

Hypothalamo–pituitary–adrenal axis and adrenal function before and after ovariectomy in premenopausal women

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Abstract

The hypothalamo–pituitary–adrenal (HPA) axis is modulated by sex hormones. Few data exist on the relation between acute estrogen deficit and HPA axis response to corticotropin-releasing hormone (CRH).

The effects of a sudden drop in estradiol levels on basal and CRH-stimulated levels of ACTH, cortisol, testosterone, androstenedione and 17-hydroxyprogesterone (17-OHP) were assessed in nine premenopausal women (44–48 years of age), before and after ovariectomy. The CRH test was performed before and 8 days after ovariectomy.

A significant reduction in ACTH and adrenal steroids but not in cortisol response to CRH was observed after ovariectomy.

The ratio of Δ max androstenedione/17-OHP after CRH stimulation was substantially the same before and after ovariectomy, whereas Δ max 17-OHP/cortisol was significantly lower in ovariectomized women showing increased 21- and 11 β -hydroxylase activity. The results show that the acute estrogen deficit induces changes in the HPA axis characterized by reduced stimulated secretion of ACTH and steroids but normal stimulated cortisol production.

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Introduction

The hypothalamo–pituitary–adrenal (HPA) axis is activated in many stress-induced neuroendocrine and metabolic responses with negative effects on the reproductive axis. Administration of adrenocorticotropin (ACTH) or glucocorticoids inhibits luteinizing hormone (LH) release and/or ovulation in animals and women (1–3). At the same time, the activity of the HPA axis is modulated by sex hormones, as shown by many studies in female rats and humans. In rats, basal and stress-stimulated concentrations of adrenal steroids are different in males and females (4). Ovariectomy reduces basal levels and stress-induced levels of corticoids, which are restored to normal by administration of estrogens (5–7). In humans, reductions in ACTH have been observed after administration of corticotropin-releasing hormone (CRH) to women on oral contraceptives (8). Basal levels of ACTH and cortisol are highest during the late follicular phase and in the middle of the menstrual cycle (9), whereas cortisol concentrations are lowest when levels of progesterone are high (10). An enhanced adrenocortical responsiveness to stress has been reported during the luteal phase of the cycle (11).

Dehydroepiandrosterone sulfate (DHAS), a C-19 steroid of exclusively adrenal origin, decreases in

concentration with age in men and women. Reduced plasma concentrations of DHAS have been found in women with hypoestrogenism and ovarian failure (12). In one interesting study (13) a close correlation between testosterone and DHAS was reported. An increase in plasma concentrations of DHAS after testosterone administration in ovariectomized women has also been reported (14).

How the sudden reduction in plasma estradiol levels occurring at menopause affects the HPA axis is unclear. The aim of the present study was to evaluate the direct effect of ovariectomy on basal levels of ACTH, cortisol, testosterone, androstenedione, 17-hydroxyprogesterone (17-OHP), DHAS and their response to CRH in premenopausal women with adequate estradiol secretion before and after ovariectomy.

Subjects and materials

Subjects

Informed consent to the study was obtained from nine fertile premenopausal women awaiting abdominal hysterectomy and ovariectomy for a variety of benign gynecological disorders. Ages ranged from 44 to 48 years and body weight from 52 to 64 kg. Body mass

index was less than 25. The normal hormonal status of the patients was ascertained on admission to hospital by determination of basal levels of gonadotropins (LH, follicle-stimulating hormone (FSH)), estradiol, progesterone, prolactin and thyrotropin. All subjects entered hospital in the follicular phase (days 6 to 9 of the menstrual cycle). The premenopausal hormone levels showed estradiol between 290–350 pmol/l and FSH below 10 IU/l. After ovariectomy estradiol fell dramatically to below 55 pmol/l and FSH rose above 35 IU/l. One week after the operation, body weight showed a loss of 2.6 ± 0.8 kg. Blood loss during the operation was calculated by counting and weighing gauze swabs, and was 140 ± 50 ml.

Experimental design

All subjects underwent the CRH stimulation test (100 µg, Nova Biochem, Zurich, Switzerland) before surgery and 8 days after the operation. One hour after intravenous catheter placement blood samples were taken, with the patients fasting, at -15, 0 and every 15 min for 2 h relative to the injection of CRH. A portion of blood sample was placed immediately in iced tubes containing EDTA-Na and centrifuged at 4 °C. Plasma was kept at -20 °C until assay.

