Impact of Gender, Menstrual Cycle Phase, and Oral Contraceptives on the Activity of the Hypothalamus-Pituitary-Adrenal Axis

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Objective: Results from animal and human studies suggest that disregulations of the hypothalamus-pituitary-adrenal (HPA) axis are involved in several behavioral, circulatory, endocrine, and immune disorders with clear-cut gender differences in disease prevalence. The aim of the present study was to investigate sex-specific HPA response patterns with a focus on the contribution of gonadal steroids as possible mediators. **Methods:** A total of 81 healthy adults were investigated in the present study. Twenty men, 19 women in the follicular phase of the menstrual cycle, 21 women in the luteal phase, and 21 women using oral contraceptives (OC) were exposed to a brief psychosocial stress test (Trier Social Stress Test; TSST) and injected with 0.25 mg $ACTH_{1-24}$ on consecutive days. Basal HPA activity was investigated by repeatedly measuring cortisol levels immediately after awakening, as well as in 30-minute intervals from 9:00 AM to 9:00 PM. Additionally, questionnaires were used to assess psychological state and trait parameters. **Results:** Results show that the TSST induced significant increases in ACTH, salivary-free cortisol, total plasma cortisol, and heart rates, as well as increased wakefulness and reduced calmness in the total group. Significant group differences emerged for ACTH and salivary-free cortisol stress responses: Although men showed higher ACTH responses to the TSST compared with each of the three groups of women, salivary cortisol responses showed the following response pattern: Luteal = Men > Follicular = OC. The salivary cortisol responses to $ACTH_{1-24}$ showed a similar response pattern: Luteal > Men > Follicular > OC. In contrast, total blood cortisol levels did not reveal any group difference between sexes or follicular versus luteal phase in either test. Although a similar salivary-free cortisol increase after awakening was found in the four groups, the circadian cortisol profile was significantly different throughout the first 4 hours of sampling. Questionnaire-derived psychological variables, as measured in the present study, could not explain the observed results. **Conclusions:** We conclude that gender, menstrual cycle phase, and OC use exert important effects on HPA responsiveness to psychosocial stress in healthy subjects. Although men seem to have a stronger hypothalamic drive in response to stressful stimulation than women, differences in salivary-free cortisol levels, at least in part, may be explained by estradiol-induced changes in corticosteroid-binding protein levels. ACTH and cortisol secretion is not affected by OC use per se but the amount of bioavailable unbound cortisol ("free") is greatly reduced in this group of women after stimulation. Inasmuch as none of these differences between the study groups emerged in total blood cortisol levels, we strongly advocate for the simultaneous measurement of free and total cortisol levels in future studies on HPA functioning. **Key words:** psychosocial stress, HPA axis, sex differences, menstrual cycle, oral contraceptives, salivary cortisol, TSST, CBG.

ACTH = adrenocorticotropic hormone; ANOVA = analysis of variance; AUC = area under curve; BDI = Beck Depression Inventory, BMI = body mass index; CBG = corticosteroid-binding globulin; CRF = corticotropin-releasing hormone; CNS = central nervous system; DHEA = dehydroepiandrosterone; FBL = Freiburger Beschwerde Liste; HPA = hypothalamus-pituitary-adrenal axis; MANOVA = multivariate analysis of variance MDBF = Mehrdimensionaler Befindlichkeitsfragebogen; OC = oral contraceptive; RIA = radioimmunoassay; TSST = Trier social stress test; VAS = visual analog scale.

INTRODUCTION

A dysfunctional hypothalamus-pituitary-adrenal axis seems to be associated with manifestations of psychosomatic and psychiatric disorders. For example, HPA *hyper*activity is often found in major depression (1–4) and also seems to be associated with susceptibility to infectious diseases (5) and cardiovascular problems (6). *Hypo*reactivity of the HPA system is associated with autoimmune processes, such as lupus erythematosus (7), multiple sclerosis (8), or neurodermatitis (9, 10). Significant gender differences in prevalence and incidence are well documented for these diseases (11).

