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Increased adrenal steroid secretion in response to CRF in women with hypothalamic amenorrhea

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Abstract

Objective: To evaluate adrenal steroid hormone secretion in response to corticotropin-releasing factor (CRF) or to adrenocorticotropin hormone in women with hypothalamic amenorrhea. *Design:* Controlled clinical study. *Setting:* Department of Reproductive Medicine and Child Development, Section of Gynecology and Obstetrics, University of Pisa, Italy. *Patient(s):* Fifteen women with hypothalamic amenorrhea were enrolled in the study. Eight normal cycling women were used as control group. *Intervention(s):* Blood samples were collected before and after an injection of ovine CRF (0.1 μg/kg iv bolus) or after synthetic ACTH (0.25 mg iv). *Main outcome measure(s):* Plasma levels of ACTH, 17-hydroxypregnenolone (17OHPe), progesterone (P), dehydroepiandrosterone (DHEA), 17-hydroxyprogesterone (17OHP), cortisol (F), 11-deoxycortisol (S) and androstenedione (A). *Result(s):* Basal plasma concentrations of ACTH, cortisol, 11-deoxycortisol, DHEA and 17OHPe were significantly higher in patients than in controls, whereas plasma levels of progesterone and 17-OHP were significantly lower in patients than in controls. In amenorrheic women the ratio of 17-OHPe/DHEA, of 17-OHPe/17-OHP and of 11-deoxycortisol/cortisol were significantly higher than in controls, while a significant reduction in the ratio of 17-OHP/androstenedione, of 17-OHP/11-deoxycortisol was obtained. In response to corticotropin-releasing factor test, plasma levels of ACTH, cortisol, 17-OHP, 11-deoxycortisol, DHEA and androstenedione were significantly lower in patients than in controls. In response to adrenocorticotropin hormone, plasma levels of 17-OHP, androstenedione and androstenedione/cortisol were significantly higher in patients than in controls. *Conclusions:* Patients suffering for hypothalamic amenorrhea showed an increased activation of hypothalamus-pituitary-adrenal (HPA) axis, as shown by the higher basal levels and by augmented adrenal hormone response to corticotropin-releasing factor administration. These data suggest a possible derangement of adrenal androgen enzymatic pathway.

Keywords: Adrenal steroids; Adrenocorticotropin hormone; Corticotropin-releasing hormone; Hypothalamic amenorrhea; Stress

1. Introduction

Hypothalamic amenorrhea is a functional disorder characterized by multiple abnormalities of the neuroendocrine control of hypothalamus-pituitary-ovarian axis. Physical and psychological stressors may lead to amenorrhea throughout a neuroendocrine derangement [1]. Alterations of hypothalamic gonadotropin-releasing hormone (GnRH) pulsatile secretion, as reflected by changes in the characteristics of pulsatile luteinizing hormone (LH) secretion, are typical findings in these patients. A decrease in frequency and amplitude [2,3], an increased frequency with a reduced amplitude [4] or even an amplification of pulsatile LH release in association with sleep [5,6] have been described. These alterations of GnRH pulsatile secretion may be the result of an impaired activity of central opioidergic or dopaminergic neurons [7,8].

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Several studies described corticotropin-releasing factor (CRF) as a possible causal factor of reproductive dysfunction in stressful conditions since in experimental animals CRF produces a dose-related decrease in GnRH release from the mediobasal hypothalamus in vitro [9] and the decrease of LH plasma levels in vivo [10–13]. Emotional, metabolic or physical stressors are associated with an activation of hypothalamus-pituitary-adrenal (HPA) axis in humans, which may lead to hypercortisolemia as in hypothalamic amenorrhea [3,14–18]. The increased cortisol (F) secretion depends from a pulse frequency higher than in healthy women during the daytime hours [18], but not during the evening (16:00–24:00 h) or sleeping hours (24:00–08:00 h) [3]. Several possible mechanisms might be behind the elevated cortisol production in hypothalamic amenorrhea, in particular an increased ACTH release and/or an increased adrenal sensitivity to ACTH. Indeed, another possibility could be a change in the adrenal steroid pattern in favor of glucocorticoids. Dehydroepiandrosterone (DHEA) and 17-hydroxyprogesterone (17OHP) are the first steps on the two biosynthetic pathways from the common substrate 17-α-hydroxy-5-pregnenolone (17OHPe) to 3-β-hydroxy-5-ene androgens and to cortisol (F), respectively. In fact, Zumoff et al. [19] demonstrated a decrease in the adrenal 17,20-lyase activity in women with anorexia nervosa with an adrenal steroid metabolism mainly involved in glucocorticoid production by a shift towards 3-oxo-4-ene C21-steroids as it has been reported in female endurance athletes [20].