Radioimmunoassay

Plasma ACTH, LH, FSH, estrogen, sex hormone-binding globulin (SHBG), cortisol-binding globulin (CBG), cortisol, testosterone, androstenedione and 17-OHP were measured by double antibody RIA using Radim kits (Rome, Italy) for LH, FSH, cortisol, CBG, androstenedione and DHAS, DPC kits (Los Angeles, CA, USA) for ACTH and 17-OHP, and Sorin kits (Saluggia, Italy) for testosterone, estrogen and SHBG. The samples were assayed in duplicate at two dilutions. All samples from each subject were assayed together. Quality control pools at low, medium and high hormone levels were included in each assay. The intra-assay and inter-assay coefficients of variation did not exceed 10 and 15% respectively.

Statistical analysis

The results are expressed as means and standard deviation. The total integrated hormonal responses to CRH were calculated by the trapezoidal method and expressed as the area under the concentration–time curve (AUC) from 0–120 min. ANOVA was performed to detect time-related differences. To compare the response before and after ovariectomy, peak values (the maximum rise above baseline values) and the AUCs were compared using Student's paired *t*-test. The ratio of maximum increment of the appropriate substrates to products in response to the CRH test were compared to detect differences in the activity of 17,20 desmolase and

Table 1 Hormonal basal values (means \pm s.d.) in blood samples obtained before and 8 days after ovariectomy.

Hormone	Baseline values		<i>P</i>
	Before	After	
ACTH (pmol/l)	3.1 ± 0.4	2.8 ± 0.4	–
Cortisol (nmol/l)	276 ± 44	281 ± 49	–
17-OHP (nmol/l)	2.1 ± 0.3	0.7 ± 0.1	<0.01
Androstenedione (nmol/l)	2.9 ± 0.4	1.1 ± 0.2	<0.01
DHAS (µmol/l)	4.3 ± 1	1.9 ± 0.8	<0.01
Testosterone (nmol/l)	1.2 ± 0.2	0.5 ± 0.1	<0.01

21- and 11 β -hydroxylase. Statistical significance was taken as $P < 0.05$.

Results

Hormonal basal values before and after ovariectomy are shown in Table 1. No statistically significant differences were found in basal concentrations of ACTH and cortisol before and after ovariectomy. The CRH test caused a significantly greater increase in ACTH at 45 min in the women before ovariectomy (7.7 ± 2.0 pmol/l) than afterwards (4.8 ± 1.0 pmol/l; $P < 0.01$) (Fig. 1). The AUC and the maximum rise above baseline were significantly lower after ovariectomy ($P < 0.01$; Fig. 2).

Cortisol reached a peak after 45 min before ovariectomy, rising from 276 ± 44 to 607 ± 71 nmol/l and decreasing to 386 ± 41 nmol/l at 120 min. After ovariectomy, basal levels of cortisol were 281 ± 49 nmol/l, reaching a peak of 598 ± 85 at 30 min and decreasing to 358 ± 60 nmol/l at 120 min after CRH administration (Fig. 3). The AUC of the cortisol response was not significantly smaller in ovariectomized women (Fig. 4).

Basal levels of the adrenal androgens DHAS, 17-OHP, androstenedione and testosterone were significantly

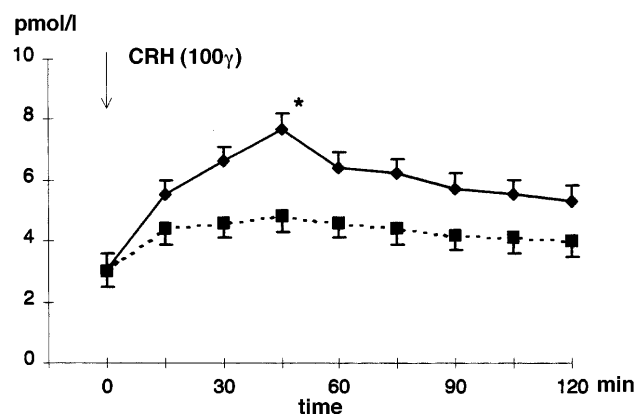


Figure 1 ACTH response to CRH after ovariectomy (■) was significantly lower (* $P < 0.01$) than before (◆). Values are means \pm s.d.

