available at www.sciencedirect.com

ScienceDirect

www.metabolismjournal.com

Altered multihormone synchrony in obese patients with polycystic ovary syndrome

Ferdinand Roelfsema[a,*], *Petra Kok*[a], *Johannes D. Veldhuis*[b], *Hanno Pijl*[a]

[a] *Department of Endocrinology and Metabolic Diseases, Leiden University Medical Center, Albinusdreef 2, 2333ZA, Leiden, the Netherlands*
[b] *Endocrine Research Unit, Mayo Medical and Graduate Schools, Clinical Translational Research Center, Mayo Clinic, Rochester, Minnesota 55901, USA*

ARTICLE INFO

Article history:
Received 12 October 2010
Accepted 20 December 2010

ABSTRACT

Luteinizing hormone (LH) concentrations and pulsatility are increased in obese women with polycystic ovary syndrome (PCOS). In addition, patients have hyperandrogenemia and insulin resistance. The mechanisms involved in aberrant hormone regulation in PCOS are still unclear. We investigated 15 obese PCOS women with a body mass index between 30 and 54 kg/m^2 and 9 healthy obese controls (body mass index, 31-60 kg/m^2) with regular menstrual cycles. Subjects underwent 24-hour blood sampling at 10-minute intervals for later measurements of LH, leptin, testosterone, and insulin concentrations. Data were analyzed with a new deconvolution program, approximate entropy (and bivariate approximate entropy), and a cross-correlation network. Patients had increased LH pulse frequency and more than 2-fold greater daily LH secretion, with diminished pattern regularity. Testosterone secretion was increased 2-fold, but pattern regularity was similar to that in controls. In the network construct, insulin was correlated positively with LH, whereas leptin and testosterone were correlated negatively with LH. Bivariate synchrony of LH with insulin was decreased. Short-term caloric restriction paradoxically increased LH secretion by 1.5-fold and pattern irregularity, and reduced interpulse variability. Testosterone secretion and fasting concentrations of estradiol and sex hormone–binding globulin levels remained unchanged. Correlations between LH and insulin, leptin, and calculated free testosterone decreased. This study demonstrates marked alterations in the control of LH secretion in PCOS in the fed and calorie-restricted states. The ensemble results point to abnormal feedback control of not only the GnRH-gonadotrope complex, but also LH's relationships with leptin, insulin, and testosterone.

1. Introduction

The polycystic ovary syndrome (PCOS), a common endocrine disorder affecting at least 4% to 8% of premenopausal women, is characterized by anovulation or oligo-ovulation, hyperandrogenism, and polycystic ovaries. Other abnormalities frequently associated with this syndrome are obesity, insulin resistance, and the metabolic syndrome [1]. The pathogenesis of PCOS has not been fully unraveled. Hypotheses include the considerations that primary neuroendocrine changes lead to increased luteinizing hormone (LH) pulsatility and ovarian testosterone secretion and that primary ovarian pathology results in excessive androgen production [2]. Another hypothesis is that insulin resistance is the primary abnormality. In this construct,

Contributions by each of the authors: design of the study: HP and FR; data collection: PK; analyses: PK and FR; data interpretation: FR, PK, JDV, and HP; writing and editing: FR, HP. and JDV.

* *Corresponding author*. Fax: +31 715248136
E-mail address: f.roelfsema@lumc.nl (F. Roelfsema).

0026-0495/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2010.12.011

hyperinsulinemia stimulates ovarian androgen production, which disrupts orchestrated gonadotropin secretion, resulting in anovulation and infertility [3]. Indeed, therapeutic interventions aiming at decreasing insulin production by increasing insulin sensitivity, including treatment with metformin and thiazolidinediones, may restore ovulation and fertility [4]. Drastic weight reduction can also restore fertility [5].

Although increased LH secretion in PCOS is well established, earlier investigations used pulse-detection programs, which were not fully validated for use in humans, including the Santen and Barden method, Pulsar, Detect, and Cluster [6-8]. Another drawback is that certain programs do not estimate both hormone secretion rates and half-life simultaneously. Moreover, earlier deconvolution programs relied on interactive operator input; and hence, the final result depended on subjective judgments. A recent new Matlab-based program is fully automated and operator independent, and is validated for estimating hormone LH secretion rates in the human [9,10]. Moreover, relationships among LH, testosterone, insulin, and leptin in PCOS have been difficult to formulate or quantify to date.

Given the foregoing limitations in the field, the present objectives were to reanalyze LH secretion in obese patients with PCOS compared with obese controls and to explore the relationships between LH secretion and other hormones, including insulin, testosterone, and leptin, which all may directly or indirectly modulate LH secretion [8,11-13]. A secondary goal was to investigate the acute effects of food restriction in patients on this network because calorie restriction decreases leptin and insulin concentrations, which could diminish androgen production and LH secretion.