On these basis, the present study aimed to evaluate plasma cortisol and adrenal androgens concentrations in women with hypothalamic amenorrhea before and after CRF or ACTH stimulating test. Enzyme activity will be evaluated as the precursor/product ratio of the estimated contributions to the circulating steroids levels as previously described [19–23].

2. Materials and methods

2.1. Subjects

Among women attending the Department of Obstetrics and Gynecology at Pisa University, 15 (aged 18–31 years) suffering for hypothalamic amenorrhea were enrolled for the study after informed consent. The criteria for the inclusion of the patients were, (a) disappearance of menses for at least 6 months before the study; (b) no evidence of pregnancy; (c) absence of metabolic or other endocrine diseases; (d) normal prolactin (PRL) and thyroid hormone levels; (e) loss of weight during the previous 18 months and a body mass index (BMI) above 20. At the anamnestic evaluation all patients had a variety of emotional-stressful events of various nature preceding the onset of amenorrhea. These events were mainly familiar and scholastic pressures, working and psycho-social problems. Psychiatric diseases were excluded using DSM-III criteria.

A group of eight normal cycling women (controls), aged 24–32 years with normal BMI was taken as controls and studied during the early follicular phase of the menstrual cycle (days 4–5). None of these subjects enrolled as control used hormonal preparations during the 6 months preceding the study with normal BMI (21.1 ± 0.35).

2.2. Study design

After an overnight fast, all subjects were inserted an indwelling catheter in an antecubital vein at 08:00 h, kept open by slow infusion of a saline solution of NaCl 0.9% to avoid the effects of venopuncture as a stressor. One hour later a baseline blood sample was collected for the determination of plasma levels of LH, follicle-stimulating hormone (FSH), PRL, 17β-estradiol (E_2), ACTH, 17OHPe, progesterone (P), DHEA, dehydroepiandrosterone-sulfate (DHEAS), 17OHP, 11-deoxycortisol (S), F and androstenedione (A). Immediately after this, all subjects received an intravenous bolus dose of ovine CRF (0.1 μg/kg, Inalco, Italy). Blood samples were taken 15, 30, 60, 90 and 120 min after the injection for ACTH, 17OHPe, P, DHEA, 17OHP, S, F and A determination.

After patients were administered 1 mg oral dexamethasone at 23:00 h, the following day an ACTH stimulation test was performed. Blood samples were done immediately before the intravenous bolus of 0.25 mg synthetic ACTH (0.25 mg, iv, Synacthen, Ciba-Geigy, Switzerland) and 60 and 120 min after the corticotropin administration. Control subjects underwent the stimulations test during the early follicular phase of the subsequent menstrual cycle (days 3–4).

All blood samples were collected, centrifuged and immediately frozen at -70 °C until assayed. For ACTH determination ethylenediaminetetraacetic acid (EDTA) containing glass tubes were used, placed on ice, centrifuged at -4 °C and immediately frozen at -70 °C until assayed.

The protocol has been approved by the Human Investigation Committee of the University of Pisa.

2.3. Hormone assays and statistical analysis

All samples from each individual were analyzed in duplicate in the same assay.

LH and FSH plasma levels were determined using a specific time-resolved fluoroimmunoassay method (Delfia, Wallac-Pharmacia & Upjhon, Milan, Italy) whose intra-assay and the inter-assay coefficients of variation (CVs) ranged from 3.7 to 4.5% and from 2.4

