Altered Morning and Nighttime Pulsatile Corticotropin and Cortisol Release in Polycystic Ovary Syndrome

Cecilia Invitti, Martina De Martin, Giuseppe Delitala, Johannes D. Veldhuis, and Francesco Cavagnini

An overdrive of the hypothalamic-pituitary-adrenal (HPA) axis has been postulated in patients with polycystic ovary syndrome (PCOS). However, little is known concerning the pulsatile modes of corticotropin (ACTH) and cortisol secretion in these patients. To further investigate this issue, spontaneous ACTH and cortisol release were evaluated in 16 normal-weight patients with PCOS and 16 control women. Nine PCOS patients and eight controls were studied between 8 AM and 12 AM (noon), and seven PCOS patients and eight controls between 11 PM and 3 AM. Venous blood samples were taken at 10-minute intervals. Cluster analysis was used to assess ACTH and cortisol pulse frequency and amplitude, deconvolution to calculate mean hormone secretion rates, and approximative entropy (ApEn) to measure the orderliness of ACTH and cortisol time-series data. PCOS patients compared with controls displayed increased ACTH and cortisol release (area under the curve [AUC] and mean plasma concentration) both in the morning and at night. This was not due to increased hormonal secretory burst frequency, but to higher hormonal interpeak valley concentrations and, in the case of ACTH, nighttime pulse amplitudes. Mean ACTH and cortisol secretion rates also were increased in PCOS patients. Further, both controls and PCOS patients exhibited significant (0 to 20 minutes lagged) concordance between individual daytime pulsatile ACTH and cortisol release episodes. As shown by increased ApEn values, PCOS patients had more disorderly daytime cortisol release. In addition, the normal daytime correlation between the amount of pulsatile ACTH and cortisol release as observed in the controls was lost in PCOS patients. Finally, cross-correlation analysis showed a more prominent negative correlation in PCOS patients versus controls between plasma cortisol and 40- to 120-minute delayed ACTH concentrations in the morning, indicating a more sustained negative feedback of cortisol on ACTH release in PCOS at this time. Taken together, these findings demonstrate the existence of multifaceted dysregulation of the HPA axis in PCOS.

POLYCYSTIC OVARY SYNDROME (PCOS) is a common disorder with a pathogenesis that likely involves a number of factors acting in a still undefined manner. Excessive androgen production is common in this syndrome. The ovary is generally considered the principal source of these steroids, but many patients with PCOS also have increased adrenal androgen secretion, and in more than half of these patients, androgens are hyperresponsive to direct (corticotropin [ACTH]) or indirect (ACTH-releasing hormone [CRH], metyrapone) stimulation of the adrenal cortex.[1-3] Increased urinary excretion of cortisol metabolites has also been reported in PCOS patients.[4-6] This alteration has been attributed to enhanced cortisol metabolism, followed by a compensatory overdrive of the hypothalamic-pituitary-adrenal (HPA) axis and hence increased androgen production. However, in many patients with PCOS, urinary free cortisol (UFC)[4,7] and plasma cortisol[4,8-11] concentrations are elevated, the cortisol response to ACTH is increased,[1] and HPA axis sensitivity to dexamethasone suppression is blunted,[12] raising the possibility of a primary activation of the HPA axis. Given the paucity of such studies and their controversial nature to date,[11,13] we further investigated the functional status of the HPA axis in patients with PCOS via a more detailed analysis of spontaneous ACTH and cortisol release.

SUBJECTS AND METHODS

Sixteen normal-weight women (body mass index [BMI], 20 to 24 kg/m^2) aged 17 to 35 years presenting with oligomenorrhea/amenorrhea, hirsutism, elevated serum levels of one or more androgens, a serum luteinizing hormone to follicle-stimulating hormone ratio greater than 1, and typical ovarian morphology of PCOS by ultrasound examination (multiple cysts at the periphery or scattered throughout the stroma and an increased volume of the latter in facultatively enlarged ovaries) and 16 normally menstruating, nonhirsute control women (BMI, 18 to 20 kg/m^2) aged 24 to 32 years were investigated. Patients of normal weight were selected to avoid the functional changes of the HPA axis described in obesity. Congenital adrenal hyperplasia was excluded in all cases. Mean 24-hour UFC concentrations were greater, although not to a statistically significant extent, in patients with PCOS than in control women (58.5 ± 7.30 *v* 38.9 ± 6.41 μg/24 h, nonsignificant [NS]). All women provided informed consent to participate in the study, the experimental nature of which had been explained and which was approved by the ethics committee of our institution.