2. Patients and methods

Fifteen obese women with the diagnosis of PCOS were studied. The diagnosis of PCOS was based on the 1990 National Institutes of Health criteria and thus on the presence of compromised fertility due to oligo- or anovulation that was not secondary to a specific underlying disease of the pituitary, ovaries, or adrenal glands and with elevated serum levels of testosterone. The mean age was 29 years (range, 20-39); mean body mass index (BMI) was 39 kg/m^2 (range, 30-54). Nine obese women (mean age, 34 years; range, 20-40 years; mean BMI, 38 kg/m^2; range, 31-60) with regular menstrual cycles comprised the control group. Volunteers were recruited through advertisements in local newspapers. Before participation, all subjects underwent a full medical screening. The purpose, nature, and possible risks of the study were explained to all subjects; and written informed consent was obtained. The study protocol was approved by the ethics committee of the Leiden University Medical Center. Results on LH secretion in the same groups of subjects were reported previously [8]. In the present analysis, we now report on new findings obtained with new analytical tools.

2.1. Study design and protocol

Patients with PCOS were studied on a random day. Ovulation had not occurred recently before admission, as evidenced by progesterone levels measured 20 days before and on the study day. Control women were sampled on days 2 to 5 of the menstrual cycles. Subjects were admitted to the research center at 7:00 AM after an overnight fast, and 24-hour blood sampling started at 9:00 AM. Blood samples were withdrawn at 10-minute intervals. Subjects remained recumbent during the study except for bathroom visits. No daytime naps were allowed. Well-being and vital signs were recorded hourly. Lights were switched off at 11:00 PM and switched on at 7:30 AM. No electroencephalographic sleep registration was performed. A second study in PCOS patients was performed after 1 week of a very low caloric diet (VLCD) (Modifast; Novartis Nutrition Benelux, Breda, the Netherlands), 2 MJ (470 kcal)/d in 3 equal proportions, with macronutrient composition of 43% protein, 15% fat, and 42% carbohydrate. During the second sampling study, the VLCD was served as meals in 3 equal portions (157 kcal each) at 9:00 AM, 2:00 PM, and 7:00 PM. Volunteers were compensated for the time spent in the study.

2.2. Body composition

Body fat was estimated by bioelectrical impedance (Bodystat, Douglas, Isle of Man, United Kingdom).

2.3. Assays

All samples from any given subject (both admissions) were measured in the same assay run. Luteinizing hormone was measured with a sensitive time-resolved fluoroimmunoassay (Wallac, Turku, Finland; interassay coefficient of variation [CV], 5.5%-6.7%). Leptin was determined by radioimmunoassay (RIA) (Linco Research, St Charles, MO; interassay CV, 3.6%-6.8%). Androstenedione (interassay CV, 9.0%), testosterone (detection limit, 0.2 nmol/L; interassay CV, 6.8%), and 17-hydroxyprogesterone (detection limit, 0.1 nmol/L; interassay CV, 2.4%) were measured by coated-tube RIAs; and progesterone (detection limit, 0.1 nmol/L; interassay CV, 6.9%), by solid-phase RIA (Diagnostic Products, Los Angeles, CA). Sex hormone–binding globulin (SHBG) was measured by a coated-tube RIA (Spectria, Espoo, Finland; detection limit, 0.5 nmol/L; interassay CV, 3.0%). Estradiol was determined by RIA (Diagnostic Systems Laboratory, Webster, TX; interassay CV, 5.1%-8.1%). Insulin was measured by immunoradiometric assay (Biosource Europe, Nivelles, Belgium; detection limit, 2 μU/L; interassay CV, 4.4%-5.9%). Basal serum glucose was measured using a fully automated Modular P 800 (Hitachi, Tokyo, Japan).

2.4. Calculations and statistics

2.4.1. Deconvolution analysis

Luteinizing hormone, testosterone, and leptin hormone concentration time series were analyzed using a recently validated deconvolution method [9,10].

2.4.2. Approximate entropy

Approximate entropy (ApEn) was used as a scale- and model-independent regularity statistic to quantify the orderliness or regularity of consecutive hormone concentration measurements over 24 hours. Normalized ApEn parameters of m = 1

(test range) and r = 20% (threshold) of the intraseries SD were used, as described previously [14]. The ApEn metric evaluates the consistency of recurrent subordinate (nonpulsatile) patterns in the data and thus yields information distinct from and complementary to deconvolution (pulse) analyses [15]. Analogous to univariate ApEn, bivariate cross-ApEn is a scale- and model-independent 2-variable regularity statistic used to quantitate the relative pattern synchrony of coupled time series. Clinical experiments establish that changes in cross-ApEn monitor feedback and/or feedforward adaptations within an interlinked axis with high sensitivity and specificity [16].

