and interassay coefficients of variation were respectively <2.2 and 5.5% for LH and <1.8 and 4.2% for FSH. Serum concentrations of 17-OHP and testosterone were measured by commercial RIA kits (Diagnostic Products Corporation, Los Angeles, CA, USA and ICN Pharmaceuticals, Costa Mesa, CA, USA). Androstenedione was measured by RIA after ether extraction, as previously described by Rey et al. (22). RIA of estradiol-17β and estrone was carried out using commercial kits (Diagnostic Products Corporation and Diagnostic Systems Laboratories Inc., Webster, TX, USA). Serum SHBG was determined by saturation analysis using tritiated dihydrotestosterone as previously described by Campo et al. (23).
Data analysis

We initially applied a conventional serum hormone concentration peak analysis method (Cluster) to evaluate pulsatile properties of spontaneous LH secretion. A 2 × 1 (test nadir 2, test peak 1) cluster size was used, and ‘t’ statistics were set at 2.0 and at 2.0 for up and down strokes (24). Thereafter, pulsatile LH secretion was appraised by deconvolution analysis, in which admixed basal and pulsatile hormone secretion rates were estimated concurrently via a two-step fitting procedure, as described previously (25, 26).

ApEn is a scale- and model-free statistical measure of the disorderliness of hormone release. Higher ApEn values denote greater irregularity or randomness of LH release (17, 27).

Statistics

Log-transformed data were analyzed by unpaired two-tailed unequal variance Student’s t-test. Relationships between variables were sought by linear regression analysis with forward selection. Results are expressed as mean ± s.e.m. P < 0.05 was considered significant.

Results

Clinical and endocrine features

The endocrine characteristics of PCOS patients and eumenorrheic adolescent controls are summarized in Table 1. PCOS and control groups were similar in both chronological (16.4 ± 0.57 and 16.5 ± 0.47 years respectively) and gynecological (post-menarcheal) age (4.8 ± 0.39 (range 3.1–8.0) and 4.0 ± 0.36 (range 3.0–6.9) years respectively). Seven of the PCOS patients were oligomenorrheic (less than eight menses/year) and five were amenorrheic (menses every 6 months or longer). The degree of hirsutism ranged from mild to severe (scores 10 to 22) in most of the PCOS patients, but two patients had a normal score. Ten of the PCOS patients had mild to severe acne and the other two had no acne.

Compared with euandrogenic controls of similar age and body mass index (BMI) (BMI 24.4 ± 1.6 and 22.2 ± 1.0 kg/m² for PCOS patients and controls respectively), PCOS adolescents had significantly (P < 0.005) elevated serum 17-OHP, androstenedione, testosterone and estrone concentrations (Table 1). Serum concentrations of SHBG were significantly (P < 0.005) decreased in PCOS patients, and estradiol and FSH levels were similar in the two groups.

Pulsatile characteristics of LH secretion

Cluster analysis disclosed a significant increase in serum LH concentration maximal peak height (IU/l) in PCOS patients, the mean value of which was 11 ± 1.3 (compared with 4.3 ± 0.48 in controls, P < 0.01; Fig. 1). Incremental amplitudes were also higher in PCOS patients (not shown). In addition, in the course of overnight blood sampling, PCOS patients exhibited a frequency of 6.2 ± 0.34 serum LH concentration pulses/12 h compared with 4.1 ± 0.64 pulses/12 h in the controls (P < 0.05). The mean serum LH concentration in interpulse nadirs (IU/l) was also markedly increased in PCOS (7.1 ± 1.1 vs 1.6 ± 0.3, P < 0.01) (Fig. 1).

Deconvolution analysis was used to evaluate the mechanisms underlying the above Cluster findings. This analysis of episodic LH secretion in four eumenorrheic controls and four adolescents with PCOS is illustrated in Fig. 2. The continuous curves through the serum LH concentration data (Fig. 2, left subpanels) represent the calculated reconvolution fits predicted by the multiple-parameter convolution method. The precision of fit of the convolution model is therefore illustrated graphically. The right subpanels show the computer-resolved underlying LH secretory bursts, i.e. the calculated LH secretion rate plotted against time.

Individual deconvolution results are shown in Fig. 3. As shown in the upper right panel, PCOS subjects exhibited a 3-fold higher mean serum LH concentration with almost no overlap between group values. The half-life of the LH secretory bursts (duration of the secretory event at half-maximal amplitude) was similar in

Table 1 Hormone levels in normal adolescents (controls) and those with PCOS. Values are means ± s.e.m.