Control and, whenever possible, PCOS women were studied during the early follicular phase of the menstrual cycle. In nine patients with PCOS and eight control women, blood samples were taken at 10-minute intervals between 8 AM and 12 AM (noon); seven additional patients with PCOS and eight control women underwent the same study protocol between 11 PM and 3 AM. The morning and nocturnal studies were performed after an overnight fast and 4 hours after the last meal, respectively. One hour before sampling, an antecubital vein was cannulated and kept open by a slow saline drip. The women remained in bed throughout the sampling study. Blood samples for ACTH and cortisol assay were collected into prechilled glass tubes containing EDTA-Trasylol and immediately centrifuged at 4°C, and the plasma was stored at −20°C until being assayed.

The plasma ACTH level was measured by a two-site immunoradiometric assay (Allegro; Nichols Institute, San Juan Capistrano, CA). The detection limit of the assay is 0.22 pmol/L; intraassay and interassay coefficients of variation for plasma ACTH concentrations of 1.3, 3.3, and 6.2 pmol/L were 6.4% (90% confidence interval [CI], 3.7% to 9.1%), 5.4% (CI, 3.1% to 7.7%), and 5.7% (CI, 3.3% to 8.1%) and 9.8% (CI, 5.6% to 13.9%), 14.2% (CI, 8.2% to 20.2%), and 8.6% (CI, 4.9% to 12.2%), respectively.

The plasma cortisol level was measured by radioimmunoassay (DPC,

From the Second Chair of Endocrinology, University of Milan, Istituto Scientifico Ospedale San Luca, Milan; Department of Medicine, University of Sassari, Sassari, Italy; and Department of Internal Medicine, University of Virginia, Charlottesville, VA.

Submitted December 31, 1996; accepted August 6, 1997.

Address reprint requests to Francesco Cavagnini, MD, Istituto Scientifico Ospedale San Luca, Via Spagnoletto, 3 20149 Milano, Italy.

0026-0495/98/4702-0004$03.00/0

Los Angeles, CA). The detection limit of the assay is 5.5 nmol/L; intraassay and interassay coefficients of variation for plasma cortisol levels of 83, 186, and 404 nmol/L were 7.0% (CI, 4.1% to 9.9%) and 10.7% (CI, 6.1% to 15.2%), 2.1% (CI, 1.2% to 2.9%) and 14.6% (CI, 8.4% to 20.8%), and 3.9% (CI, 2.2% to 5.5%) and 4.6% (CI, 2.6% to 6.5%), respectively. The cross-reactivity of the cortisol antibody with cortisone and 11-deoxycorticosterone is less than 1%.

The Cluster analysis program, which serially scans data series for clusters of significantly increased or decreased hormone values,[14] was used to identify ACTH and cortisol pulses and to quantify pulse frequency and amplitude. Significant pulsatile events were detected by a moving 2 × 2 (test nadir and peak sample numbers) cluster configuration for ACTH and a 2 × 1 cluster for cortisol, with t statistics of 2.0 and 2.0 for significant upstrokes and downstrokes to limit false-positive rates to less than 5%. Values for the areas under the curve (AUCs) were calculated using the trapezoidal rule. Basal nonpulsatile hormone concentrations were calculated by subtracting the sum of the burst areas from the total area of hormone release. This latter value is defined as the increase in hormone concentration associated with a secretory peak. A waveform-independent deconvolution program (Pulse) was used to calculate mean hormone secretion rates allowing for variably admixed basal and pulsatile hormone secretion and a nominal half-life of 85 minutes for cortisol and 14 minutes for ACTH.[15] Secretion rates denote the calculated release of ACTH and cortisol mass per unit of distribution volume per unit of time.