2.4.3. *Cross-correlation*

Calculation of the correlation between hormone pairs viewed as part of a hormone network used an adaptation of Spearman correlation, which analyzes the whole group of subjects simultaneously. By taking the hyperbolic tangent of a correlation, skewing is alleviated and convergence is enhanced. Fisher Z transformation was applied before averaging the correlation coefficients, and the average was translated back to the correlation space by taking the inverse of the function [17].

2.5. *Statistical analysis*

Data are presented as mean ± SEM, unless otherwise specified. Statistical comparisons were made with the 2-tailed Student *t* test or analysis of variance, where required. Statistical calculations were performed with Systat software, version 11 (Systat Software, San Jose, CA). Significance level was set at .05.

3. Results

Testosterone, androstenedione, and 17-hydroxyprogesterone concentrations were higher in patients than in controls. Furthermore, estradiol concentrations were higher in patients than in controls; but progesterone and SHBG concentrations did not differ. The anthropomorphic measures were similar in patients and controls (Table 1).

Table 1 – Fasting hormone concentrations and anthropomorphic data in 15 obese patients with PCOS and 9 obese healthy controls

	Patients	Controls	P value
Testosterone (nmol/L)	2.6 ± 0.4	0.6 ± 0.1	.0002
Androstenedione (nmol/L)	10.4 ± 1.2	5.1 ± 1.2	.007
17-Hydroxyprogesterone (nmol/L)	4.3 ± 0.5	2.2 ± 0.2	.007
Estradiol (pmol/L)	167 ± 22	98 ± 10	.016
Progesterone (nmol/L)	2.32 ± 0.60	1.45 ± 0.20	.20
SHBG (nmol/L)	22 ± 3	27 ± 3	.23
Waist-hip ratio	0.93 ± 0.02	0.96 ± 0.02	.46
Fat percentage	47 ± 1	49 ± 2	.26

Data are shown as mean ± SEM. Statistical comparisons were made with the 2-tailed Student *t* test for unpaired data.

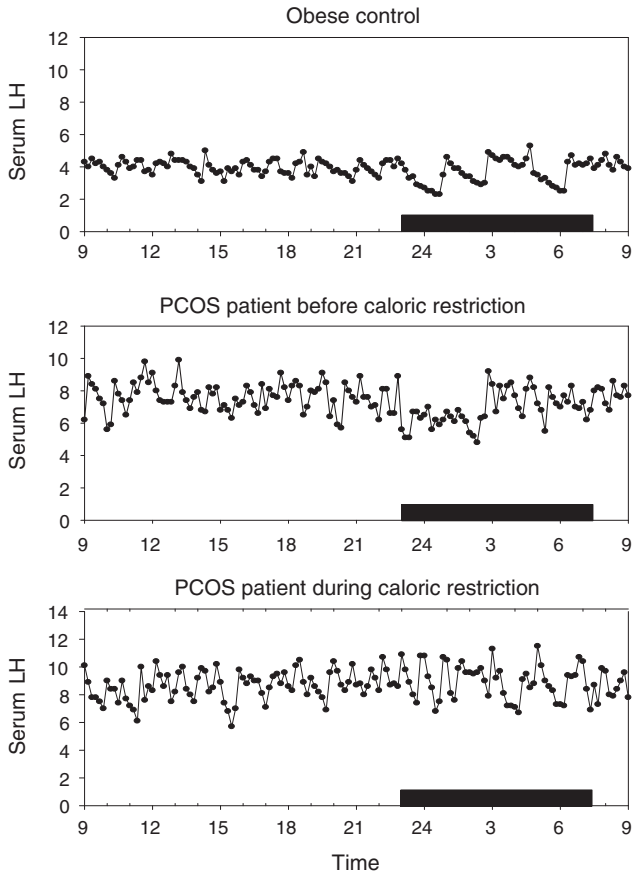

Fig. 1 – Representative serum LH concentration profiles in a healthy obese control woman and a PCOS patient before and during short-term caloric restriction. The black horizontal bar represents the period with lights off.

Representative serum LH profiles are displayed in Fig. 1. The outcomes of LH deconvolution analyses are summarized in Table 2. Patients had increased LH secretory-burst number compared with controls, which was confirmed by the Weibull λ (frequency) parameter. The LH secretion was increased because of 4-fold amplification of basal secretion and almost 2-fold increase of pulsatile release. The slow component of LH disappearance did not differ between patients and controls. However, the mode of LH secretory bursts was larger in patients, indicating a longer delay between pulse onset and maximal secretion rate. A remarkable finding was also the increased regularity of the interpulse intervals in PCOS patients, denoted by higher Weibull γ parameter. The results of deconvolution of serum testosterone profiles are shown in Table 2. Testosterone secretion was enhanced in patients because of 5-fold increased basal release, without changes in burst frequency, hormone half-life, or mode of secretion bursts.

