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Menstrual Abnormalities in Women with Cushing's Disease Are Correlated with Hypercortisolemia Rather Than Raised Circulating Androgen Levels

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ABSTRACT

Menstrual irregularity is a common complaint at presentation in women with Cushing's syndrome, although the etiology has been little studied. We have assessed 45 female patients (median age, 32 yr; range, 16–41 yr) with newly diagnosed pituitary-dependent Cushing's syndrome. Patients were subdivided into 4 groups according to the duration of their menstrual cycle: normal cycles (NC; 26–30 days), oligomenorrhea (OL; 31–120 days), amenorrhea (AM; >120 days), and polymenorrhea (PM; <26 days). Blood was taken at 0900 h for measurement of LH, FSH, PRL, testosterone, androstenedione, dehydroepiandrosterone sulfate, estradiol (E_2), sex hormone-binding globulin (SHBG), and ACTH; cortisol was sampled at 0900, 1800, and 2400 h. The LH and FSH responses to 100 μg GnRH were analyzed in 23 patients. Statistical analysis was performed using the nonparametric Mann-Whitney U and Spearman tests.

Only 9 patients had NC (20%), 14 had OL (31.1%), 15 had AM (33.3%), and 4 had PM (8.8%), whereas 3 had variable cycles (6.7%). By group, AM patients had lower serum E_2 levels (median, 110 pmol/L) than OL patients (225 pmol/L; $P < 0.05$) or NC patients (279 pmol/L; $P < 0.05$), and higher serum cortisol levels at 0900 h (800 *vs.* 602 and 580 nmol/L, respectively; $P < 0.05$) and 1800 h (816 *vs.* 557 and 523 nmol/L, respectively; $P < 0.05$) and higher mean values from 6 samples obtained through the day (753 *vs.* 491 and 459 nmol/L, respectively; $P < 0.05$). For the whole group of patients there was a negative correlation between serum E_2 and cortisol at 0900 h ($r = -0.50$; $P < 0.01$) and 1800 h ($r = -0.56$; $P < 0.01$) and with mean cortisol ($r = -0.46$; $P < 0.05$). No significant correlation was found between any serum androgen and E_2 or cortisol. The LH response to GnRH was normal in 43.5% of the patients, exaggerated in 52.1%, and decreased in 4.4%, but there were no significant differences among the menstrual groups. No differences were found in any other parameter.

In summary, in our study 80% of patients with Cushing's syndrome had menstrual irregularity, and this was most closely related to serum cortisol rather than to circulating androgens. Patients with AM had higher levels of cortisol and lower levels of E_2, while the GnRH response was either normal or exaggerated. Our data suggest that the menstrual irregularity in Cushing's disease appears to be the result of hypercortisolemic inhibition of gonadotropin release acting at a hypothalamic level, rather than raised circulating androgen levels. (*J Clin Endocrinol Metab* **83:** 3083–3088, 1998)

CUSHING'S syndrome is 4 times more common in women than men, and menstrual disturbance is a frequent complaint at presentation (1–5). The mechanism responsible for this alteration has not yet been established, although a relationship to excessive adrenal androgen levels is often presumed (6). However, it is known that in primates, chronic hypercortisolemia suppresses gonadotropin release by inhibiting the secretion of GnRH from the hypothalamus (7). GnRH neurons are known to express glucocorticoid receptors (8), thus indicating a possible mechanism by which hypercortisolemia can block GnRH/gonadotropin release and be responsible for the menstrual disorders associated with Cushing's syndrome.

This paper reports the menstrual and endocrine features in 45 women with newly diagnosed pituitary-dependent Cushing's syndrome studied at the time of diagnosis and investigates the relationships between gonadal dysfunction and circulating cortisol and androgen levels to address whether any such interaction is at the hypothalamic or the pituitary level.

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Subjects and Methods

Patients

We have reviewed 45 premenopausal women (median age at diagnosis, 32 yr; range, 16–41 yr) with Cushing's disease diagnosed at St. Bartholomew's Hospital between 1974–1995. Transsphenoidal surgery was performed in 36 patients. Pituitary radiotherapy and/or adrenalectomy were performed in the 9 remaining patients. All patients were postpubertal at diagnosis.