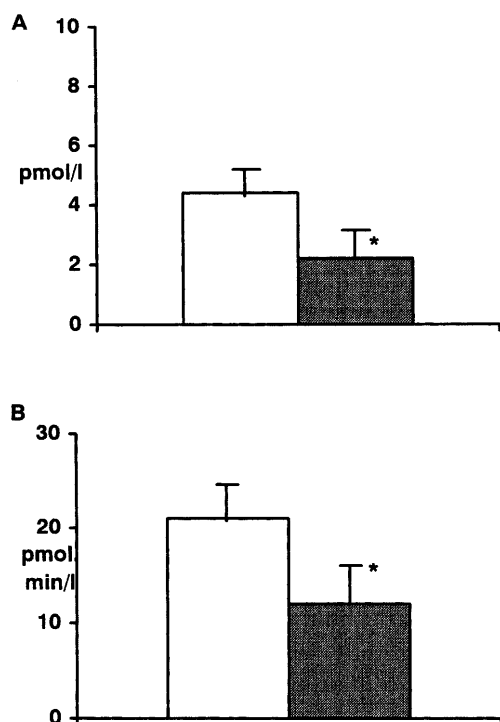


Figure 2 The maximum increase in ACTH (A) and the cumulated response (AUC) of ACTH to CRH (B) were significantly higher (* $P < 0.01$) before (open bars) than after ovariectomy (filled bars). Values are means \pm s.d.

lower after ovariectomy. Testosterone decreased from 1.2 ± 0.2 to 0.5 ± 0.1 nmol/l ($P < 0.01$), DHAS from 4.3 ± 1.0 to 1.9 ± 0.8 μ mol/l ($P < 0.01$), androstenedione from 2.9 ± 0.4 to 1.1 ± 0.2 nmol/l ($P < 0.01$) and 17-OHP from 2.1 ± 0.3 to 0.7 ± 0.1 nmol/l ($P < 0.01$). SHBG plasma levels were significantly lower after ovariectomy (36 ± 6 vs 31 ± 5 nmol/l; $P < 0.05$). CBG plasma levels did not change significantly after ovariectomy (33 ± 9 vs 30 ± 11 mg/l).

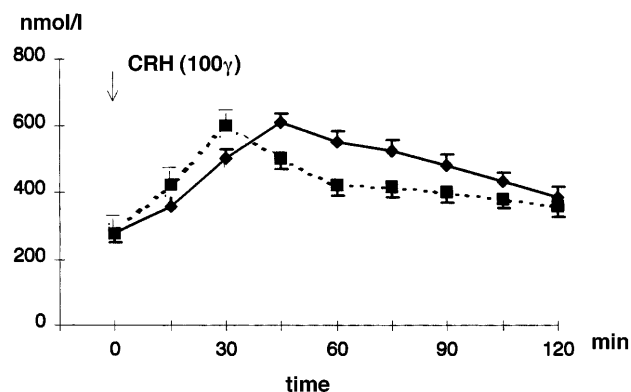


Figure 3 Cortisol response to CRH before (\diamond) and after (\blacksquare) ovariectomy. Significant difference in the timing of the peak response was present. Values are means \pm s.d.

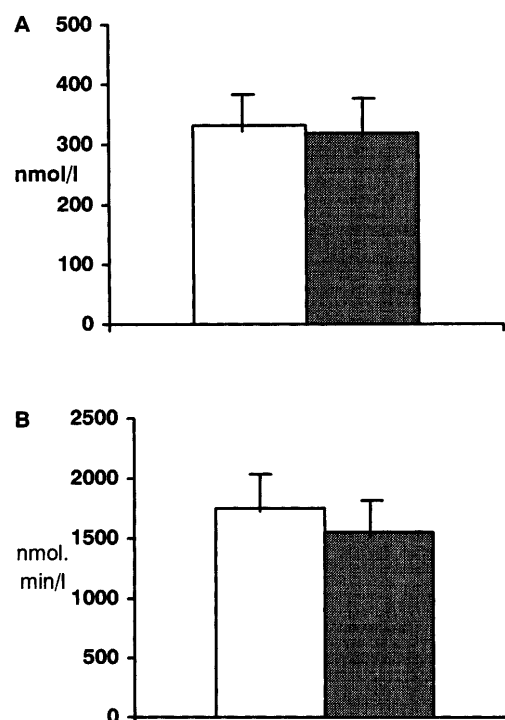


Figure 4 No significant differences were present in the maximum increase in cortisol (A) and AUC of cortisol to CRH (B) before (open bars) and after ovariectomy (filled bars). Values are means \pm s.d.