Concordance between ACTH and cortisol pulses was determined by counting the computer-identified pulses of cortisol occurring simultaneously with and within the 10 or 20 minutes immediately preceding or following an ACTH pulse and then comparing this coincidence with expected random peak associations via the hypergeometric probability distribution, as previously described.[16] The significance of simultaneous or time-delayed coordinate variation in ACTH and cortisol concentrations was established by cross-correlation analysis. The cross-correlation coefficient, r_k, measures the correlation between two values at a distance (lag) k time units apart.[16] Approximative entropy (ApEn), which represents a relative, model-free, and scale-invariant measure of process regularity,[17] was used to assess ACTH and cortisol time-series data. Because of the brevity of the data series, only ApEn1 was calculated.

Correlations between ACTH and cortisol pulse characteristics were established by the Spearman rank test.

Diurnal variations in plasma ACTH and cortisol concentrations were evaluated by comparing mean hormonal values recorded in the daytime and nighttime hours. The Mann-Whitney test was used to assess differences between PCOS and control parameters of ACTH/cortisol release, diurnal variation, and ApEn. A P value less than .05 was considered statistically significant.

RESULTS

Mean 4-hour plasma ACTH and cortisol profiles for PCOS patients and control women are illustrated in Fig 1. During both daytime and nighttime sampling, ACTH and cortisol concentrations evaluated either by Cluster analysis or by comparison of single time points were higher in patients with PCOS than in controls. Mean 4-hour ACTH and cortisol AUC and interpeak valley (and nadir) concentrations were increased in PCOS patients compared with controls during the diurnal and nocturnal periods (Tables 1 and 2). When basal and pulsatile concentrations of the two hormones were analyzed separately, it appeared that the increase in mean ACTH and cortisol concentrations, as well as their AUC values, in PCOS patients was chiefly the result of an increase in interpulse hormone concentrations. A positive correlation between the amount of pulsatile ACTH and cortisol release was present in control women during the day ($r = .82$, $P < .05$) and the night ($r = .92$, $P < .005$); in patients with PCOS, this pattern was maintained in the nocturnal period ($r = .91$, $P < .005$), but not in the daytime ($r = .54$, NS). No correlations were found between the amounts of nonpulsatile hormonal release in either group of women. In the daytime, but not in the nocturnal period, PCOS patients showed a significantly lower mean ACTH secretory burst frequency compared with that defined in controls. The number of cortisol release episodes did not differ between the two groups. The mean duration of ACTH and cortisol pulses was not significantly different in PCOS patients compared with