The patients were subdivided into four groups according to the duration of their menstrual cycle: normal cycles (NC; 26–30 days), oligomenorrhea (OL; 31–120 days), amenorrhea (AM; >120 days), and polymenorrhea (PM; <26 days).

Diagnosis of Cushing's disease

All patients were studied during their initial admission for confirmation of the diagnosis of Cushing's disease. Basal blood samples, for determinations of androgen, estradiol (E_2), LH, FSH, and PRL levels, were collected at 0900 h on the first day of investigation regardless of the stage of the menstrual cycle, as only 20% of the subjects had regular menstrual cycles. The order of the subsequent investigations to confirm the diagnosis of Cushing's disease was: circadian rhythm of serum cortisol, low dose dexamethasone suppression test (LDDST), and high dose dexamethasone suppression test (HDDST). The circadian rhythm of serum cortisol entailed blood being taken at 0900, 1800, and 2400 h

(asleep) not less than 48 h after admission to the hospital. Loss of the normal circadian rhythm of serum cortisol was identified by a sleeping midnight cortisol level greater than 50 nmol/L (9, 10). LDDST involved the administration of 0.5 mg dexamethasone at 6-h intervals from 0900 h for eight doses, with measurement of serum cortisol at 0900 h before dexamethasone administration and at 48 h. In normal subjects the 48 h cortisol level should be suppressed to less than 50 nmol/L (9). The HDDST was performed as described for the LDDST, except that the dose of dexamethasone was 2 mg every 6 h. In patients with Cushing's disease a fall in serum cortisol to less than 50% of the basal predexamethasone level is expected (11). From 1985, inferior petrosal sinus sampling was performed in all patients, at least 10 days after the last dose of dexamethasone.

CRH test. The CRH test involved the administration of 100 μg synthetic CRH, iv, with measurements of ACTH and cortisol every 15 min for 2 h. In patients with Cushing's disease, serum cortisol usually rises excessively in response to stimulation with CRH (12, 13).

GnRH test. Twenty-three patients underwent a GnRH stimulation test. After an overnight fast, 100 μg GnRH were administered as an iv bolus injection, and serum LH and FSH were measured at 0, 20, and 60 min. The normal range for LH is 15–42 IU/L at 20 min and 12–35 IU/L at 60 min; for FSH, the normal range is 1–11 IU/L at 20 min and 1–25 IU/L at 60 min (14, 15).

Hormone assays

Serum cortisol was assayed by an in-house RIA (16). The intra- and interassay coefficients of variation (CVs) were below 8%. Values less than 50 nmol/L were reported as undetectable. The minimum detectable concentration was 50 nmol/L. Serum androstenedione (A_4) was measured by an in-house RIA after extraction with ether. The intra- and interassay CVs were less than 10% and less than 12%, respectively. The assay sensitivity was 0.5 nmol/L. Serum dehydroepiandrosterone sulfate (DHEAS) was assayed with an in-house RIA (17). The intra- and interassay CVs were less than 10% and less than 14%, respectively. The assay sensitivity was 0.5 μmol/L. Serum testosterone was determined with an in-house RIA after organic solvent extraction. The intra- and interassay CVs were less than 11%. The assay sensitivity was 0.25 nmol/L. Serum E_2 was directly measured using the Diagnostic Products RIA (Llanberis, Wales). The intra- and interassay CVs were below 8%. Assay sensitivity was less than 20 pmol/L. Serum SHBG was measured by a saturation analysis based upon the binding of [^{3}H]dihydrotestosterone to SHBG. The intra- and interassay CVs were less than 6% (18). The assay sensitivity was 10 nmol/L. Plasma ACTH concentrations were determined by RIA (19). The intra- and interassay CVs were 10% and 12%, respectively. Serum LH and FSH were measured by RIA (NETRIA, London, UK) up until 1989 and subsequently by immunoradiometric assay (IRMA; NETRIA). The interassay CV for LH was 3.5% at 2.2 mU/L and 2.3% at 9.6 mU/L; that for FSH was 7.0% at 2.6 mU/L and 4.4% at 9.6 mU/L. Serum PRL was measured using the NETRIA RIA method until 1990. After that, the NETRIA IRMA was used. The interassay CV for the RIA method was 8%; that for the IRMA method was 5%. The assay sensitivity was 40 mU/L. The free androgen index (FAI) was calculated as FAI = (testosterone/SHBG) $\times$ 100.