The (sample-to-sample) regularity of LH secretory patterns was decreased (higher ApEn) in patients compared with controls (1.52 ± 0.03 vs 1.35 ± 0.06, P = .02), but no difference was present for testosterone secretion. Cross-ApEn in either direction (forward or reverse) was similar in the groups. However, cross-ApEn in the direction of LH to testosterone

Table 2 – Deconvolution of serum LH and testosterone profiles in 15 PCOS patients and 9 obese controls

LH	PCOS patients	Obese controls	P value
Burst frequency (n/24 h)	24.1 ± 0.8	19.2 ± 1.6	.02
Half-life (min)	104 ± 9	106 ± 9	.75
Mode (min)	13.4 ± 0.5	11.5 ± 2.0	.01
Basal secretion (IU/[L 24 h])	36.3 ± 7.5	8.6 ± 3.4	.01
Pulsatile secretion (IU/[L 24 h])	51.7 ± 6.6	29.5 ± 3.1	.01
Total secretion (IU/[L 24 h])	88 ± 11	38 ± 5	.0004
Mean pulse mass (IU/L)	2.10 ± 0.24	1.59 ± 0.18	.20
Weibull λ (n/24 h)	21.6 ± 0.7	16.8 ± 1.3	.01
Weibull γ	3.03 ± 0.69	2.20 ± 0.11	.0001
Testosterone			
Burst frequency (n/24 h)	15.2 ± 1.0	16.1 ± 2.1	.97
Half-life (min)	44.8 ± 2.8	44.3 ± 4.2	.84
Mode (min)	31.0 ± 6.5	19.1 ± 4.1	.37
Basal secretion (nmol/[L 24 h])	34.3 ± 4.9	6.1 ± 2.1	.005
Pulsatile secretion (nmol/[L 24 h])	11.2 ± 2.3	15.8 ± 5.7	.59
Total secretion (nmol/[L 24 h])	45.5 ± 6.2	22.0 ± 5.1	.005
Mean pulse mass (nmol/L)	0.73 ± 0.13	1.01 ± 0.30	.57
Weibull λ (n/24 h)	13.9 ± 0.9	16.8 ± 1.3	.94
Weibull γ	2.88 ± 0.19	2.20 ± 0.11	.51

Data are shown as mean and SEM. Statistical comparisons were made with the Student t test for unpaired data after logarithmic transformation.

was smaller than in the reverse direction in both groups (both $P < .0001$), suggesting a tighter coupling of the hormone ensemble in the feedforward than feedback direction.

Control subjects showed the characteristic slowing of LH pulse frequency rate during the sleep period (Fig. 2); and patients also displayed this phenomenon, although with a higher pulse frequency rate than controls, both during the dark period and period with lights on.

The influence of restricting calorie intake for 1 week was investigated in patients under identical conditions. Total LH secretion increased from 88 ± 11 to 130 ± 14 IU/(L 24 h) ($P = .008$) caused by amplification of both basal and pulsatile secretion, without a change in burst frequency (Fig. 3). Other changes included a decrease of LH half-life from 104 ± 9 to 78 ± 7 minutes ($P = .03$) and a further increase in regularity of the LH interpulse intervals ($P = .009$). The nocturnal decrease in pulse frequency rate remained unchanged (Fig. 2). Estradiol and SHBG concentrations did not change (data not shown). Approximate entropy of LH secretion increased further from 1.526 ± 0.032 to 1.635 ± 0.038 ($P = .02$). In contrast, secretion of total testosterone remained unchanged under calorie restriction (Fig. 3). Approximate entropy for testosterone was similar before and during calorie restriction (1.169 ± 0.045 vs 1.164 ± 0.075, $P = .90$). Comparable findings were found for the bidirectional cross-ApEn between LH and testosterone (data not shown). The directional difference in cross-ApEn remained highly significant ($P = .0001$). Consistent with caloric restriction, mean 24-hour insulin concentrations decreased from 601 ± 101 to 144 ± 17 pmol/L ($P < .001$); and estimated leptin secretion decreased from 520 ± 55 to 305 ± 50 μg/(L 24 h) $P < .0001$). Mean weight loss was 3 kg.

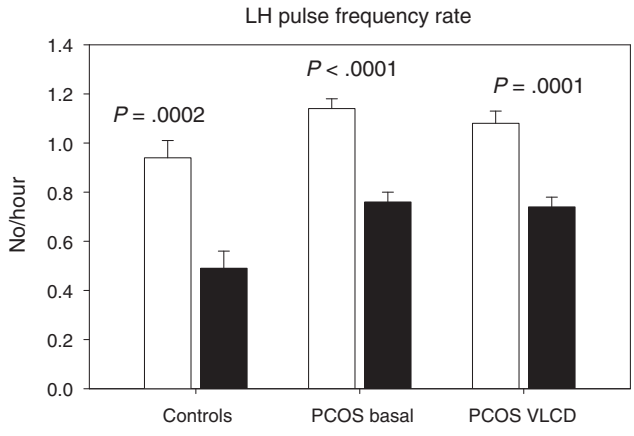

Fig. 2 – Luteinizing hormone pulse frequency rates during the period with lights on and off. Pulse frequency is expressed as number of pulses per hour. The black bars represent the dark period (from 11:00 PM to 7:30 AM); and the open bars, the light period. Error bars represent SEM. Statistical comparisons were made with the Student 2-tailed t test.