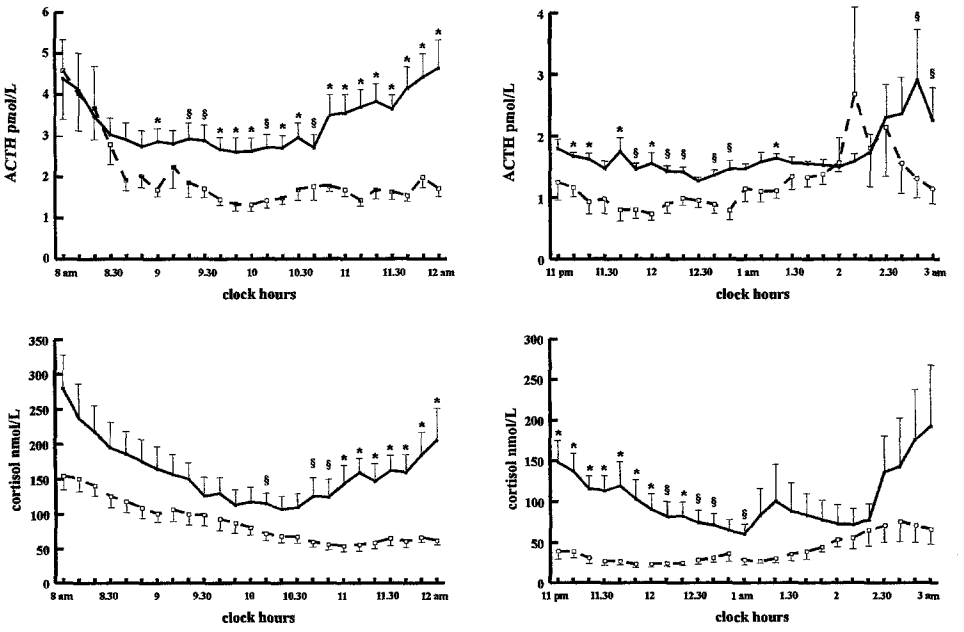

Fig 1. Four-hour profiles of plasma ACTH and cortisol concentrations in samples collected at 10-minute intervals during the day (left) and night (right) in PCOS (■—■) and control (□---□) women. Data are the mean ± SEM for PCOS patients (n = 9 or 7) and controls (n = 8). §$P < .05$, *$P < .01$.

Table 1. Cluster Analysis of ACTH Release in Patients With PCOS and Control Women

Parameter	ACTH Release			
	8 AM-12 AM (noon)		11 PM-3 AM	
	PCOS	Control	PCOS	Control
4-h mean concentration (pmol/L)	3.3 ± 0.36†§	2.0 ± 0.17§	1.7 ± 0.09*	1.2 ± 0.14
AUC (pmol/L · min)	760 ± 85.3*§	472 ± 40.6‡	399 ± 19.7*	295 ± 35.1
Peak frequency (n/240 min)	1.8 ± 0.22†§	3.1 ± 0.30	3.1 ± 0.26	3.1 ± 0.22
Interpeak interval (min)	87.1 ± 10.85	79.4 ± 8.04	65.0 ± 7.48	60.6 ± 5.55
Basal AUC (pmol/L · min)	597 ± 71.4†‡	317 ± 29.7§	331 ± 9.2†	163 ± 19.5
Pulsatile AUC (pmol/L · min)	147 ± 36.3‡	145 ± 16.3	59.9 ± 18.12*	126 ± 23.9
Maximal peak amplitude (pmol/L)	3.9 ± 0.63‡	2.7 ± 0.47§	2.0 ± 0.10†	1.3 ± 0.15
Incremental height (pmol/L)	1.4 ± 0.40‡	1.3 ± 0.40	0.56 ± 0.08	0.60 ± 0.12
Pulse area (pmol/L · min)	43.6 ± 15.27‡	28.2 ± 7.04	10.2 ± 3.14	20.9 ± 5.55
Valley mean (pmol/L)	2.7 ± 0.35‡	1.5 ± 0.11§	1.6 ± 0.04†	0.8 ± 0.08

NOTE. Data are the mean ± SE.
*$P < .05$, †$P < .01$: *v* control women.
‡$P < .05$, §$P < .01$: *v* nocturnal value.

controls, whereas the mean pulse amplitude appeared higher in women with PCOS, although statistical significance was attained only for ACTH nocturnal values (Tables 1 and 2). Also, the mean ACTH, but not cortisol, maximal peak amplitude underwent a nighttime reduction in both PCOS and control women.

Mean ACTH secretion rates were significantly higher in PCOS compared with control women both during the day (0.15 ± 0.01 *v* 0.09 ± 0.01 pmol/L/min, $P < .01$) and at night (0.08 ± 0.004 *v* 0.05 ± 0.01 pmol/L/min, $P < .05$). However, mean cortisol secretion rates displayed a similar trend without reaching statistical significance, due to larger variability within the PCOS group (Table 3).

Significant (approximately twofold above chance expectations) ACTH and cortisol peak concordance existed in both control and PCOS women. This nonrandom pulse coincidence was observed both in the morning and at night, when cortisol peaks were considered simultaneously with and 10 or 20 minutes after (lagged) ACTH peaks. In contrast, compared with purely random associations, there was a twofold reduction in the number of observed coincidences between cortisol peaks and 20-minute delayed ACTH peaks in both study groups. This indicates that cortisol pulses in both controls and PCOS patients can significantly suppress the emergence of ACTH peaks 20 minutes later.

By cross-correlation analysis, we observed a significant positive correlation between plasma ACTH and cortisol concentrations at various positive lags in the two series of both controls and patients with PCOS (Fig 2). Specifically, increased plasma ACTH concentrations preceded increases in cortisol by up to 40 to 60 minutes in both study groups, denoting the feedforward drive of ACTH on cortisol. Moreover, at negative lags of 40 to 120 minutes increases in cortisol correlated with later suppression of ACTH, denoting negative feedback (negative *r* values). The negative correlations were more prominent in PCOS patients versus controls in the morning.