Statistical analysis

The data were not normally distributed, and the results have been expressed as medians and ranges. Between-group comparisons were performed using the Mann-Whitney U test, and correlations have been expressed as Spearman's correlation coefficients (r). $P < 0.05$ was considered statistically significant.

Results

All patients had cortisol dynamics studies during investigation diagnostic of Cushing's disease. Histological confirmation of an ACTH-secreting pituitary adenoma was obtained in 32 of the 36 patients who underwent transsphenoidal surgery; in another 3 subjects, no tumor was identified, but the presence of Crooke's hyaline confirmed prolonged hypercortisolemia. The remaining patient who underwent transsphenoidal surgery had postoperative cortisol levels of less than 50 nmol/L, indicative of complete excision, and remains in long term remission (20). Nine patients did not undergo hypophysectomy, all of whom all had ACTH-dependent Cushing's syndrome, a high dose dexamethasone suppression test in keeping with Cushing's disease, and no evidence of ectopic ACTH secretion during prolonged follow-up (median, 16 yr; range, 7–21 yr).

The median age at menarche was 13 yr (range, 10–16 yr), with one patient having primary amenorrhea. Initial menstrual cycles were regular in 81%, with 9% of patients reporting oligomenorrhea from menarche, and 7% reporting polymenorrhea.

Eighty percent of the 45 patients studied had a history of menstrual abnormalities at diagnosis: 15 had AM (33.3%), 14 had OL (31.1%), and 4 had PM (8.8%). In another 3 patients (6.6%), cycles were irregular, but did not meet the criteria for the above groups; in only 9 patients (20%) was the menstrual cycle regular.

Table 1 contains medians and ranges for each hormone studied for the entire cohort of 45 patients. The median serum A_4 level was elevated above the normal range, as were the

TABLE 1. Basal hormonal values (median and range) at diagnosis of 45 women with Cushing's disease

	Median	Minimum	Maximum
Age (yr)	32	16	41
BMI (kg/m^2)	27.0	16.8	46.8
Serum:			
LH (IU/L; NR, 1–13)	4.8	0.8	15.2
FSH (IU/L; NR, 0.3–10)	3.6	0.3	7.2
PRL (mU/L; NR, up to 360)	283	107	768
Estradiol (pmol/L; NR, 200–1000)	196	39	590
Testosterone (nmol/L; NR, 0.5–3)	2.4	0.6	4.6
Androstenedione (nmol/L; NR, 3–8)	12.5	2.7	24.6
DHEAS (μmol/L; NR, 1.9–9.4)	8	1.7	30.7
SHBG [nmol/L; NR (females), 38–103]	17.5	8	43.3
Cortisol, 0900 h (nmol/L; NR, 200–700)	660	183	1300
Cortisol, 1800 h (nmol/L; NR, 100–300)	598	110	1600
Cortisol, 0000 h (nmol/L; NR, <50)	460	210	1530
Mean cortisol (nmol/L)	560.5	211.3	1366.6
Mean plasma ACTH (ng/L)	57.8	21.6	185

NR, Normal range.

TABLE 2. Basal hormonal values presented by menstrual history, normal cycles, oligomenorrhea, and amenorrhea, [median (range)], in 45 women with Cushing's disease