Interrelationships among hormone systems, which could potentially regulate LH secretion, were sought for leptin, insulin, and free testosterone index. Fig. 4 summarizes the dynamic differences in magnitude and direction of cross-correlations between hormone concentration series, for example, insulin, leptin, and testosterone as leading systems and LH as secondary, in patients before and during VLCD and in obese controls. Individual hormone pairs were clustered in particular quadrants. The distance between each point and the horizontal zero-axis reflects the strength of the correlation; and that for the vertical zero-axis, the time delay. Ellipses for the 95% confidence intervals are shown for each group's mean correlation coefficient. Thus, all 3 insulin-LH correlation groups (control fed, PCOS fed, PCOS food restricted) were significant, the leptin-LH correlation was significant only in controls; and the LH-free testosterone correlation was significant in fed controls and PCOS. Moreover, during food restriction, the strength of the correlations (positive or negative) diminished in absolute magnitude.

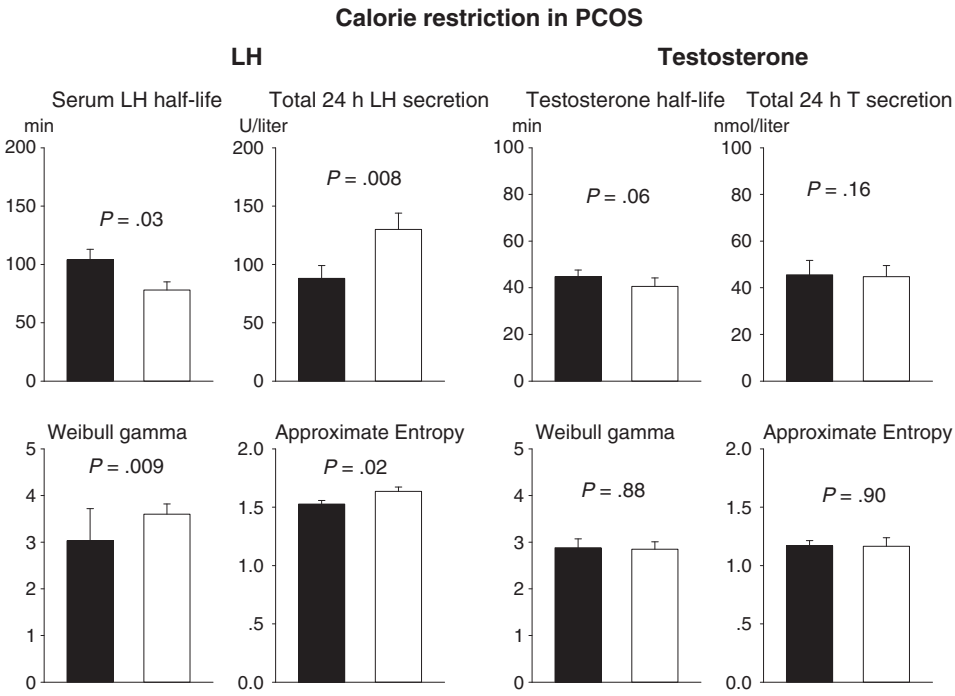

Fig. 3 – Effects of calorie restriction on LH and testosterone secretion in PCOS patients. Black bars represent the basal study, and the open bars represent the second study under calorie restriction. Error bars represent SEM. Statistical comparisons were made with the Student 2-tailed *t* test.

4. Discussion

This study confirms that pulsatile 24-hour LH secretion in obese PCOS women is increased compared with that found in obese women with normal menstrual cycles. New findings include unchanged hormone half-life, elevated basal LH secretion, prolonged LH secretory bursts, and increased regularity of the LH interpulse intervals. Testosterone secretion was elevated mainly because of increased basal (nonpulsatile) secretion. Caloric deprivation in patients further increased LH secretion and the regularity of the interpulse intervals, diminished pattern regularity, and left pulse frequency unchanged. Testosterone secretion during food restriction was unaltered. The correlation-network construct in PCOS patients showed the largest absolute correlations between LH and insulin (positive), LH and leptin (negative), and LH and testosterone (negative), which all diminished absolutely during food restriction. Joint synchrony was decreased in patients compared with controls for LH and insulin pairs.