Unlike controls, who exhibited a physiological nocturnal decrease of ACTH and cortisol, PCOS patients displayed a reduction only in ACTH levels, while daytime and nighttime cortisol values were not significantly different (Tables 1 and 2).

To measure the serial orderliness of hormone release, ApEn values were calculated for ACTH and cortisol concentrations in the two groups of subjects. Patients with PCOS showed ACTH ApEn values comparable to those of controls (0.77 ± 0.04 *v*

Table 2. Cluster Analysis of Cortisol Release in Patients With PCOS and Control Women

Parameter	Cortisol Release			
	8 AM-12 AM (noon)		11 PM-3 AM	
	PCOS	Control	PCOS	Control
4-h mean concentration (nmol/L)	157 ± 26.2*	88.8 ± 9.52†	102 ± 23.0*	41.1 ± 5.06
AUC (nmol/L · min) × 10^3	36.9 ± 6.25*	21.1 ± 2.31†	23.7 ± 5.45*	9.74 ± 1.21
Peak frequency (n/240 min)	2.06 ± 0.37	2.0 ± 0.16	1.9 ± 0.23	2.5 ± 0.31
Interpeak interval (min)	71.7 ± 15.4	81.2 ± 17.2	78.3 ± 10.8	82.5 ± 12.9
Basal AUC (nmol/L · min) × 10^3	25.6 ± 4.39*	13.4 ± 1.75†	15.7 ± 3.69*	5.67 ± 0.81
Pulsatile AUC (nmol/L · min) × 10^3	11.4 ± 2.24	7.69 ± 1.91	8.05 ± 2.15	4.06 ± 1.17
Maximal peak amplitude (nmol/L)	116 ± 14.7	99.9 ± 18.3	116 ± 42.4	71.8 ± 22.3
Incremental height (nmol/L)	39.2 ± 5.55	29.1 ± 12.6	49.6 ± 26.8	51.9 ± 21.9
Pulse area (nmol/L · min) × 10^3	1.67 ± 0.94	2.61 ± 0.88	1.53 ± 0.92	1.43 ± 0.82
Valley mean (nmol/L)	106 ± 17.1*	59.3 ± 9.0†	61.5 ± 15.2*	24.1 ± 4.20

NOTE. Data are the mean ± SE.
*$P < .05$ *v* control women.
†$P < .01$ *v* nocturnal value.

Table 3. Mean ACTH and Cortisol Secretion Rates in Patients With PCOS and Control Women

	8 AM-12 AM (noon)		11 PM-3 AM	
	PCOS	Control	PCOS	Control
ACTH (pmol/L/min)	0.15 ± 0.01†‡	0.09 ± 0.01§	0.08 ± 0.01*	0.05 ± 0.01
Cortisol (nmol/L/min)	0.86 ± 0.14	0.5 ± 0.12	1.0 ± 0.28	0.5 ± 0.09

NOTE. Data are the mean ± SEM.
*$P < .05$, †$P < .01$: *v* control women.
‡$P < .05$, §$P < .01$: *v* nocturnal value.

0.79 ± 0.05 daytime and 0.79 ± 0.07 *v* 0.76 ± 0.08 nighttime), while the corresponding cortisol ApEn values were significantly higher during the day (0.73 ± 0.05 *v* 0.58 ± 0.02, $P < .05$), indicating greater disorderliness or irregularity of cortisol release in PCOS versus control women. Cortisol ApEn values were comparable in the two groups during the night (0.54 ± 0.08 in PCOS *v* 0.63 ± 0.06 in controls).