Among the multitude of factors contributing to individual differences in HPA activity, a complex sexual dimorphism seems to exist. Evidence from animal as well as human studies suggests that there are marked differences in HPA response patterns between males and females. Whereas in rodents, basal ACTH and corticosterone levels, as well as responses to various stimuli, are uniformly greater in females (12, 13), the picture is more complex in humans. Men seem to secrete more ACTH than women with comparable total cortisol levels under basal conditions, which suggests an increased sensitivity of the female adrenal cortex (14). At the pituitary level, no gender differences are observed after injection of synthetic human corticotropin-releasing factor (h-CRF) with or without pretreatment with dexamethasone (15–17). However,

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women may be more responsive to ovine CRF (18) or to a combination of h-CRF and vasopressin with respect to ACTH secretion (17). At a suprapituitary level, consistent gender differences in the HPA response to psychosocial stress have been observed in our laboratory. Concerning similar subjective or emotional responses, men always showed enhanced salivary (ie, unbound or "free") cortisol responses to a public speaking and mental arithmetic task in many studies (eg, 15, 19, 20). However, neither ACTH nor total blood cortisol levels have been measured in these studies, which would have allowed a more detailed localization of the observed sexual dimorphism.

Gonadal steroids, especially estradiol, seem to exert a substantial influence on the reactivity of the HPA axis responsiveness in rodents as well as in humans. Animal studies show that ovariectomy leads to attenuated HPA responses, whereas estradiol substitution induces HPA stimulation (21–23). Similarly, a short-term estradiol treatment leads to an enhanced ACTH and cortisol stress response in healthy young men (24) and a 2-week treatment with DHEA, a sex steroid precursor, increases the ACTH stress response in elderly postmenopausal women (25).

Pronounced changes in estradiol levels occur over the course of the menstrual cycle with low levels in the early follicular phase, which peak shortly before or during ovulation and slowly decrease throughout the luteal phase. Studies investigating HPA functions with respect to the menstrual cycle found that basal as well as stimulated ACTH and corticosterone levels are highest around the time of ovulation in the rat (26, 27). In contrast, human studies have produced inconsistent results with respect to possible changes of HPA (re)activity over the menstrual cycle (28–31).

Another consistent finding is rather puzzling and calls for explanation: women using ethinyl-estradiol-containing oral contraceptives (OC user) were found repeatedly to show blunted free cortisol responses to psychosocial or physical stress (32–33). It is unknown whether the lower free cortisol responses reflect a hyporeactive HPA axis in these women. Alternatively, OC users may secrete similar amounts of cortisol, but due to the increased production of CBG induced by OC medication, the biologically active free cortisol fraction is largely reduced.

The aim of the present study, therefore, was to investigate pituitary and adrenal responses to a potent psychosocial laboratory stress protocol (the TSST) (34) in different phases of the menstrual cycle. These endocrine, heart rate, and subjective responses were compared with OC users and men, respectively. In addition, a Synacthen ($ACTH_{1-24}$) test was performed to measure the capacity of the adrenal cortex in the experimental groups. Moreover, basal HPA activity was investigated with salivary-free cortisol levels after wakening in the morning and a 12-hour salivary-free cortisol profile.

METHODS

Subjects

A total of 81 healthy men and women between 18 and 32 years of age participated in this study. The study sample was composed of 20 men, 19 women in the follicular phase (days 4–7) of the menstrual cycle, 21 women in the luteal phase (days 21–25), and 21 women who had used oral contraceptives (monophasic formulas, ethinyl-estradiol content: <50 μg) for at least 6 months. Menstrual cycle phase was validated post hoc endocrinologically by measurement of estradiol and progesterone levels. Because the available resources did not allow us to assess women repeatedly for assessment of ovulation, no midcycle group was included.

Before entering the study, all subjects provided written consent and underwent a comprehensive medical examination for past and current health problems. Smokers, subjects suffering from allergies, women with irregular menstrual cycles, or using multiphasic contraceptives were excluded. The study protocol was approved by the ethics committee of the University of Trier. Table 1 shows the number of subjects, mean age, BMI, sex steroid, and CBG levels for the four experimental groups.

Experimental Protocol

Each subject reported three times to the laboratory. The first appointment consisted of a medical examination and subjects received the material and detailed instructions for saliva collection at home (Salivette; Sarstedt, Rommelsdorf, Germany). On the second day, a Synacthen ($ACTH_{1-24}$) (Ciba, Wehr, Germany) test was per-

TABLE 1. Sociodemographic Variables, Sex Steroid, and Corticosteroid-Binding Globulin Levels in the Four Experimental Groups