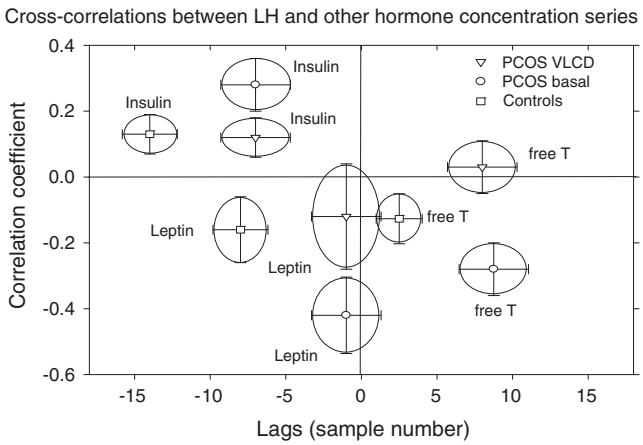

Fig. 4 – Diagram of the correlations and lag periods (sample number) between free testosterone, insulin, and leptin (leading) and LH concentration profiles in PCOS patients before and during caloric restriction and healthy obese controls. Data are shown as the mean ± 1.96 SD (95%) in both directions. Confidence intervals crossing the horizontal zero-line are not significant.

The present operator-independent deconvolution technique confirmed increased LH burst frequency in PCOS patients. Novel findings were the increased duration (mode) of LH secretory bursts and the greater regularity of interpulse intervals in patients compared with obese controls, as denoted by the dimensionless Weibull γ parameter. More prolonged LH release during a burst suggests more sustained GnRH-LH coupling in PCOS. Enhanced pulse regularity and increased pulse frequency could reflect either a primary or secondary change in the hypothalamic GnRH pacemaker. These changes were also present in lean adolescents with PCOS [7,18]. However, PCOS patients retain the phenomenon of LH pulse slowing during sleep [19]. In other investigations, the euglycemic-hyperinsulinemic clamp increased LH pulse frequency in controls, but not in PCOS patients [11]. Treatment with drugs aimed at improving insulin sensitivity has sometimes reduced LH secretion, but has never normalized LH pulse frequency [20,21]. Interestingly, treatment with

gonadotropin-releasing hormone or synthetic agonists decreases LH secretion and LH pulse frequency so long as treatment is continued [22]. Progesterone and estradiol (temporarily) diminish LH amplitude and frequency in lean and obese PCOS patients, although sensitivity to these steroids appears to be reduced [23]. In contrast, testosterone administration paradoxically increased LH secretion in nonobese adolescents with PCOS [24]. Because BMI is negatively correlated with LH pulse mass but unrelated to LH pulse frequency [25], collectively, these observations suggest that the GnRH pulse generator is under heightened drive and/or attenuated feedback.

Insulin resistance occurs frequently in lean and obese patients with PCOS, raising the question of whether this hormone might play a pathogenetic role in the syndrome. Cultured rat pituitary cells exposed to insulin exhibit increased basal and GnRH-stimulated LH release in a dose-dependent way [26]. However, the euglycemic-hyperinsulinemic clamp failed to influence LH pulse frequency or amplitude in PCOS patients [27]. The same was true for treatment with pioglitazone for 20 weeks [28].

Under in vitro conditions, insulin and IGF-I potentiate LH-stimulated androgen secretion by theca cells [2]. Troglitazone dose-dependently antagonizes stimulation of androstenedione and testosterone during combined LH/insulin stimulation in vitro [29]. In the present study, estimated testosterone production was increased 2-fold because of amplification of the nonpulsatile component. A possible explanation is that insulin and IGF-I preferentially stimulate nonpulsatile secretion. Thus, one might anticipate not finding irregular testosterone secretion (increased ApEn) in PCOS patients, which was found as predicted.

Hormone systems mutually influence one another. Available studies have assessed 2 or at most 3 interacting systems, namely, LH-androgens or estrogens, ACTH and cortisol, or CRH-ACTH-cortisol in the conscious horse [16]. Here, a simplified linear correlation network was used to evaluate how several hormonal systems are related to the regulation of LH secretion in PCOS. The hormone systems connected to LH were insulin (positive) and leptin and testosterone (negative), whose correlations decreased during food restriction. Nevertheless, a direct role for insulin in the regulation of LH secretion is not established [11,26-30]. In contrast, leptin deficiency–associated hypogonadism and hypothalamic amenorrhea are restored by leptin administration [31,32]. The negative interaction of (free) testosterone and LH is consistent with detectable feedback.