DISCUSSION

In our patients with PCOS, circulating hormonal concentrations and calculated secretion rates predict significantly greater ACTH and cortisol release compared with control women both during the day and at night, but especially in the late morning and early night. PCOS patients were typified by an attenuation of the physiologically expected nocturnal decrease in cortisol, a reduced daytime correlation between the calculated amounts of pulsatile ACTH and cortisol release compared with controls, and a greater disorderliness of daytime cortisol release (higher ApEn values). On the whole, these findings favor the existence of a primary activation of the HPA axis in patients with PCOS with altered coordinate-feedback control. The former view is supported by the observation of increased UFC concentrations exceeding the upper limit of the normal range in approximately 50% of such patients.[7] Also in the present series of patients, mean 24-hour UFC excretion was moderately higher compared with that recorded in a group of control women. Luppa et al[4] also reported, in patients with PCOS, slightly elevated UFC levels that, at variance with values recorded in the controls, increased after administration of a gonadotropin-releasing hormone agonist. Likewise, increased plasma cortisol concentrations in patients with PCOS under basal conditions,[8,9,11] as well as in response to stimulation of the HPA axis with CRH,[18] ACTH,[1] and psychological stress,[10] have been reported. On the other hand, others have considered enhancement of the HPA axis to be of a compensatory nature in PCOS based on elevated urinary cortisol metabolites due to increased hepatic 5α-reduction of cortisol[5] and/or increased conversion of cortisol to cortisone by enhanced activity of 11β-hydroxysteroid dehydrogenase.[4,6] The enhanced drive of the HPA axis observed in our patients cannot be certainly accounted for by their body weight, which was slightly higher than that of control women but well within the normal range. Indeed, this alteration has been described only in some patients with frank obesity of the visceral type.[19]

The higher plasma ACTH and cortisol concentrations seen in our patients with PCOS were associated with an increase in interpulse valley hormone concentrations, as well as (for ACTH) maximal peak amplitudes, compared with healthy controls. In PCOS patients, plasma ACTH levels, but not plasma cortisol levels, decreased significantly at night. This appears to be due to a nighttime reduction of ACTH peak amplitude in both control and PCOS patients, consistent with the fact that the physiological ACTH circadian rhythmicity is mainly dependent on variations in the amplitude of the secretory bursts.[16] The cortisol pattern is likely attributable to the

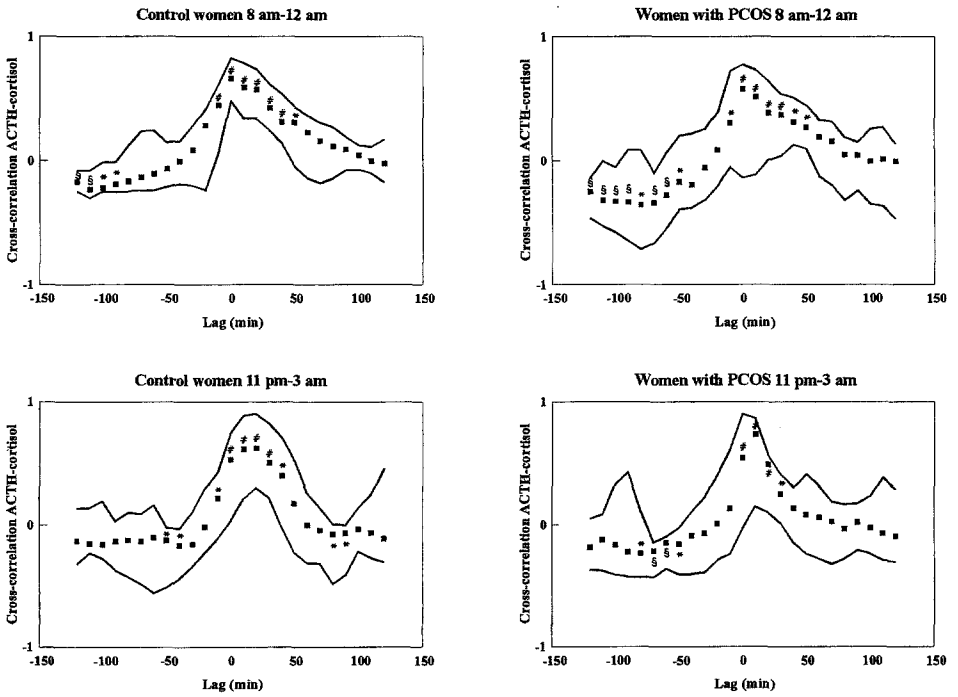

Fig 2. Cross-correlation plots evaluating the correlation of plasma ACTH and cortisol concentrations at various time lags (minutes). Negative lags indicate that cortisol changes precede ACTH changes; conversely for positive lag times, ACTH changes precede cortisol changes. (—) Represent the lowest and highest *r* values recorded; (■) group mean *r* values. *$P < .05$, §$P < .01$, #$P < .001$.