	Normal cycles	Oligomenorrhoea	Amenorrhoea
LH (IU/L; NR, 1–13)	5.6 (2.6–12.6)	4.8 (1.4–15.2)	2.5 (0.8–14.5)
FSH (IU/L; NR, 0.3–10)	3.8 (0.8–5)	3.2 (1.1–6.6)	2.6 (0.3–7.2)
PRL (mIU/L; NR, up to 360)	309.5 (193–768)	246.5 (107–649)	231 (118–440)
Testosterone (nmol/L; NR, 0.5–3)	2.5 (1.6–3.1)	2.3 (0.6–3.4)	2.1 (1.3–4.6)
Androstenedione (nmol/L; NR, 3–8)	11.9 (6.1–21)	14.4 (2.7–23)	13 (7.6–17.5)
DHEAS (μmol/L; NR, 1.9–9.4)	8.9 (1.8–14.4)	4.8 (2.3–28)	10 (1.7–20)
SHBG [nmol/L; NR females), 38–103]	24 (15–43.3)	18.5 (10–40)	13.25[a] (10–31)
FAI [(T/SBHG) × 100]	11.5 (4.2–18)	14.5 (2–31)	14 (6–46)
Estradiol (pmol/L; NR, 200–1000)	279 (99–444)	225 (117–500)	110[b] (39–290)
Cortisol, 0900 h (nmol/L; NR, 200–700)	580 (390–930)	602 (183–1100)	800[b] (439–1258)
Cortisol, 1800 h (nmol/L; NR, 100–300)	523.5 (275–830)	557.5 (110–1060)	816[b] (503–1600)
Cortisol, 0000 h (nmol/L; NR, <50)	320.5 (250–711)	453 (292–1060)	611 (237–1530)
Mean cortisol (nmol/L)	459 (313–780)	491.1 (212–1073)	753[b] (393–1367)

NR, Normal range.
[a] $P < 0.05$ *vs.* normal.
[b] $P < 0.05$ *vs.* normal and oligomenorrhea.

individual results from 74% of the patients. Serum testosterone and DHEAS levels were elevated in 29% and 39% of the patients, respectively, whereas SHBG was below the normal range in 94% of the patients. The median serum E_2 level was below the normal value for the follicular phase in normally menstruating women. The circadian rhythm of serum cortisol was absent in all patients, with elevated median values for 1800 and 2400 h.

By groups (Table 2), AM patients had lower serum E_2 levels than OL or NC patients. The AM group had higher serum cortisol levels at 0900 and 1800 h, and higher mean serum cortisol levels (Figs. 1 and 2). The AM group had lower SHBG levels than the NC group, but there was no difference in FAI, serum androgens, or gonadotropins between the groups (Fig. 3).

There was a negative correlation between serum E_2 and cortisol at 0900 h ($r = -0.50$; $P < 0.01$) and 18.00 h ($r = -0.56$; $P < 0.01$) and with mean cortisol ($r = -0.46$; $P < 0.05$). No correlation was found between any serum androgen (A_4, DHEAS, or testosterone) and either E_2 or cortisol. Serum testosterone significantly correlated with A_4 ($r = 0.80$; $P < 0.001$), DHEAS ($r = 0.46$; $P < 0.05$), and body mass index ($r = 0.55$; $P < 0.05$). There was a significant correlation between DHEAS and A_4 ($r = 0.46$; $P < 0.05$). There was a highly significant correlation between LH and FSH ($r = 0.76$; $P < 0.001$). No correlation was found between serum gonadotropin values and any other parameter. The median serum PRL values were normal (<360 mU/L) in all groups and did not differ among the three groups. Serum PRL was raised in 36% of patients with OL, 13% with AM, and 22% with regular cycles. Serum PRL was not correlated with any other variable.

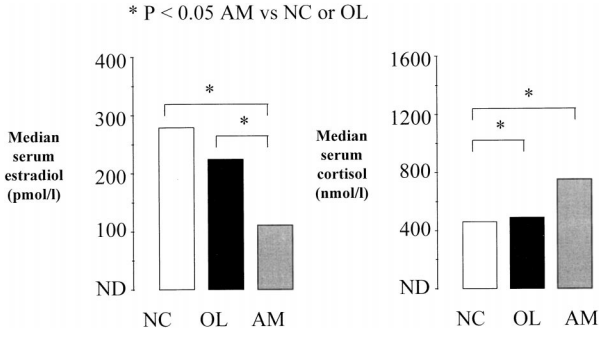

FIG. 1. Comparison between median serum levels of E_2 (*left*) and median serum cortisol levels (*right*) in females with Cushing's disease and NC, OL, or AM.