Weight reduction in obese women with PCOS can restore normal ovulatory menstrual cycles [30,31]. Although insulin and leptin decreased considerably in the present study, basal and pulsatile LH secretion further increased (paradoxically) with unchanged burst frequency, demonstrating the fixed LH frequency in this disorder. Interestingly, the regularity of the LH pulses also increased, as denoted by the Weibull γ parameter, whereas testosterone secretion was unchanged. The basis for altered LH outflow is not yet clear. Other hormone systems reacted as expected in nonstressed subjects during calorie deprivation; that is, leptin secretion decreased by 42%, and mean insulin concentrations decreased by 60%. In contrast, similar calorie deprivation in healthy obese subjects did not impact LH pulse frequency or amount secreted [33]; but complete 3-day fasting in normal-weight women diminished LH pulse frequency, which was prevented by leptin administration [34]. In PCOS, an inferred decrease in serum half-life of LH could suggest a lesser degree of sulfation and sialylation of this hormone [35].

Multisite monitoring of hormone systems in the human is not possible, but indirect methods can be used to appraise feedback control, namely, ApEn and an analogous bivariate statistic, cross-ApEn [36]. The latter metric quantitates pattern synchrony of paired hormone time series. Patients with PCOS had irregular LH secretion as well as decreased pattern synchrony between LH and insulin, but not leptin and testosterone. One earlier study reported disruption of synchrony between LH and androstenedione or testosterone in young nonobese patients with PCOS [12]. Irregular LH secretion in obese PCOS patients was not normalized by food restriction, nor did cross-ApEn values change.

In summary, in obese women with PCOS, LH secretion exhibits decreased pattern regularity, disrupted synchrony, changed nodal strength between LH and insulin and leptin, and enhanced pulsing frequency and regularity. Under caloric restriction, some of the abnormalities paradoxically increased (pattern irregularity, LH secretion, pulsing regularity), whereas others decreased (nodal network strength) or remained unchanged (bivariate pattern synchrony). These observations define more complex multisystem disturbances in PCOS.

REFERENCES

[1] Ehrmann DA. Polycystic ovary syndrome. N Engl J Med 2005;352:1223–36.
[2] Gilling-Smith C, Story H, Rogers S, et al. Evidence for a primary abnormality of theca cell steroidogenesis in the polycystic ovary syndrome. Clin Endocrinol 1997;47:93–9.
[3] Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. Endocr Rev 1997;18:774–800.
[4] Dunaif A, Scott D, Finegood D, et al. The insulin-sensitizing agent troglitazone improves metabolic and reproductive abnormalities in the polycystic ovary syndrome. J Clin Endocrinol Metab 1996;81:3299–306.
[5] Pasquali R, Antenucci D, Casimirri F, et al. Clinical and hormonal characteristics of obese amenorrheic hyperandrogenic women before and after weight loss. J Clin Endocrinol Metab 1989;68:173–9.
[6] Yen SSC, Vela P, Rankin J. Inappropriate secretion of follicle-stimulating hormone and luteinizing hormone in polycystic ovarian disease. J Clin Endocrinol Metab 1970;30: 435–41.
[7] Garcia-Rudaz MC, Ropelato MG, Escobar ME, et al. Augmented frequency and mass of LH discharged per burst are accompanied by marked disorderliness of LH secretion in adolescents with polycystic ovary syndrome. Eur J Endocrinol 1998;139:621–30.
[8] van Dam EWCM, Roelfsema F, Veldhuis JD, et al. Increase in daily LH secretion in response to short-term calorie restriction in obese women with PCOS. Am J Physiol Endocrinol Metab 2002;282:E865–72.
[9] Keenan DM, Roelfsema F, Biermasz N, et al. Physiological control of pituitary hormone secretory-burst mass, frequency, and waveform: a statistical formulation and analysis. Am J Physiol 2003;285:R664–73.