absence of normal day-night variation in cortisol peak amplitudes, as shown by pulse analysis. The relatively increased ACTH burst frequency at night versus daytime observed in our PCOS patients, as well as an increased nighttime adrenal sensitivity to ACTH in PCOS,[1] might be involved.

In agreement with our results, Miller et al[11] observed increased 24-hour cortisol concentrations in normal-weight (but not in obese) patients with PCOS. However, at variance with our findings, they reported in both PCOS groups an increase in cortisol pulse frequency and no alterations in pulse amplitude and duration as assessed by 15-minute blood sampling. These findings, together with a tendency toward an elevated cortisol response to a meal, led them to suggest dysregulation of the HPA axis in their patients. On the contrary, Stewart et al,[13] who also analyzed ACTH pulsatility over a 12-hour period, did not observe any difference in ACTH and cortisol release between PCOS and control women but, in keeping with the diurnal pattern seen by us, recorded a reduction in ACTH but not cortisol pulse frequency. The different sampling and food-intake paradigms, mathematical analyses, and patient populations evaluated by various investigators might partly explain the discrepancies of the results.

Finally, the increased diurnal ApEn cortisol values, denoting greater serial irregularity of hormone release, observed in patients with PCOS compared with control women point to a disordered mode of daytime cortisol secretion in these patients. Cross-correlation analysis showed a more substantial window of morning cortisol-ACTH negative feedback (negative r values at negative lags) in PCOS, suggesting preserved or actually enhanced auto–negative-feedback regulation by endogenous cortisol of daytime cortisol and ACTH release in such patients. This finding, combined with higher absolute plasma ACTH concentrations, favors an interpretation of primary ACTH overdrive during daytime hours in PCOS patients.

Whereas prolonged sampling over 24 hours in the same individual would be required to capture the full ultradian and circadian rhythmicity of ACTH and cortisol release,[16] our use of two 4-hour sampling windows still disclosed significant differences in pituitary-adrenal activity. In addition, while blood sampling every 1 to 5 minutes would have detected a higher absolute frequency of pulsatile ACTH events, the present paradigm of 10-minute sampling detected significant frequency differences. Further studies with even more intensive and/or prolonged sampling schedules will be useful in confirming our inferences. Importantly, other measures of pituitary-adrenal activity, such as the mean serum hormone concentration or ApEn, also revealed significant contrast among the study groups, thus corroborating our inference of excessive ACTH secretory drive, particularly in the daytime, independently of pulse analysis in PCOS.

In conclusion, our data point further to multifaceted dysregulation of the HPA axis in patients with PCOS. In keeping with this possibility, hirsute women with PCOS often present with physical and biochemical changes suggestive of Cushing's syndrome.[20] Moreover, Cushing's disease itself is accompanied by reduced ACTH-cortisol coupling and more disorderly patterns of hormone release.[21] The neuroendocrine mechanisms underlying these alterations in PCOS patients are not yet defined, but likely entail some disruption in physiological bidirectional ACTH-adrenal feedback control. Studies aimed at further exploring the feedback and feedforward control of ACTH secretion, and the network function of the CRH-ACTH axis by the use of cross-entropy statistics on long-term sampling may help to better understand the pathophysiology of PCOS.