Twelve patients (52.1%) had an exaggerated LH response to the GnRH test, 10 patients (43.5%) had a normal response, and only 1 patient had a subnormal response (4.4%). Nine patients (39.1%) had an exaggerated FSH response to the GnRH test; the remaining 14 patients (60.8%) had a normal response. There were no significant differences in the LH or FSH responses to GnRH among patients with NC, OL, and AM.

Discussion

Menstrual irregularity is a common finding in women with Cushing's syndrome, although the underlying mechanisms are poorly understood (21). In this study, 80% of patients with pituitary-dependent Cushing's syndrome had menstrual irregularities at diagnosis, with AM being the most common problem observed.

Many of the presenting features of women with Cushing's syndrome are similar to those observed in patients with the polycystic ovarian syndrome (PCOS): obesity, oligomenorrhea or amenorrhea, hirsutism, low serum SHBG levels, increased circulating androgen levels, and an exaggerated gonadotropin response to GnRH (22, 23). However, the histological appearances of the ovaries in Cushing's disease are distinct from those in PCOS; in Cushing's syndrome there is a marked reduction in all phases of primordial follicles, an absence of cortical stromal hyperplasia and luteinization, significant fibrosis, and a tendency toward a reduction in size, suggestive of a lack of gonadotroph stimulation (24). In our study the levels of gonadotropins were inappropriately low for the observed serum E_2 levels, indicative of hypogonadotropic hypogonadism as the cause of menstrual disturbance, contrasting with the elevation in LH usually observed in patients with PCOS (25). Our patients had a median age at menarche of 13 yr (range, 10–16 yr), with only 9% giving a history of OL, an incidence in keeping with the general population (26).

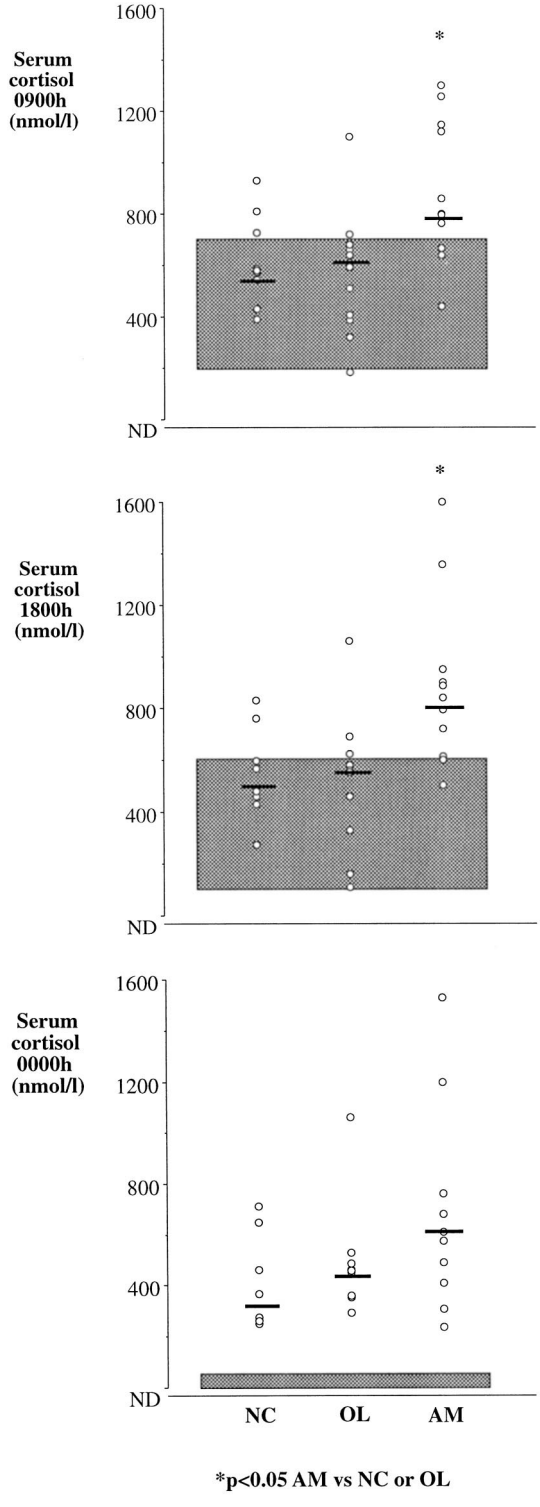

FIG. 2. Serum cortisol levels at 0900, 1800, and 2400 h in women with Cushing's disease and NC, OL, and AM. The *black line* represents the median, and the *shadowed area* shows the normal range.