<table>
<thead>
<tr>
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<th>Controls (n = 11)</th>
<th>PCOS (n = 12)</th>
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<tr>
<td>17-OHP (ng/ml (nmol/l))</td>
<td>0.59 ± 0.10 (1.79 ± 0.30)</td>
<td>2.0 ± 0.31* (6.05 ± 0.94)</td>
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<tr>
<td>Androstenedione (ng/ml (nmol/l))</td>
<td>1.30 ± 0.11 (4.54 ± 0.38)</td>
<td>2.62 ± 0.33* (9.15 ± 1.15)</td>
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<tr>
<td>Testosterone (ng/ml (nmol/l))</td>
<td>0.45 ± 0.06 (1.56 ± 0.21)</td>
<td>1.50 ± 0.25* (5.20 ± 0.86)</td>
</tr>
<tr>
<td>Estrone (pg/ml (pmol/l))</td>
<td>11.0 ± 1.4 (40.7 ± 5.2)</td>
<td>32.5 ± 4.0* (120.0 ± 14.8)</td>
</tr>
<tr>
<td>Estradiol (pg/ml (pmol/l))</td>
<td>40.0 ± 5.0 (146.8 ± 18.4)</td>
<td>48.0 ± 3.6 (176.2 ± 13.2)</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>42.0 ± 4.3</td>
<td>23.1 ± 3.2*</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>4.3 ± 0.28</td>
<td>3.7 ± 0.17</td>
</tr>
<tr>
<td>LH/FSH ratio</td>
<td>0.65 ± 0.07</td>
<td>2.46 ± 0.38*</td>
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* P < 0.005 vs controls.
adolescents with PCOS and their controls (data not shown). The mean interburst interval was lower (92.4 ± 5.3 and 137 ± 15.8 min in PCOS patients and controls respectively, P < 0.05) and the frequency of spontaneous LH secretory bursts was significantly higher in the PCOS group (7.9 ± 0.38 and 5.7 ± 0.63 pulses/12 h in the PCOS group and controls respectively, P < 0.05). The mass of LH released per secretory event (calculated as the area under the computer-resolved LH secretory pulse) was also significantly greater in PCOS subjects (5.4 ± 0.57 IU/I compared with 3.4 ± 0.56 IU/I in controls, P < 0.05). Since the pulsatile production rate is the product of the mean mass of hormone secreted per pulse and the number of pulses per day, there was a significantly higher mean pulsatile LH production rate in the PCOS group (P < 0.01, Fig. 3). As illustrated further in the upper left panel of Fig. 3, only one value for the LH production rate in the PCOS group overlapped those in the control group. The mean estimated half-life of endogenous LH disappearance was also significantly higher in patients with PCOS (Fig. 3, lower left panel).

To quantify the orderliness of LH release, we used ApEn. As shown in Fig. 3 (lower right panel), PCOS patients have markedly increased disorderliness (higher ApEn) of LH release (P = 0.002).

Stepwise regression analyses in the PCOS patients demonstrated that the main correlates of 12 h mean LH secretion are the mass of LH secreted per burst (P < 0.0002), the half-life of LH molecules (P < 0.001) and the pulse frequency (P < 0.03).

In the PCOS group, baseline 17-OHP levels were positively related to the 12 h mean LH concentration (r = 0.57, P < 0.05), the mass of LH secreted per burst (r = 0.64, P < 0.02, Fig. 4), and the 12 h LH production rate (r = 0.61, P < 0.05). These correlations were not found in the normal adolescent group. Increased androstenedione concentrations in PCOS adolescents were also related to the mean serum 12 h LH concentration (r = 0.69, P < 0.02), the mass secreted per burst (r = 0.66, P < 0.02, Fig. 4) and the production rate (r = 0.54, P < 0.05). Serum concentrations of testosterone, estradiol, estrone and SHBG were not related to any parameters of LH secretion in either of the groups.

The relationships of LH secretory properties with BMI in PCOS adolescents are shown in Fig. 5. Stepwise linear regression analysis in the PCOS patients indicated a negative influence of BMI on mean 12 h spontaneous LH secretion, affecting specifically both the mass of secretory bursts (r = -0.77, P < 0.005) and the 12 h LH production rate (r = -0.70, P < 0.01).