REFERENCES

1. Ehrmann DA, Barnes RB, Rosenfield RL: Polycystic ovary syndrome as a form of functional ovarian hyperandrogenism due to dysregulation of androgen secretion. Endocr Rev 16:322-353, 1995
2. Cunningham SK, Loughlin T, Bertagna X, et al: Plasma pro-opiomelanocortin fragments and adrenal steroids following administration of metyrapone to normal and hirsute women. J Endocrinol Invest 11:247-253, 1988
3. Ditkoff EC, Fruzzetti F, Chang L, et al: The impact of estrogen on adrenal androgen sensitivity and secretion in polycystic ovary syndrome. J Clin Endocrinol Metab 80:603-607, 1995
4. Luppa P, Müller B, Jacob K, et al: Variations of steroid hormone metabolites in serum and urine in polycystic ovary syndrome after nafarelin stimulation: Evidence for an altered corticoid excretion. J Clin Endocrinol Metab 80:280-288, 1995
5. Stewart PM, Shackleton CHL, Beastall GH, et al: 5 Alpha-reductase activity in polycystic ovary syndrome. Lancet 335:431-433, 1990
6. Rodin A, Thakkar H, Taylor N, et al: Hyperandrogenism in polycystic ovary syndrome. Evidence of dysregulation of 11β-hydroxysteroid dehydrogenase. N Engl J Med 7:460-465, 1994
7. Invitti C, Pecori Giraldi F, Dubini A, et al: Increased urinary free cortisol and decreased serum corticosteroid-binding globulin in polycystic ovary syndrome. Acta Endocrinol (Copenh) 125:28-32, 1991
8. Mongioi A, Macchi M, Vicari E, et al: Pituitary and adrenal response to ovine corticotropin-releasing hormone in women with polycystic ovarian syndrome. J Endocrinol Invest 11:637-640, 1988
9. Coiro V, Volpi R, Capretti L, et al: Arginine vasopressin secretion in non-obese women with polycystic ovary syndrome. Acta Endocrinol (Copenh) 121:784-790, 1989
10. Modell E, Goldstein D, Reyes FI: Endocrine and behavioral responses to psychological stress in hyperandrogenic women. Fertil Steril 53:454-459, 1990
11. Miller JF, Bray MA, Faiman C, et al: Characterization of 24-h cortisol release in obese and non-obese hyperandrogenic women. Gynecol Endocrinol 8:247-254, 1994
12. Atkinson AB, McAteer EJ, Hadden DR, et al: A weight-related intravenous dexamethasone suppression test distinguishes obese controls from patients with Cushing's syndrome. Acta Endocrinol (Copenh) 120:753-759, 1989
13. Stewart PM, Penn R, Holder R, et al: The hypothalamo-pituitary-adrenal axis across the normal menstrual cycle and in polycystic ovary syndrome. Clin Endocrinol (Oxf) 38:387-391, 1993
14. Veldhuis JD, Johnson ML: Cluster analysis: A simple, versatile, and robust algorithm for endocrine pulse detection. Am J Physiol 250:E486-E493, 1986
15. Veldhuis JD, Johnson ML: Deconvolution analysis of hormone data. Methods Enzymol 210:539-575, 1992
16. Veldhuis JD, Iranmanesh A, Johnson ML, et al: Amplitude, but not frequency, modulation of adrenocorticotropin secretory bursts gives rise to the nyctohemeral rhythm of the corticotropic axis in man. J Clin Endocrinol Metab 71:452-463, 1990
17. Pincus SM, Gevers E, Robinson ICA, et al: Females secrete growth hormone with more process irregularity than males in both human and rat. Am J Physiol 270:E107-E115, 1996

18. Lanzone A, Petraglia F, Fulghesu AM, et al: Corticotropin-releasing hormone induces an exaggerated response of adrenocorticotropic hormone and cortisol in polycystic ovary syndrome. Fertil Steril 63:1195-1199, 1995

19. Pasquali R, Cantobelli S, Casimirri F, et al: The hypothalamo-pituitary-adrenal axis in obese women with different patterns of body fat distribution. J Clin Endocrinol Metab 77:341-346, 1993

20. Bals-Pratsch M, Hanker JP, Helhammer DH, et al: Intermittent Cushing's disease in hirsute women. Horm Metab Res 28:105-110, 1996

21. van den Berg G, Frölich M, Veldhuis JD, et al: Combined amplification of the pulsatile and basal modes of adrenocorticotropin and cortisol secretion in patients with Cushing's disease: Evidence for decreased responsiveness of the adrenal glands. J Clin Endocrinol Metab 80:3750-3757, 1995