to 3.8%, respectively, for LH and from 3 to 4% and from 3.7 to 4.3%, respectively, for FSH. The sensitivity of the fluoroimmunoassay method was 0.005 U/l. Estradiol plasma levels were determined using a standard RIA (DPC-Medical Systems, Genova, Italy) whose intra-assay and inter-assay CVs were 7.0 and 8.1%, respectively, with a sensitivity of 8 pg/ml. Prolactin determination was performed using an immunoradiometric assay (Biodata-Biochem Immunosystems Company, Milan, Italy) with a magnetic solid phase. The intra-assay and the inter-assay CVs ranged from 3.2 to 5% and from 5.4 to 8%, respectively. The sensitivity was 0.3 g/ml. Plasma P concentrations were determined using a standard RIA (Radim, Pomezia, Italy) and 17OHP (Pantex, USA) were measured by RIA. The intra- and inter-assay CVs were 4.8 and 9.2% for P and 4.2 and 5.2% for 17OHP, respectively. The sensitivities were 0.1 and 0.2 nmol/l for P and 17OHP determination, respectively. The S and 17OHPe plasma determination were performed using a RIA technique (DRG Instruments, Marburg, Germany) after ether extraction and celite columns purification. The intra- and inter-assay CVs were 6.3 and 10.5% for S and 8.1 and 11.5% for 17OHPe, whereas the sensitivities were 0.3 and 0.07 nmol/l, for S and 17OHPe, respectively. Plasma DHEAS and A were determined using a standard RIA technique (Radim) whose intra-assay and inter-assay CVs were 6 and 8%, respectively, for DHEAS and 5.1 and 8.9% for A. Plasma levels of ACTH, F and DHEA were assayed using a RIA technique (ICN, Pharmaceutical, Orangeburg, NY, USA for ACTH; Radim for F; DSL, Webster, TX, USA for DHEA). The intra and inter-assay CVs were 6.3 and 10.8, 3.7 and 5.8, 5.4 and 8.9% for ACTH, F and DHEA, respectively. The assay sensitivities were 2.0 pmol/l, 2.7 and 0.06 nmol/l for ACTH, F and DHEA, respectively.

Table 1
Baseline hormonal values of women participating in the study

	Hypothalamic amenorrhea	Controls
Number of subjects	15	8
BMI	21.9 ± 0.28	21.1 ± 0.35
LH (IU/l)	1.3 ± 0.25*	7.5 ± 1.3
FSH (IU/l)	3.1 ± 0.89*	7.4 1.05
E_2 (pmol/l)	39.33 ± 12.35*	113.8 ± 24.2
PRL (μg/l)	12.2 ± 2.4	14.0 ± 2.1
ACTH (nmol/l)	5.20 ± 0.32*	2.48 ± 0.52
17OHPe (nmol/l)	13.03 ± 1.56*	3.48 ± 0.51
P (nmol/l)	1.35 ± 0.81*	2.24 ± 0.92
DHEA (nmol/l)	18.61 ± 0.91*	11.67 ± 0.82
DHEAS (μmol/l)	2.75 ± 0.43	3.68 ± 0.27
17OHP (nmol/l)	1.12 ± 0.22*	2.29 ± 0.31
S (nmol/l)	6.36 ± 0.74*	3.5 ± 0.76
F (nmol/l)	444 ± 37*	338 ± 26
A (nmol/l)	6.38 ± 0.67	8.03 ± 0.45

All values are reported as mean ± S.E. *, $P < 0.01$ vs. control subjects.

To evaluate adrenal response after CRF and ACTH injections all data were normalized and analyzed as Δ increase over basal values (Δ). The maximum increase (Δmax) above baseline values of ACTH, 17OHPe, P, DHEA, 17OHP, S, F and A in response to CRF and the Δmax over dexamethasone-suppressed baseline values of A, DHEAS, 17OHP and F in response to ACTH were calculated to evaluate the adrenal androgen production and cortisol secretion.

The activities of 3β-hydroxysteroid dehydrogenase and of 17,20-lyase and the other adrenal enzymatic activities were calculated as the precursor/product ratio of the estimated contributions to the circulating steroid levels as previously described [19–22]. The adrenal contribution for each steroid was taken as the net difference in hormone concentration between either the morning value (basal) and after a bolus of 0.1 μg/kg CRF (max) and between that after a bolus of 0.25 mg ACTH (max) and the concentration after overnight dexamethazone suppression.

Data were compared using Student's *t*-test for paired and unpaired data where appropriate after analysis of variance to establish omogeinity of data using *F*-test.