Discussion

The present clinical investigation in adolescent girls used an LH IFMA, deconvolution analysis, and ApEn calculations to provide evidence for the first time that increased LH concentrations in PCOS girls arise mechanistically from an increased frequency and mass of LH secreted per burst and secretion of LH molecules with a prolonged half-life, and that LH profiles of adolescents with PCOS show a marked disorderliness of LH release. To our knowledge, no previous study has demonstrated that PCOS adults or adolescents secrete LH with markedly disrupted orderliness, as shown here by ApEn calculations.

Available studies of LH pulsatility in PCOS have used discrete peak detection methods exclusively (3, 7–9). These earlier studies indicated that the high circulating LH concentrations, as estimated mostly by RIA, in PCOS are largely maintained by an exaggerated pulsatile discharge of LH, especially evident as enhanced amplitude and frequency (3, 7–9). Even during the night, when in both PCOS and early-follicular-phase controls sleep slows LH pulsatility (28), a higher pulse frequency has been demonstrated in PCOS women (7).

Cluster analysis, applied initially in our study to characterize spontaneous LH profiles, also showed increased serum LH concentration peak height, frequency
Marked disorderliness of LH secretion in PCOS adolescents

Figure 2: Illustrative 12 h serum LH concentration profiles in four eumenorrheic controls and four adolescents with PCOS (PCO). Serum LH concentrations were measured by IFMA in blood collected every 20 min for 12 h. Left panel, the measured (within sample dose-dependent s.e.) serum LH concentrations, and the deconvolution-predicted fits; right panel, the calculated LH secretory rates.
and interpulse nadirs in PCOS girls, confirming previous observations in other pertinent populations of adults. We therefore applied deconvolution analysis to test the mechanisms by which maximal LH pulse amplitude and interpulse nadir serum LH concentrations are both elevated in PCOS. In principle, several mechanisms could pertain in PCOS: an increased mass of LH secreted per burst; a prolonged LH half-life; and/or a higher basal LH secretion rate. The combined use of a highly specific LH IFMA and deconvolution analysis showed that two of these mechanisms are applicable in adolescents with PCOS, namely augmentation of LH secretory burst mass (the calculated amount of LH secreted per burst), accompanied by a slight but significant prolongation of apparent (endogenous) LH half-life. In contrast, estimated basal LH secretion rates appeared to be similar in PCOS and controls, with the proviso that this parameter is more difficult to estimate numerically (29). The increased LH secretory pulse mass calculated here by deconvolution analysis is more accurate and reproducible than LH secretory pulse amplitude, since the latter co-varies with burst duration (29, 30). Importantly, the greater mass of LH secreted per burst as observed in this study is probably not related to the secretion of free α-subunit reported in PCOS (8, 31), since this is not measured by the IFMA.

Figure 3 Individual values (and mean ± S.E.M.) of the episodic LH secretion, its half-life estimated by deconvolution analysis, and quantification of the orderliness (ApEn) within 12-h serum LH concentration time series obtained in 11 normal girls and 12 PCOS (PCO) adolescents. Higher ApEn denotes greater disorderliness or randomness of the hormone release process. PCOS patients have a higher LH production rate (P < 0.01), mean serum LH concentration (P < 0.001) and LH half-life (P < 0.01) than controls. PCOS patients also have increased disorderliness of LH release (P = 0.0016 vs controls).
in plasma (33). The latter is controlled by underlying secretory event frequency, duration and amplitude, as well as hormone half-life (32). An increase in LH pulse frequency in adults with PCOS has also been inferred in earlier RIA studies (7–9). As our study shows that LH half-life in both PCOS patients and controls is more than 60 min, being more prolonged in the PCOS group, and PCOS patients also showed a higher pulse frequency, the sampling interval of 20 min used in this study as opposed to the 10 min used in other studies (3, 7–9) means that our results are more relevant.

Taken together, the observations suggest that at least part of the gonadotropin secretory abnormality in adolescent PCOS occurs as the result of excessive hypothalamic GnRH drive. Moreover, our novel finding of greater disorderliness of 12 h LH release endorses the new notion of altered feedback regulation and/or increased complexity of neuroregulatory inputs in

Figure 4 Serum 17-OHP (top) and androstenedione (A, bottom) concentrations vs LH pulse mass estimated by deconvolution analysis in 11 normal (○) and 12 PCOS (■) adolescents. In PCOS patients 17-OHP and androstenedione concentrations were positively and significantly related to LH pulse mass ($r = 0.64, P < 0.02$ and $r = 0.66, P < 0.02$ respectively). The solid line represents the linear regression of the PCOS data.