	Men (M)	Folliular Phase (F)	Luteal Phase (L)	Oral Contraceptive Users (OC)	*F*	*p*	Differences
Number	20	19	21	21	$F < 1$	NS[a]	
Age (yr)	23.5 ± 0.6	23.7 ± 0.7	23.2 ± 0.8	22.8 ± 0.4	$F < 1$	NS	
BMI	22.2 ± 0.5	21.5 ± 0.6	21.9 ± 0.5	21.1 ± 0.5	$F < 1$	NS	
Estradiol (pg/ml)	82.9 ± 3.9	87.7 ± 4.8	117.7 ± 7.9	63.6 ± 3.5	18.22	.0001	L > F = M > OC
Progesterone (ng/ml)	0.52 ± 0.1	0.42 ± 0.1	5.14 ± 1.4	0.27 ± 0.1	11.69	.0001	L > M = F = OC
CBG (μg/ml)	42.6 ± 1.6	48.4 ± 3.4	39.9 ± 0.8	73.7 ± 4.1	30.69	.0001	OC > F = M = L

[a] NS = not significant.

formed, followed by the psychosocial stress test on day 3. All experimental sessions started between 3:00 PM and 5:00 PM. A venous catheter was inserted and kept patent with a lock and subjects rested for 45 to 60 minutes. Thereafter, they filled in questionnaires (see below) before a baseline blood sample and a first saliva sample were collected. Then volunteers received an intravenous injection of 0.25 mg Synacthen on day 2 and additional blood and saliva samples were obtained 20, 30, 45, 60, and 90 minutes after injection. After baseline collection on day 3, subjects were exposed to the psychosocial stress test (TSST). The TSST, which mainly consists of a free speech and mental arithmetic task of 15 minutes duration performed in front of an audience, has been found repeatedly to induce endocrine and cardiovascular responses in 70% to 80% of all subjects tested (34). Because the endocrine responses to the TSST show a different pattern compared with the Synacthen test, blood and saliva samples were obtained 1, 10, 20, 30, 45, and 60 minutes after cessation of stress.

For assessment of basal free cortisol levels, participants collected saliva samples on the morning of day 2. Immediately after waking up and 15, 30, 45, and 60 minutes thereafter, saliva samples were obtained to measure the individual cortisol response to awakening. Recently, this laboratory has reported that salivary-free cortisol levels increase 50% to 150% within the first 30 minutes after waking up in the morning, independent of sleep duration, time of day, or other variables (35). Moreover, all subjects collected saliva from 9:00 AM to 9:00 PM at 30-minute intervals in their natural environment for assessment of the daytime circadian rhythm. The subjects were free to choose a day for collection of the circadian profile after completion of all laboratory tests. Accuracy of ambulatory saliva collection was not validated, but relied on the compliance of the subjects.

Psychological Assessment

Four psychological questionnaires were used to measure depression, physical complaints, mood changes, and perceived stressfulness of the TSST:

1. To control for a possible impact of depression on the HPA response (36), the Beck Depression Inventory (37) was used. Scores higher than 18 are assumed to represent clinically relevant depressive symptoms.
2. The short version of the Freiburger Beschwerdeliste (FBL) (38) measures physical complaints on five different scales (cardiac and blood pressure problems, gastrointestinal problems, tension, pain, total scale) with a total of 32 items. Subjects must choose one of six answers ranging from "not existent" to "very strong."
3. Momentary mood was assessed before and after the TSST with the MDBF, a German mood scale (39). This multidimensional questionnaire measures "elevated vs. depressed mood," "wakefulness vs. sleepiness," and "calmness vs. restlessness" on a five-point rating scale ranging from 1 = not at all to 5 = very much.
4. Six VAS were used for participants' ratings of the stressfulness of the TSST. After cessation of the stress task, subjects were required to rate the extent of their personal involvement, how stressful, new, uncontrollable, and unpredictable the task was, and whether they anticipated negative consequences.

The Depression Inventory and the FBL were administered at baseline, mood was assessed before and after the stress task, and the VAS was applied only after the stress test.

Biochemical Analyses

Basal blood samples were used to measure estradiol (RIA; Biermann, Bad Nauheim, Germany), progesterone (RIA; IBL, Hamburg, Germany), and CBG (IBL, Hamburg, Germany). Salivary-free cortisol concentration in saliva was measured using a time-resolved immunoassay with fluorometric detection, as described in detail elsewhere (40). Total plasma cortisol was measured with a radioimmunoassay (RIA; IBL, Hamburg, Germany). ACTH was determined with a two-site chemiluminescence assay (Nichols Institute, Bad Nauheim, Germany). Interassay and intraassay coefficients of variance were below 12% and 10%, respectively, for all analyses.