3. Results

Hormonal characteristics of all groups of subjects are summarized in Table 1 (mean ± S.E.). Basal plasma concentrations of ACTH, 17OHPe, DHEA, S and F were significantly ($P < 0.01$) higher in women with hypothalamic amenorrhea than in control subjects (Table 1). Conversely, basal plasma levels of P and 17OHP were significantly ($P < 0.01$) lower in amenorrheic women than in healthy women (Table 1). No significant difference between patients and normal women was detected for A and DHEAS plasma levels (Table 1).

Women with hypothalamic amenorrhea showed a ratio of 17OHPe/DHEA (marker of 17,20-lyase of Δ^5 pathway), of 17OHPe/17OHP (marker of 3β-hydroxysteroid-oxidoreductase-Δ^{5-4}-isomerase) and of S/F (marker of 11β-hydroxylase) significantly ($P < 0.01$) higher than control subjects (Table 2). Moreover, ratio of 17OHP/A and A/F (markers of 17,20-lyase of Δ^4 pathway), of 17OHP/S and 17OHP/F (markers of 21-hydroxylase) were significantly ($P < 0.01$) lower in amenorrheic patients than in healthy women (Table 2). No significant difference in the P/17OHP (marker of 17α-hydroxylase of Δ^4 pathway), DHEA/A (marker of 3β-hydroxysteroid-oxidoreductase-Δ^{5-4}-isomerase) and DHEAS/DHEA (sulfatase) ratios were observed between amenorrheic and normal women (Table 2).

After CRF administration, the adrenal response was significantly lower ($P < 0.01$) in amenorrheic patients than in control subjects. In fact amenorrheic women showed a Δmax response of ACTH, 17OHP, A, S,

Table 2
Adrenal precursor/product ratio in women with hypothalamic amenorrhea and in control subjects during the early follicular phase

	Hypothalamic amenorrhae	Controls
Number of subjects	15	8
17OHPe/DHEA (17,20-lyase of Δ^5 pathway)	0.73 ± 0.11*	0.31 ± 0.06
17OHPe/17OHP (3β-hydroxysteroid-oxido-reductase-Δ^{5-4}-isomerase)	7.77 ± 0.56*	2.12 ± 0.30
S/F (11β-hydroxylase)	0.015 ± 0.002*	0.009 ± 0.001
17OHP/A (17,20-lyase of Δ^4 pathway)	0.28 ± 0.13*	0.92 ± 0.18
A/F (17,20-lyase of Δ^4 pathway)	0.014 ± 0.002*	0.025 ± 0.003
17OHP/S (21-hydroxylase)	0.31 ± 0.11*	0.79 ± 0.12
17OHP/F (21-hydroxylase)	0.003 ± 0.001*	0.007 ± 0.001
P/17OHP (17α-hydroxylase of Δ^4 pathway)	1.56 ± 0.28	1.22 ± 0.39
DHEA/A (3β-hydroxysteroid-oxido-reductase-Δ^{5-4}-isomerase)	3.04 ± 0.66	4.13 ± 0.43
DHEAS/DHEA (sulfatase)	0.14 ± 0.05	$0.31 + 0.08$

All values are reported as mean ± S.E. *, $P < 0.01$ vs. control subject.

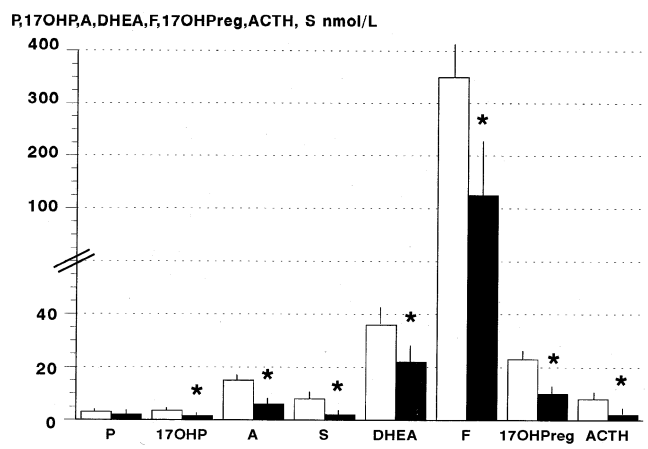

Fig. 1. Δmax Increase over basal values of plasma levels of progesterone (P), 17OHP, androstenedione (A), 11-deoxycortisol (S), DHEA, cortisol (F), 17OHPe and ACTH after CRF (0.1 μg/kg) bolus. All hormones but P responses resulted reduced in amenorrheic patients. Values are expressed in nmol/l as mean ± S.E. *, $P < 0.01$ hypothalamic amenorrhea (black column) vs. control subjects (white column).