[10] Liu PY, Keenan DM, Kok P, et al. Sensitivity and specificity of pulse detection using a new deconvolution method. Am J Physiol 2009;297:E538–44.
[11] Moret M, Stettler R, Rodieux F, et al. Insulin modulation of luteinizing hormone secretion in normal female volunteers and lean polycystic ovary syndrome patients. Neuroendocrinol 2009;89:131–9.
[12] Veldhuis JD, Pincus SM, Garcia-Rudaz MC, et al. Disruption of joint synchrony of luteinizing hormone, testosterone, and androstenedione secretion in adolescents with polycystic ovary syndrome. J Clin Endocrinol Metab 2001;86:72–9.
[13] Licinio J, Negrao AB, Mantzoros C, et al. Synchronicity of frequently sampled, 24-h concentrations of circulating leptin, luteinizing hormone, and estradiol in healthy women. Proc Natl Acad Sci USA 1998;95:2541–6.
[14] Pincus SM. Irregularity and asynchrony in biologic network signals. Meth Enzymol 2000;321:149–82.
[15] Veldhuis JD, Straume M, Iranmanesh A, et al. Secretory process regularity monitors neuroendocrine feedback and feedforward signaling strength in humans. Am J Physiol 2001;280:R721–9.
[16] Veldhuis JD, Keenan DM, Pincus SM. Motivation and methods for analyzing pulsatile hormone secretion. Endocr Rev 2008;29:823–64.
[17] Vis DJ. Dynamic endocrine networks, revealing dynamic differences between health and disease. Abstract. Metabolomics Society 4th Annual Conference, Boston, MA; 2008.
[18] Venturoli S, Porcu E, Fabbri R, et al. Episodic pulsatile secretion of FSH, LH, prolactin, oestradiol, oestrone, and LH circadian variations in polycystic ovary syndrome. Clin Endocrinol 1988;28:93–107.
[19] Rossmanith WG. The impact of sleep on gonadotropin secretion. Gynecol Endocrinol 1998;12:381–9.
[20] Genazzani AD, Battaglia C, Malavasi B, et al. Metformin administration modulates and restores luteinizing hormone spontaneous episodic secretion and ovarian function in nonobese patients with polycystic ovary syndrome. Fertil Steril 2004;81:114–9.
[21] Glintborg D, Hermann AP, Andersen M, et al. Effect of pioglitazone on glucose metabolism and luteinizing hormone secretion in women with polycystic ovary syndrome. Fertil Steril 2006;86:385–97.
[22] Graňa-Barcia M, Liz-Leston J, Lado-Abeal J. Subcutaneous administration of pulsatile gonadotropin-releasing hormone decreases serum follicle-stimulating hormone and luteinizing hormone levels in women with polycystic ovary syndrome: a preliminary study. Fertil Steril 2005;83:1466–72.
[23] Pastor CL, Griffin-Korf ML, Aloi JA, et al. Polycystic ovary syndrome: evidence for reduced sensitivity of the gonadotropin-releasing hormone pulse generator to inhibition by estradiol and progesterone. J Clin Endocrinol Metab 2008;83:582–90.
[24] Ropelato MG, Garcia Rudaz MC, Escobar ME. Acute effects of testosterone infusion on the serum luteinizing hormone profile in eumenorrheic and polycystic ovary syndrome adolescents. J Clin Endocrinol Metab 2009;94: 3602–10.
[25] Arroyo A, Laughlin GA, Morales AJ. Inappropriate gonadotropin secretion in polycystic ovary syndrome: influence of adiposity. J Clin Endocrinol Metab 1997;82: 3728–33.
[26] Xia YX, Weiss JM, Polack S, et al. Interactions of insulin-like growth factor, insulin and estradiol with GnRH-stimulated luteinizing hormone release from female rat gonadotrophs. Eur J Endocrinol 2001;144:73–9.
[27] Patel K, Coffler MS, Dahan MH, et al. Increased luteinizing hormone secretion in women with polycystic ovary syndrome is unaltered by prolonged insulin infusion. J Clin Endocrinol Metab 2003;88:5456–61.
[28] Mehta RV, Patel KS, Coffler MS, et al. Luteinizing hormone secretion is not influenced by insulin infusion in women with polycystic ovary syndrome despite improved insulin sensitivity during pioglitazone treatment. J Clin Endocrinol Metab 2005;90:2136–41.
[29] Veldhuis JD, Zhang G, Garmey JC. Troglitazone, an insulin-sensitizing thiazolidinediones, represses combined stimulation by LH and insulin of de novo androgen biosynthesis by theca cells in vitro. J Clin Endocrinol Metab 2002;87:1129–33.
[30] Lawson MA, Jain S, Sun S, et al. Evidence for insulin suppression of baseline luteinizing hormone in women with polycystic ovary syndrome and normal women. J Clin Endocrinol Metab 2008;93:2089–96.
[31] Oral EA, Ruiz E, Andewelt A, et al. Effect of leptin replacement on pituitary hormone regulation in patients with severe lipodystrophy. J Clin Endocrinol Metab 2002;87:3110–7.
[32] Welt CK, Chan JL, Bullen J, et al. Recombinant human leptin in women with hypothalamic amenorrhea. N Engl J Med 2004;351:987–97.
[33] Drezgic M, Penezic Z, Zarkovic M, et al. Influence of three-week fasting on gonadotropin pulsatility in obese menstruating women. Intl J Obes Rel Metab Disord 1996;20: 608–12.
[34] Chan JL, Matarese G, Shetty GK, et al. Differential regulation of metabolic, neuroendocrine, and immune function by leptin in humans. Proc Natl Acad Sci U S A 2006;103:8481–6.
[35] Ropelato MG, Garcia-Rudaz MC, Castro-Fernandez C, et al. A preponderance of basic luteinizing hormone (LH) isoforms accompanies inappropriate hypersecretion of both basal and pulsatile LH in adolescents with polycystic ovary syndrome. J Clin Endocrinol Metab 1999;84:4629–36.
[36] Pincus SM, Mulligan T, Iranmanesh A. Older males secrete luteinizing hormone and testosterone more irregularly, and jointly more asynchronously, than younger males. Proc Natl Acad Sci USA 1996;93:14100–5.