Heart Rate

Heart rates were measured continuously at 1-minute intervals with ECG precision using wireless transmission (Sport Tester Profi, Polar Instruments, Gross-Gerau, Germany). Heart rate responses were computed from 7 minutes before stress exposure to 9 minutes after cessation of stress.

Statistical Analyses

ANOVAs for repeated measures were computed to analyze endocrine and heart rate responses to the stressor. To control for different baseline levels, hormone samples obtained directly before the stress exposure were treated as covariates. All reported results were corrected by the Greenhouse-Geisser procedure where appropriate. Newman-Keuls post hoc tests were applied for significant effects. Correlations were computed by Pearson product-moment correlations. Psychological parameters were analyzed by ANOVAs. Furthermore, factor analyses (principal component, varimax oblique) and reliability analyses were used to create the scale "stressfulness" based on items of the VAS. For all analyses, the significance level was $\alpha = 5\%$. For multiple comparisons the nominal α level was adjusted by Bonferroni correction. All results shown are means ± SEM.

RESULTS

No subject had to be excluded from statistical analyses due to depression. BDI scores ranged between 0 and 18 and there were no differences among the four groups. Eleven subjects were classified as slightly depressed. Additional analyses could not reveal any significant correlations between depression scores and endocrine parameters (expressed as area under the curve). The physical complaint questionnaire (FBL) revealed that all subjects reported only minor physical impairments. As shown in Table 1, estradiol, progesterone, and CBC levels differed significantly among the four groups (F values = 18.22, 11.69, and 30.96; all p values $< .0001$). Newman-Keuls post hoc tests revealed that women in the luteal phase of the menstrual cycle had the highest estradiol and progesterone concentration with no differences between women in the follicular phase and men. OC users had the lowest levels of sex steroid levels with concurrent high CBG levels.

TABLE 2. Momentary Mood Assessed Before and After TSST With the Three Scales of the MDBF

MDBF	Before TSST	After TSST	Time Effect	Time by Group Effect
Elevated vs. depressed mood	3.9 ± 0.2	3.6 ± 0.1	NS[a]	NS
Wakefulness vs. sleepiness	3.3 ± 0.1	3.7 ± 0.1	$F = 8.35$; $p < .005$	NS
Calmness vs. restlessness	3.5 ± 0.1	3.2 ± 0.1	$F = 8.83$; $p < .004$	NS

[a] NS = not significant.

The psychosocial stress protocol caused significant endocrine, cardiovascular, and psychological responses. In the total group, ACTH, salivary-free cortisol, and total plasma cortisol increased significantly with 60% to 100% changes from baseline values. ACTH concentrations peaked 1 minute after cessation of the TSST with continuously decreasing hormone concentrations thereafter (ACTH: $F = 48.28$; $p < .0001$). Cortisol levels were highest 10 minutes after cessation of stress (salivary-free cortisol: $F = 32.90$; $p < .0001$; total plasma cortisol: $F = 47.78$; $p < .0001$). Maximum heart rate responses were observed between 6 and 13 minutes of stress exposure ($F = 29.0$; $p < .0001$). Correlations between ACTH and cortisol stress responses (expressed as areas under the response curves) were significant with $r = .57$ for total plasma cortisol ($p < .001$) and $r = .53$ for salivary-free cortisol ($p < .001$). Total plasma cortisol and salivary-free cortisol showed a correlation of $r = .62$ ($p < .001$).

MANOVAs revealed significant response differences between groups for ACTH stress responses ($F = 2.52$: $p = .05$; Figure 1) and salivary-free cortisol ($F = 2.96$; $p = .007$). Newman-Keuls post hoc tests confirmed that men showed significantly larger ACTH responses than women in the follicular phase at 1 and 10 minutes after stress exposure (both p values $< .004$) and at 1, 10, and 20 minutes after stress than both OC users and women in the luteal phase (all p values $< .01$). Concerning cortisol responsiveness, men had significantly higher salivary-free cortisol reactions than women in the follicular phase (all p values $< .03$) 10, 20, and 30 minutes after cessation of stress, and higher than OC users 1, 10, 20 and 30 after stress (all p values $< .025$). No differences in saliva cortisol emerged between men and women in the luteal phase (all not significant). Furthermore, women in the luteal phase showed higher salivary-free cortisol responses compared with women in the follicular phase 10 minutes after cessation of stress ($p = .016$) and higher saliva free cortisol concentrations than OC users 10 and 20 minutes after stress (both p values $< .002$). Women in the follicular phase and OC users did not differ in their salivary-free cortisol responses. Concerning total plasma cortisol, we only found significantly increased hormone concentrations 1 minute before stress in OC users compared with the other groups ($F = 5.38$; $p < .002$). No differences between groups emerged in total cortisol response patterns ($F < 1$). Likewise, heart rate responses did not differ among groups ($F < 1$).