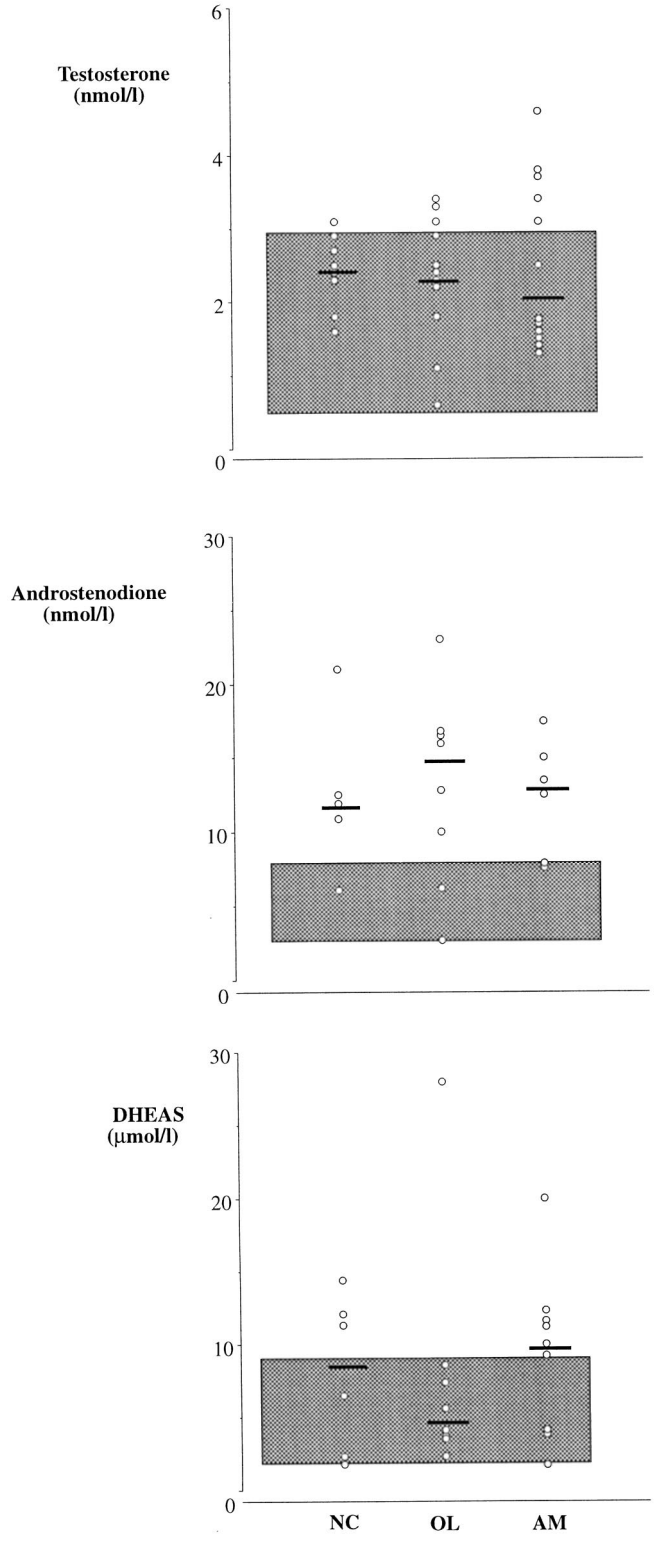

FIG. 3. Serum testosterone, A_4, and DHEAS levels in patients with Cushing's disease and NC, OL, or AM. The *black line* represents the median, and the *shadowed area* shows the normal range.