DHEA, F, 17OHPe significantly lower ($P < 0.01$) than healthy subjects. No significant difference for P response to CRF injection was detected between amenorrheic and normal women (Fig. 1). CRF injection did not induce any significant differential response of any adrenal precursor/product ratio between patients and control subjects (data not shown).

After ACTH administration, amenorrheic women showed a Δmax increase of A and 17OHP significantly higher ($P < 0.01$) than healthy women (Fig. 2). No significant difference for F and DHEAS responses to ACTH injection was detected between amenorrheic and normal women (data not shown). After ACTH administration, the adrenal ratio of A/F (marker of 17,20-lyase of Δ^4 pathway) was ($P < 0.01$) higher in patients than in control subjects (Fig. 3). A slight, but not significant, decrease was observed for the Δmax response of 17OHP/A in amenorrheic women after ACTH stimulation test (Fig. 4). No significant difference for 17OHP/F response to ACTH injection was detected between amenorrheic and normal women (Fig. 3).

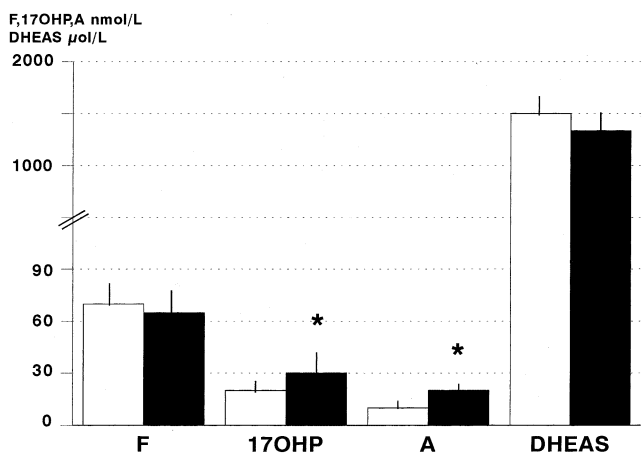

Fig. 2. Δmax Increase over basal values of plasma levels of 17OHP, androstenedione (A), DHEAS and cortisol (F) after ACTH (0.25 mg) bolus. Amenorrheic subjects showed higher 17OHP and A responses. Values are expressed in nmol/l as mean ± S.E. *, $P < 0.01$ hypothalamic amenorrhea (black column) vs. control subjects (white column).

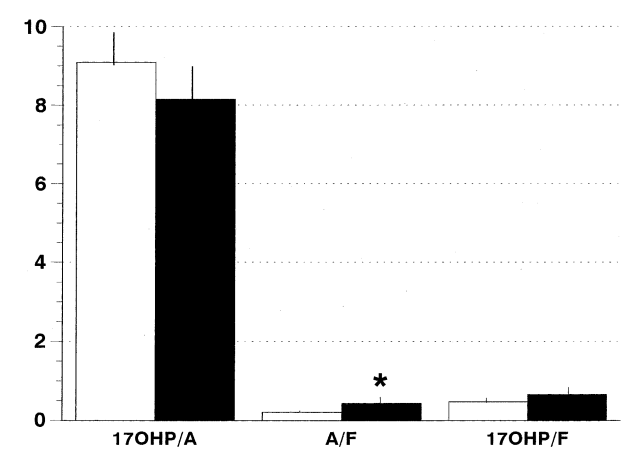

Fig. 3. Net change (Δ) of adrenal precursor/product ratio after ACTH (0.25 mg) bolus in women with hypothalamic amenorhea (black column) and in control subjects (white column). Only the A/F ratio resulted higher in amenorrheic subjects. Values are expressed as mean ± S.E. *, $P < 0.01$ hypothalamic amenorrhea (black column) vs. control subjects (white column).

4. Discussion

The present study showed that women with hypothalamic amenorrhea have a higher adrenal activity than healthy subjects and suggested that such higher activity reflects an altered function of specific adrenal enzymatic pathways.