After administration of CRH, the maximum increase in 17-OHP was 2.7 ± 0.3 nmol/l before and 1.2 ± 0.2 nmol/l after ovariectomy ($P < 0.01$); AUC, the cumulated response, was significantly reduced after ovariectomy (Fig. 5).

The maximum increase in androstenedione after CRH was 1.4 ± 0.2 nmol/l before and 0.6 ± 0.1 nmol/l after ovariectomy ($P < 0.01$). AUC was significantly reduced (Fig. 6).

There was no significant difference in the maximum increment in DHAS or the cumulated response of DHAS to CRH before and after ovariectomy. No significant difference was found in the testosterone response to CRH (data not show).

Adrenal sensitivity to ACTH, evaluated as the ratio of the maximum increments of cortisol and ACTH (Δ max cortisol/ACTH) was 0.75 ± 0.16 before and 1.45 ± 0.25 after ovariectomy; the difference was significant ($P < 0.01$). The Δ max 17-OHP/ACTH and androstenedione/ACTH were 0.60 ± 0.16 and 0.30 ± 0.06 before and 0.55 ± 0.14 and 0.27 ± 0.70 after surgery respectively; the difference was not significant (Fig. 7).

Adrenal enzyme activity was evaluated in terms of the ratio of maximum increment of the various steroid hormones in response to the CRH test. The activity of 17,20-desmolase is expressed by the ratio Δ max androstenedione/17-OHP (Fig. 8A). The activity of 21- and 11 β -hydroxylase is expressed by the ratio Δ max

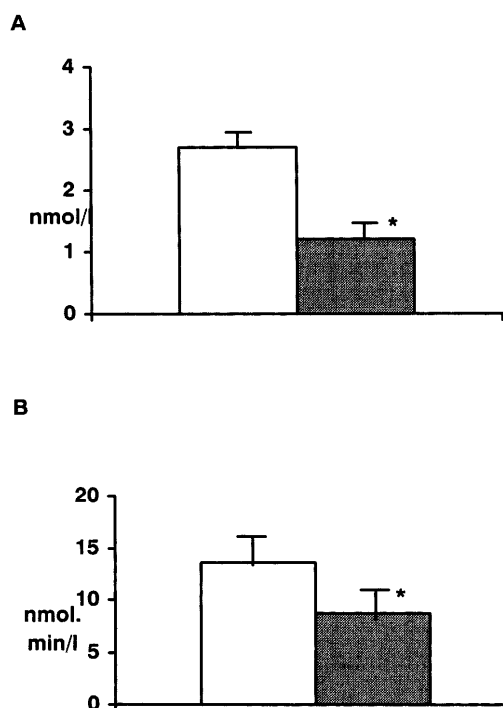


Figure 5 The maximum increase in 17-OHP (A) and the cumulated response (AUC) of 17-OHP to CRH (B) were significantly higher ($*P < 0.01$) before (open bars) than after ovariectomy (filled bars). Values are means \pm s.d.

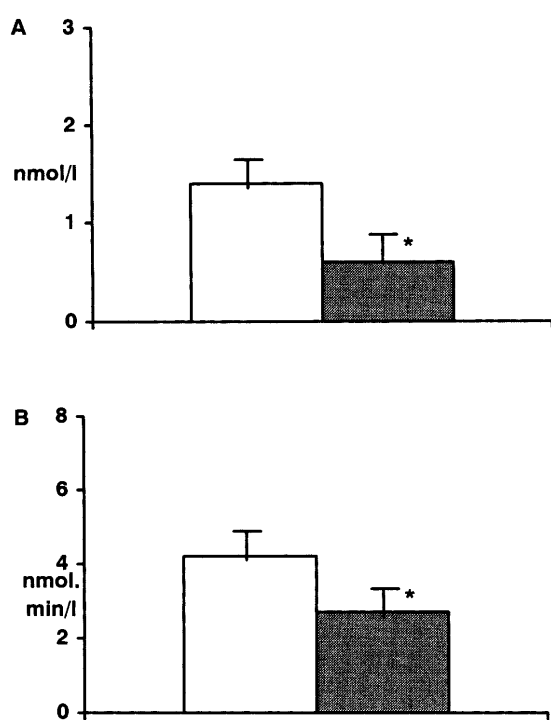


Figure 6 The maximum increase in androstenedione (A) and the cumulated response (AUC) of androstenedione to CRH (B) were significantly higher ($*P < 0.01$) before (open bars) than after ovariectomy (filled bars). Values are means \pm s.d.