Exogenous stimulation with $ACTH_{1-24}$ caused significant cortisol responses in the total group (total plasma cortisol: $F = 30.96$; $p < .0001$; salivary-free cortisol: $F = 250.33$; $p < .0001$; Figure 2). After $ACTH_{1-24}$ challenge, both total plasma cortisol and salivary-free cortisol showed continuously increasing hormone concentrations in the observed time period. Concerning total plasma cortisol, MANOVAs did not reveal significant group differences ($F = 1.66$; $p > .13$). In contrast, salivary-free cortisol responses showed significant differences among the four groups of subjects ($F = 2.28$; $p < .028$). Women in the luteal phase of the menstrual cycle had the highest salivary-free cortisol responses followed by men, follicular phase women, and OC users, respectively. Post hoc tests revealed significantly higher reactions in the luteal phase compared with men 60 and 90 minutes after Synacthen injection (both p values $< .01$), compared with women in the follicular phase 30, 60, and 90 minutes after $ACTH_{1-24}$ injection (all p values $< .004$), and compared with OC users 30, 45, 60, and 90 minutes after injection (all p values $< .0001$). Men showed higher levels than OC users 15, 30, 45, 60, and 90 minutes after injection (all p values $< .05$) with a similar response pattern as women in the follicular phase. The latter group showed higher salivary-free cortisol levels than OC users 30, 45, and 90 minutes after $ACTH_{1-24}$ challenge.

Salivary-free cortisol responses after stimulation with exogenous ACTH (expressed as AUC) correlated significantly with CBG levels ($r = -.44$; $p < .0001$), whereas after TSST-stimulation salivary-free cortisol levels (AUC) tended to correlate with CBG concentrations ($r = -.21$; $p = .084$; 2-tailed tests).

Analyses of the multidimensional mood scale, MDBF, revealed that stress exposure did not generally worsen mood ($F = 1.99$; $p < .16$), but significantly increased wakefulness ($F = 8.35$; $p < .005$) and re-