Hypothalamic amenorrhea is often associated with stress-induced changes induced by metabolic, physical, psychological stressors [15,24]. Indeed, loss of weight for dieting and/or exaggerated training has been demonstrated to alter several hormonal parameters affecting not only the reproductive axis [4,24] but also other endocrine glands such as thyroid [25,26], the GH-RH-GH and IGF-1 axis [26] and the HPA axis [15,18]. This last one has been extensively reported to be modified during acute and/or chronic stress [10,11,13,15,18] as well as in psychiatric disorders [27], both in plasma and in cerebrospinal fluid [27]. Previous studies [19] proposed that such modification of adrenal function might be related to some altered enzymatic pathway. Indeed cortisol and androgens are biosynthetically derived from a common precursor, 17OHPe: the step to cortisol is mediated by 3β-hydroxysteroid dehydrogenase while the initial step to DHEA is mediated by 17,20-lyase. Since these two pathways are true alternative branches from the common precursor, the precursor/product ratio can be considered as an index of the relative activities of the two enzymes. Our data clearly showed that patients with hypothalamic amenorrhea have a decreased 17,20-lyase Δ^5 and Δ^4 enzymatic activity, confirming previous reports in anorexia nervosa [19]. Indeed, these authors suggested also that the decreased adrenal 17,20-lyase activity, typical in prepubertal children, may constitute a specific parameter to support the hypothesis that hypothalamic amenorrhea is a sort of functional regression to a prepubertal state. The hypothesis, that a reduced activity of 17,20-lyase of adrenal Δ^5 pathway may explain the increased 17OHPe plasma levels in hypothalamic amenorrhea, is strongly supported. Such excess in 17OHPe concentrations has a specific biological relevance since is finalized towards cortisol secretion. This hypothesis is strongly supported by the increased enzymatic activity of 21-hydroxylase we observed in amenorrheic subjects.

The present data suggest that in women with hypothalamic amenorrhea the adrenal steroid metabolism is shifted in favor of cortisol secretion, as demonstrated by the higher cortisol levels. The adrenal androgen concentrations in presence of hypoestrogenism and reduced plasma SHBG concentrations may explain the signs of moderate hirsutism that are often observed in amenorrheic patients.

The adrenal hypersecretion of cortisol is likely to be mediated through an increased hypothalmic CRF drive [18]. In fact, our data confirmed a blunted ACTH and F response to CRF test in women with hypothalamic amenorrhea [16,18] and showed that the Δmax increase over basal values of DHEA, 17OHP, S and A in women with hypothalamic amenorrhea was lower than in control subjects. Two hypotheses could be proposed to explain such reduced response of adrenal steroids in amenorrheic women, (1) a defect in enzymatic activity at the adrenal gland level; (2) an increased functional activation of the HPA axis via CRF overproduction. In support to this last hypothesis, there is the observation that hypothalamic amenorrhea shows a relatively high response of plasma levels of ACTH to acute CRF injection in relation to an increased endogenous CRF tone that reduces anterior pituitary sensitivity to endogenous CRF stimulation. Moreover, this leads to adrenal ACTH receptors down-regulation and to a blunted cortisol and androgen responses to CRF administration [16].

Under ACTH test, no significant difference in the Δmax response of F and DHEAS was observed between amenorrheic and normal women, whereas the Δmax increase over basal values of A and 17OHP was significantly higher in women with hypothalamic amenorrhea than in control subjects. Interestingly such data support a 17,20-lyase activity of Δ^4 pathway significantly higher than healthy women. Thus, our data show that the ACTH stimulation test in amenorrheic women reveals an increased adrenal cortex activity. Thus supporting that adrenal response to ACTH stimulation test seems to induce more androgen production rather than cortisol secretion. However, it cannot be excluded that minimal modifications of clearance rate of steroids might participate to determine the increase of androgen plasma levels.

In conclusion, the present data showed that in hypothalamic amenorrhea the basal activity of HPA axis was increased and report a reduced sensitivity of the anterior pituitary to CRF stimulation, in absence of any altered sensitivity of the adrenal cortex. Moreover, the reduced adrenal androgen secretion we observed in basal conditions in women with hypothalamic amenorrhea suggests a redistribution of adrenal steroid metabolism in favor of cortisol. Whether this physiopathological situation depends or not from a genetically based predisposition remains to be determined in following studies.

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