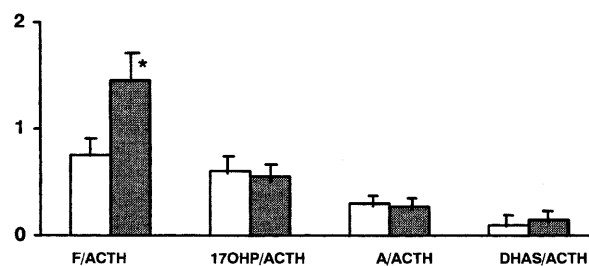


Figure 7 The ratio of maximum increment of cortisol ('F'), 17-OHP, androstenedione ('A') and DHAS to ACTH in response to CRH before (open bars) and after ovariectomy (filled bars). The ratio cortisol/ACTH was significantly higher ($*P < 0.01$) after ovariectomy. Values are means \pm s.d.

17-OHP/cortisol (Fig. 8B). The former ratio was 0.52 ± 0.11 before and 0.50 ± 0.10 after ovariectomy, indicating no significant difference in 17,20-desmolase activity after ovariectomy. The second ratio was 0.81 ± 0.16 and 0.37 ± 0.90 ($P < 0.05$) before and after ovariectomy respectively.

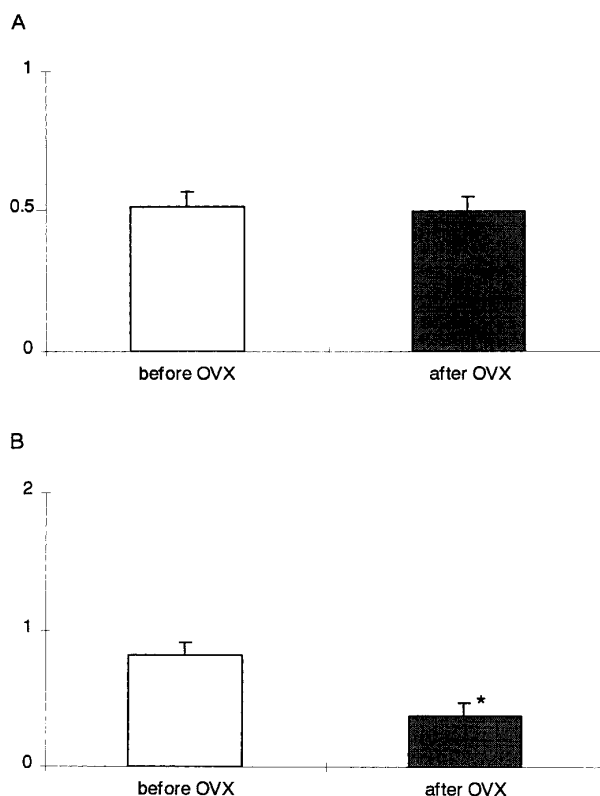


Figure 8 The Δ max androstenedione/17-OHP (A) and Δ max 17-OHP/cortisol (B) response to the CRH test before (open bars) and after ovariectomy (filled bars). The ratio 17-OHP/cortisol was significantly lower ($*P < 0.05$) after ovariectomy. Values are means \pm s.d.

Discussion

This study shows a lower ACTH response to CRH after ovariectomy, whereas the response of cortisol was substantially the same, with only a difference in the timing of the peak response. That the cortisol response was unchanged after ovariectomy may be due to increased adrenal sensitivity to ACTH, to reduced ACTH plasma levels and to increased adrenal levels. The deficit of estrogens could therefore determine the reduced response of ACTH to CRH on the one hand and an increase in adrenal sensitivity to ACTH on the other. The ratio Δ_{max} cortisol/ACTH increased significantly after ovariectomy, showing that the adrenal is in fact more sensitive to ACTH. The unchanged Δ_{max} 17-OHP/ACTH and androstenedione/ACTH ratios suggest that the increase in adrenal sensitivity is limited to cortisol production. The finding of estrogen receptors of uncertain function on adrenal cells (15–17) makes this hypothesis seem likely. These receptors may therefore be the site at which estrogens modulate adrenal activity. In one study a direct stimulation of the adrenals by estrogens is mentioned (18).