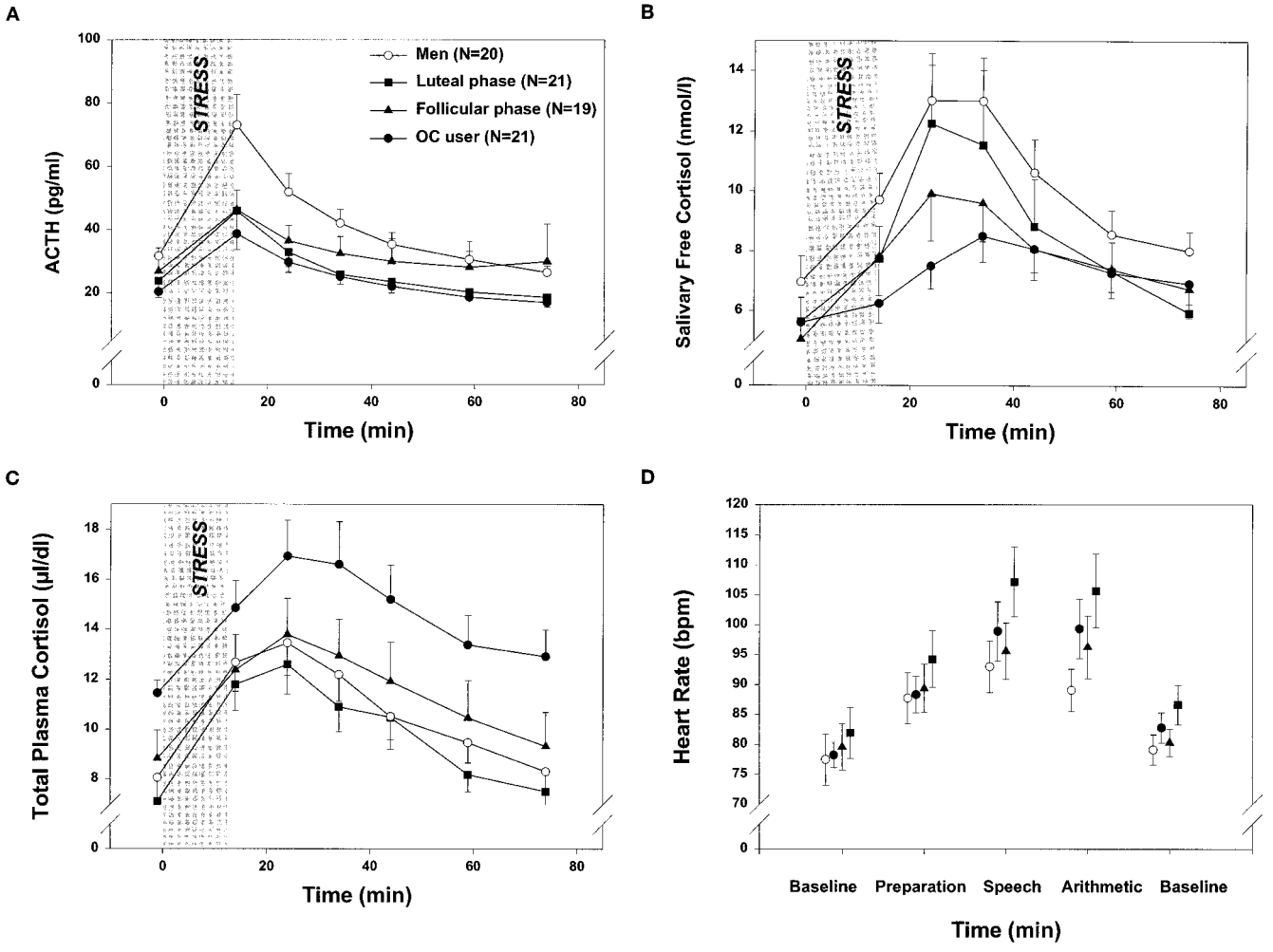

Fig. 1. *A*, ACTH level; *B*, salivary-free cortisol level; *C*, total plasma cortisol level; and *D*, heart rate responses before and after psychosocial stress (TSST) in men, women in the luteal or follicular phase of the menstrual cycle, and oral contraceptive users. Values are mean ± SEM.

duced calmness ($F = 8.83$; $p < .004$) in the total group. No group differences in any of the three scales were observed.

The participants' ratings of the stressfulness of the TSST were measured by six VASs. A factor analysis clustered the items to the factor "perceived stressfulness." Reliability of the resulting factor was $\alpha = .65$ (Cronbach's α). No differences emerged among groups, neither by comparing the clustered factor nor by comparing the single items. Also no group differences were found in any of the five scales of the questionnaire on physical complaints (FBL). Comparing sexes, women in general reported more strain than men (scale 3: $F = 6.73$; $p < .011$). However, after adjustment of nominal α level by Bonferroni correction for multiple comparisons, this result could be considered as a trend only.

Referring to basal HPA activity in their natural environment, the four groups did not show a clear-cut difference in salivary-free cortisol profiles after awakening ($F = 1.33$; $p > .22$). There was only a trend toward higher morning cortisol levels in women in the luteal phase ($F = 2.70$; $p < .07$) when the area under the response curve was compared. In 12-hour salivary-free cortisol profiles, ANOVA results indicated a small but statistically significant difference ($F = 1.61$; $p > .044$). However, the cortisol levels showed a rather inconclusive picture (Figure 3).

DISCUSSION

On the basis of findings from animal and human studies showing a significant impact of gonadal steroids on the activity of the HPA axis, the present study set out to investigate HPA responses to a potent laboratory stress test in subjects differing in endogenous gonadal steroid levels. Women in the follicular or lu-