Figure 5 BMI vs mass of LH secreted per burst (top) and production rate (bottom) in PCOS (■) and normal cycling adolescents (○). In PCOS adolescents BMI was negatively and significantly related to the mass of LH secreted per burst ($r = -0.77, P < 0.005$) and to the LH production rate ($r = -0.70, P < 0.01$). The solid line represents the linear regression of the PCOS data.
PCOS (17, 34–35). This is intuitively consistent with the combined increases in LH and ovarian sex steroids, suggesting defective negative feedback (35).

The present study also suggests that LH molecules with a prolonged half-life are released in PCOS patients, which could be accounted for alternatively by an increase in basal (non-pulsatile) LH release or a prolongation or asymmetry of the LH secretory pulse shape (29). It is also possible that altered LH isoforms are released by the pituitary gland in PCOS. Indeed, the nature of the oligosaccharide side chains on LH molecules affects their metabolic clearance and bioactivity (36), and LH is rapidly removed from the circulation by a receptor present in the liver that specifically recognizes sulfated oligosaccharides (37). Since estrogens modulate the expression of the glycosyltransferases that synthesize (sulfated) oligosaccharides (38), the longer estimated half-life of LH in PCOS adolescents may indicate, at least at the onset of the disorder, altered LH isoforms in the circulation. An increase in (LH) distribution volume in PCOS would also influence the apparent hormone half-life.

Whether the abnormal pulsatile and entropic patterns of LH secretion in PCOS could primarily induce increased androgen secretion by the ovary, or conversely whether the deranged gonadal steroid secretion (tonic estrogen and/or excessive androgen secretion) that characterizes PCOS could secondarily disrupt LH release and alter its half-life remains to be determined. However, our results show that levels of 17-OHP and androstenedione in PCOS are positively related to mean serum LH levels. Indeed, two pulsatile properties of LH secretion were related to androgen levels, namely the mass of LH secreted per burst and its 12 h production rate. Two independent studies have demonstrated positive correlations between serum 17-OHP and 24 h LH levels. Apter et al. (13) also found that mean serum LH levels were positively related not only to 17-OHP but also to androstenedione, testosterone and estrone levels in a group of hyperandrogenic girls.

Abnormal LH-driven regulation (dysregulation) of ovarian androgen secretion, particularly at the level of the enzyme cytochrome P450C17 which has both 17-hydroxylase and 17,20-lyase activities, has been hypothesized to underlie the pathophysiology of PCOS (39). The strong correlations we find between LH secretion and both 17-OHP and androstenedione levels are consistent with this hypothesis. Neither this study nor that of Taylor et al. (10) found any relation between parameters of LH pulsatile secretion and either serum estradiol or estrone concentrations. These reports contrast with two other studies (3, 40) suggesting that estrogen levels may be associated with gonadotropin abnormalities in PCOS.

Obesity is observed in 30–50% of women with PCOS (41–42). Several studies have investigated the relationship between body fat and inappropriate gonadotropin secretion in PCOS (9–11, 43). One proposal is that an obesity-associated factor suppresses GnRH pulse amplitude or pituitary responsiveness, but not pulse frequency in this syndrome (10, 11). In agreement with other studies in adults (10, 11), we found here in adolescents a negative influence of BMI on mean LH concentration. Our use of deconvolution analysis to estimate LH secretion properties per se allowed us to discern that the lower mean serum LH concentrations in more obese PCOS patients are related to a negative effect of BMI on LH secretory burst mass (and hence the LH production rate). Arroyo et al. (11) recently reported that LH concentrations and pulse amplitude in PCOS women are inversely related to both 24 h insulin levels and the degree of insulin resistance. However, to date, the mechanism of the postulated link between insulin levels and gonadotropin secretion in PCOS is unclear.

Another mechanism proposed to explain the effect of obesity on LH secretion is an increase in renal gonadotropin clearance (10). However, this is unlikely to be relevant here, as we did not find any relation between BMI and the estimated LH half-life in either PCOS or normal adolescents.

In conclusion, the present clinical study of PCOS adolescents shows hypersecretion of LH due to amplified LH secretory burst frequency and mass, associated with markedly disrupted orderliness of 12 h LH release profiles. A putative rise in basal (non-pulsatile) LH release, more basic LH isoforms, and/or a prolongation or asymmetry of the LH secretory pulse shape could account for the apparently prolonged LH half-life. To discover whether disorderliness of the pituitary LH release process is an intrinsic abnormality in PCOS or reflects androgen (or estrogen or GnRH) excess will require further study.

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