Studies, in human and in animals, have obtained results similar to the present (19, 20). In female rats ovariectomy reduced stress-induced ACTH concentrations which were restored by administration of estrogen but not by progesterone, whereas there was no substantial change in adrenal steroid levels (20). The response of ACTH to stress in ovariectomized rats was less than in normal rats, and the same was found for the steroids responses (21). In ovariectomized monkeys, administration of interleukin-1, an interleukin that activates the HPA axis, caused lower ACTH and cortisol responses than in controls, and these responses were subsequently restored by estradiol administration (22). These and other studies (5, 23, 24) provide evidence of the facilitatory effect of estrogens on the HPA axis, not only in animal models but also in humans. A reduction in the synthesis and release of pituitary ACTH after ovariectomy, restored by estradiol administration, has also been observed (25, 26).

Carey *et al.* (20) showed that estradiol has a facilitatory effect on the HPA axis, mediated by a reduction in the number and binding capacity of pituitary mineralocorticoid receptors and glucocorticoid receptors. Estrogen deficit, especially if acute (as after ovariectomy), induces profound modifications in the pituitary, including reduced activity of corticotrophic cells detectable through reduced levels of β -endorphins and β -lipotropin (27).

CRH receptors are found in the anterior lobe of the pituitary and also in melanocytes of the intermediate lobe. In 1992, a paper (28) reported that dopamine (DA) has a facilitatory effect on the intermediate lobe, characterized by up-regulation of CRH receptors. On the other hand, acute estrogen deficit caused a central reduction in DA which could lead to a reduction in CRH

receptors on corticotrophic cells. Similar observations were made by other researchers (25) who used hypothalamic extracts to stimulate pituitary release of ACTH and found a reduction in pituitary sensitivity after ovariectomy; this sensitivity was partially restored by administration of estrogens.

Ovariectomy also caused a reduction in basal levels of adrenal steroids such as 17-OHP, androstenedione, DHAS and testosterone, as well as reduced adrenal secretion of 17-OHP and androstenedione in response to CRH stimulation. The reduction in basal adrenal androgens observed after ovariectomy is probably the direct consequence of castration, though a secondary reduction in adrenal function in the immediate post-operative period cannot be excluded. The adrenal androgens measured have weak binding to SHBG and are largely bound to albumin (29), therefore changes in levels of carrier proteins cannot account for the effects of sex steroids.

The reduction in stimulated hormone levels on the other hand suggests that estrogens can affect adrenal steroidogenesis. Estrogens have been postulated to affect the enzyme activities of the adrenal gland (30, 31). No significant differences in 17,20-desmolase activity were revealed by the various ratios of adrenal steroid secretion. The Δ_{max} androstenedione/17-OHP ratio was not substantially altered by the reductions in the secretion peaks of androstenedione and 17-OHP after ovariectomy.

On the other hand, the significant reduction in the Δ_{max} 17-OHP/cortisol ratio indicates an increase in 21- and 11 β -hydroxylase activity. The possibility that this enzyme could be affected by estrogens is interesting. This could explain the normal, adrenal secretion of cortisol in women in the presence of low concentrations of 17-OHP, during acute absence of estrogens.

Our results show: (1) 17,20-desmolase activity is conserved while stimulated androstenedione and 17-OHP undergo similar percentage reductions; (2) CRH-induced concentrations of 17-OHP are considerably reduced after ovariectomy; and (3) there is a simultaneous increase in stimulated 21- and 11 β -hydroxylase activity with normal levels of cortisol and a significant reduction in adrenal production of 17-OHP. This suggests that acute estrogen deficit determines a slowing of the first enzymatic stages of steroidogenesis, including cleavage of the lateral chain of cholesterol, which is, among other things, the rate-limiting step of this metabolic pathway.

We can therefore conclude that the acute estrogen deficit is accompanied by changes in the HPA axis characterized by reduced pituitary secretion of ACTH but normal cortisol levels. Changes in adrenal steroidogenesis determine reduced stimulated production of androgens such as 17-OHP and androstenedione on the one hand and increased 21- and 11 β -hydroxylase activity to maintain cortisol production on the other.

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