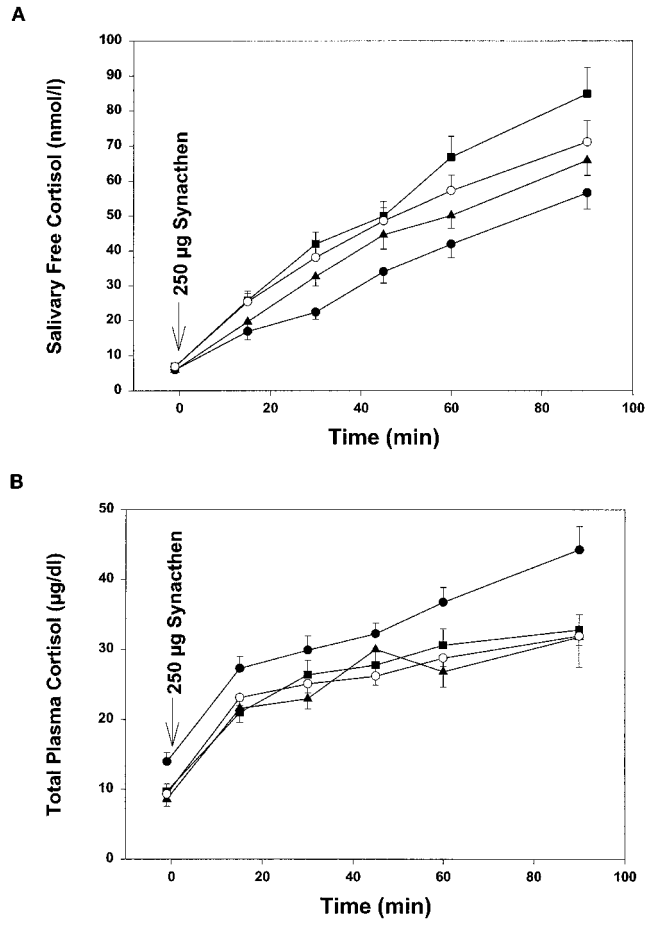

Fig. 2. *A*, salivary-free and *B*, total plasma cortisol responses to an injection of 0.25 mg $ACTH_{1-24}$.

teal phase of their menstrual cycle were compared with women using estrogen-containing contraceptive pills (OC). Inasmuch as previous studies conducted in this laboratory have consistently found a clear-cut gender difference in the salivary-free cortisol response to psychosocial stress (15), an age and BMI-matched group of healthy men was also included. Inasmuch as possible group differences could have emerged because of alterations in adrenal cortex capacity or sensitivity, a Synacthen test was performed as a second challenge to the axis.

The results demonstrate that, whereas gender differences or changes in HPA functioning over the course of the menstrual cycle are barely reflected in basal parameters, they are rather prominent when the system is activated by a potent psychosocial stressor. Although two studies reported cyclic variations of basal ACTH levels throughout the menstrual cycle (41, 42), no differences in prestress ACTH concentrations were observed in the present study. This difference can be explained by the timing of blood sampling: Whereas in the previous studies samples were obtained early in

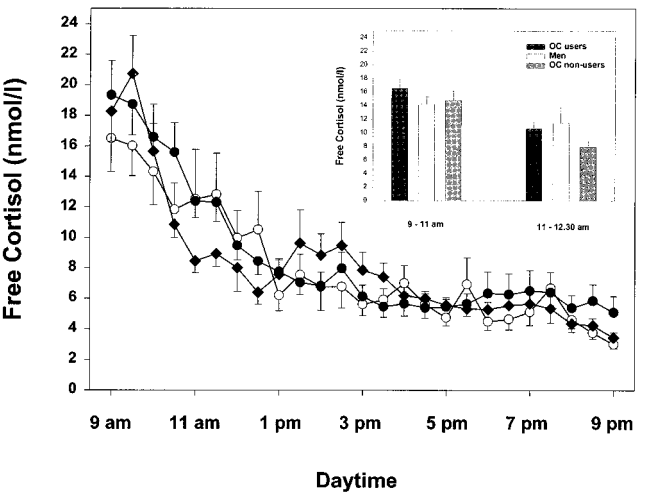

Fig. 3. Circadian saliva-free cortisol profiles obtained at 30-minute intervals between 9:00 AM and 9:00 PM. For better comparison, the insert shows the mean cortisol levels for two intervals with the largest differences among the four groups of subjects studied: 9:00 to 11:00 AM and 11:00 AM to 12:30 PM).

the morning or values collapsed over a 12-hour period (41, 42), we measured baseline ACTH levels in the late afternoon hours only. Like ACTH, basal cortisol levels before stimulation or immediately after awakening in the morning did not seem to show cyclic variation as measured in the follicular versus luteal phase, this observation agrees with several previous reports (42–44). There are only small, if any, differences in the circadian rhythm (9:00 AM-11:00 AM) between these subgroups.

In contrast to the findings of basal HPA activity, exposure to psychosocial stress resulted in different endocrine response profiles: Men showed larger ACTH increases compared with all three groups of women, lending support to the idea of an enhanced hypothalamic drive in men (45). It seems rather unlikely that differences in pituitary sensitivity to CRF are responsible for this result, inasmuch as challenge tests with human CRF result in similar ACTH responses in men and women in the same age group (16, 17).