Previous reports of GnRH tests in women with Cushing's syndrome have variously concluded the gonadotropin response to be poor (27, 28), normal (29, 30), or exaggerated (31–33). All of our patients had a normal FSH reserve, and a normal or exaggerated LH response was observed in 22 of 23 patients. These data indicate that pituitary gonadotropin reserve is normal or even increased, and taken together with the inappropriately low basal gonadotropin levels of the

amenorrheic patients, they suggest that the menstrual disturbances associated with Cushing's syndrome may be a consequence of the disturbed hypothalamic GnRH secretion induced by chronic hypercortisolemia (7). Glucocorticoid receptors have been localized to granulosa cells and GnRH neurons (8). Chronic hypercortisolemia blocks both the action of gonadotropins on the gonads and the secretion from the hypothalamus of GnRH, an effect not consistently found with acute administration of glucocorticoids (7, 34–37). In acute stress, CRH is a potent inhibitor of gonadotropin secretion in ovariectomized nonhuman primates, an action mediated by β-endorphin but independent of the adrenals (38–40). Studies in humans have produced conflicting results (40–42); however, it is improbable that CRH is important in the etiology of the menstrual disturbance seen in women with Cushing's disease, as the negative feedback of hypercortisolemia on the hypothalamus should inhibit CRH secretion, in contrast to the high CRH drive encountered in conditions associated with pseudo-Cushing's, such as depression and alcoholism (43).

Twenty percent of our patients have marginally raised serum PRL levels, a consistent observation in patients with Cushing's syndrome (44, 45), although the mechanism is not well understood. It is unlikely that high serum PRL levels are responsible for the menstrual differences in our patients because the serum PRL values did not correlate with any other variable, serum PRL levels were not statistically different among three groups, and the amenorrheic group had the lowest incidence of hyperprolactinemia (13%). Transsphenoidal surgery was performed on five of the nine patients with hyperprolactinemia, and in none did the tumors stain for PRL, a finding in conflict with the reported presence of distinct ACTH- and PRL-staining cell populations within ACTH adenomas (46).

In our study serum cortisol was significantly inversely correlated with serum E_2 levels, but not with serum androgens. As amenorrheic patients had the highest serum cortisol levels but no difference in circulating androgens and FAI, we suggest that in women with Cushing's disease, AM relates to the high circulating cortisol levels and not to the circulating levels of androgens. In support of the hypothesis is our observation that normalization of serum cortisol levels with long term metyrapone therapy in women with Cushing's syndrome is associated with restoration of regular menstruation despite the marked rise in serum androgen levels associated with this therapy (47, 48). The low gonadotropin levels and menstrual irregularities observed in Cushing's syndrome do not appear to be the result of elevated serum androgen levels, an observation in agreement with the belief that androgens do not play a major role in the neuroendocrine regulation of the menstrual cycle (49, 50). Also, testosterone administration to female to male transsexuals induces morphological features of polycystic ovarian disease (51, 52). However, these alterations have only been produced when serum levels of testosterone are elevated, higher than the levels encountered in normal males, females with virilizing tumors, or the patients studied here (53–56). Administration of testosterone to women at doses that raised serum levels of testosterone to those seen in normal men does not alter serum gonadotropin levels or LH pulsatility or result in menstrual cycle regularity (57, 58). Further evidence for the minor role of androgens in the regulation of gonadotropin secretion comes from the observation in a man with estrogen resistance secondary to a mutation of the estrogen receptor that despite normal circulating androgen levels his serum gonadotropin levels were elevated, indicating the main feedback mechanism to be via estrogens rather than androgens (59).

In summary, we have confirmed that menstrual disturbances are a frequent feature on presentation in women with Cushing's disease. Serum E_2 significantly and inversely correlates with serum cortisol levels, but not with serum androgens or FAI. This suggests that the reduced basal gonadotropins and E_2 levels and menstrual irregularities observed in Cushing's disease result from the high circulating cortisol levels acting at the hypothalamic level, rather than from any elevation in serum androgen levels.

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