Interestingly, the stress-induced cortisol patterns deviated to some degree from the ACTH picture: Men and women in the luteal phase had the largest salivary-free cortisol responses, which were clearly enhanced compared with women in the follicular phase. This was actually the first time that we have observed similar salivary-free cortisol responses to psychosocial stress in men and (one group of) women. In contrast to the biologically active free hormone fraction, total blood cortisol levels masked these differences with similar levels in the three groups (for discussion of the OC results, see below). This supports findings that

suggest that the female adrenal cortex may be more sensitive to ACTH (45, 46) with no apparent change of net cortisol secretion in follicular versus luteal phases. The capacity of the female adrenal cortex to synthesize and secrete cortisol in response to maximal stimulation, however, is similar to men, as shown in the Synacthen test. However, women in the luteal phase clearly have higher circulating salivary-free cortisol levels after $ACTH_{1-24}$ stimulation. These findings agree with a previous report (47).

The consistent differences between salivary-free and total plasma cortisol levels in response to psychosocial stress and synthetic $ACTH_{1-24}$ in the present study may explain discrepant results on gender response differences described in the literature. The measurement of total cortisol levels only suggest that there is a similar cortisol response in the sense of cortisol *secretion* in men and women. However, this research strategy might miss a biologically important message: There seems to be a significant gender and menstrual cycle phase difference in the *availability of free cortisol* (ie, biologically active steroid). In our view, these results call for simultaneous measurement of free and total cortisol levels whenever feasible. Only this enables the investigator to relate hyporesponsive versus hyperresponsive patterns to differences in cortisol production or tissue availability of the endocrine signal. Whether the target tissue counterregulates intracellular signal cascades in response to a changing free glucocorticoid signal in vivo is completely unknown.

We have previously reported that women using oral contraceptives show blunted salivary-free cortisol responses to physical and psychosocial stress (32, 33) and suggested that this was due to the well-documented CBG-enhancing effect of ethinyl-estradiol (43, 48, 49). The present results strengthen this hypothesis: OC users can produce and secrete similar amounts of ACTH and total plasma cortisol in response to psychosocial stress and $ACTH_{1-24}$; however, the salivary-free cortisol levels were clearly lower under both conditions. The notion that higher CBG levels may lead to lower salivary-free cortisol responses is supported by the negative correlations between cortisol and CBG. Although this might help to explain the group difference in saliva free cortisol levels, the observed gender difference in ACTH stress responsiveness without apparent influence of the menstrual cycle or OC use requires additional regulatory factors. Likely candidates are obviously gonadal steroids. Among them, estradiol is known to exert a strong stimulatory influence on the axis in several animal species (22, 23, 27, 50–52) with important modulatory effects on mineralocorticoid and glucocorticoid receptors (26, 53–56). Moreover, estradiol may directly enhance CRF gene transcription in the hypothalamus through binding to estrogen responsive elements on the CRF gene (57). But why should men have a stronger estrogen-driven CRF signal (as indicated by larger ACTH baselines and response levels), given that women usually produce significantly more estradiol over the course of the menstrual cycle than men? What seems to be a paradox may be explained by the metabolism of sex steroids in the brain. Most, but not all, CNS effects of testosterone are, in fact, estradiol-induced. In several tissues, it has been shown that testosterone must be aromatized to estradiol to modulate brain processes (58–63). Thus, the relative abundance of free testosterone in men favors a stronger stimulation of hypothalamic or hippocampal structures after conversion into estradiol compared with women.

Although estradiol seems to be the best candidate for explaining the different HPA responses observed in this study, changes in progesterone levels also might have contributed to these findings. Data on the impact of progestins on the HPA axis in humans are sparse; however, the available results do not suggest a significant mediation of ACTH and cortisol stress responses (64, 65).

The present results raise a number of questions concerning the consequences of high/low HPA activity. Are subjects who vigorously respond to psychosocial stress in the laboratory more susceptible or more resilient with respect to health outcome measures? Are lower free cortisol levels in the follicular phase or in OC users prospectively associated with an increased number of physical complaints? Or does their organism adapt to this endocrine situation by increasing intracellular responses to a given cortisol signal by, eg, upregulation of receptors or transcription factors? Future psychobiological studies most probably will include the intracellular components of the HPA axis signal cascade in order to understand in more detail how this multilevel regulated endocrine system helps the individual to fight off diseases and remain healthy, despite ever-changing environmental strains and demands.

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