

ORAL CONTRACEPTIVES AND THE DEXAMETHASONE SUPPRESSION TEST

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SUMMARY

Among other confounding factors, the influence of the intake of estrogen-containing oral contraceptives on the dexamethasone suppression test (DST) results has never been specifically studied. Therefore, we performed 1 mg DSTs in 14 healthy women taking oral contraceptives and 14 age-matched women taking no oral contraceptives. Mean 0800h basal total cortisol was significantly higher among the women taking contraceptives than in the control group, whereas mean free cortisol did not significantly differ. At 1600h following DST, no significant difference existed between the two groups. Two subjects taking contraceptives and one control subject were considered DST nonsuppressors. These results confirm the powerful influence of oral contraceptives on basal total cortisol levels but suggest a lack of significant influence on DST results.

INTRODUCTION

THE SPECIFICITY OF the dexamethasone suppression test (DST) for the diagnostic confirmation of major or endogenous depression remains a controversial issue, even with regard to normal control subjects (Braddock, 1986). Among other confounding factors, the influence of estrogen-containing oral contraceptives has been mentioned (Holsboer *et al.*, 1984). Estrogens raise the level of total cortisol through an increase in corticosteroid binding globulin (CBG) (Demey-Ponsart *et al.*, 1977; Stahl and Dörner, 1982), whereas the unbound (free) fraction is not influenced (Sandberg *et al.*, 1964). In a previous preliminary study, we found little influence of oral contraceptives on DST results in five healthy volunteers and two presurgical patients (D'Haenen *et al.*, 1987). The purpose of the present study was, therefore, to further assess the influence of oral contraceptives on DST results, addressing the relevance of using free cortisol levels in these subjects.

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TABLE I. CHARACTERISTICS OF THE SAMPLE

	Women taking oral contraceptives (n = 14)	Women not taking oral contraceptives (n = 14)	t	p
Age range, years	22-43	23-42		
Mean age, years (SD)	29.0 (6.3)	30.4 (5.7)	-0.6	NS
Weight, kg (SD)	55.6 (6.2)	59.8 (5.4)	-1.9	NS
Height, cm (SD)	162.9 (4.6)	164.4 (4.1)	-0.9	NS
Systolic blood pressure, mmHg (SD)	120.0 (7.8)	117.9 (8.2)	0.7	NS
Diastolic blood pressure, mmHg (SD)	75.0 (7.6)	68.9 (6.6)	2.3	0.03
Pulse rate (SD)	79.9 (13.0)	76.5 (6.2)	0.7	NS
Beck Depression Inventory (SD)	4.4 (2.8)	3.4 (3.1)	0.7	NS
Number of pregnancies (SD)	0.7 (0.8)	0.6 (0.9)	0.4	NS
Daily use of alcoholic beverages, glasses (SD)	0.9 (1.5)	0.0 (0.0)	2.2	NS
Daily use of tobacco, g (SD)	8.6 (10.8)	4.6 (8.4)	1.1	NS
Duration of use of oral contracep- tives, years (SD)	5.0 (3.2)	—	—	—

NS = not significant.

METHODS

Subjects

Fourteen healthy female volunteers, aged 22 to 43 years (mean age = 29.0 ± 6.3 years) taking oral contraceptives were recruited for this study. All subjects were carefully screened for any medical illness through history and medical examination.

Moreover, the subjects were free of present and past psychiatric illness as well as in their first degree relatives. They also scored less than 8 on the 21-item Beck Depression Inventory (Beck *et al.*, 1961). Daily estrogen content (ethinyl estradiol) was 30 μg in eight cases and 50 μg in six cases. All subjects were individually matched for age (within 3 years) with 14 healthy female volunteers not taking oral contraceptives also screened through the same procedure. The clinical characteristics of the two samples of subjects are given in Table I.

The protocol was approved by the Ethical Committee of the University of Liège Medical School and all subjects gave written informed consent.

DST Procedure

DSTs were performed randomly throughout the menstrual cycle according to the simplified procedure described by Carroll (1982). At 0800h on day 1, a basal 10 cc blood sample was collected. Subjects were given dexamethasone 1 mg orally on the same day at 2300h. A second 10 cc blood sample was collected the following day at 1600h. Blood was immediately centrifuged and serum was stored at -20°C until analysis. DST nonsuppression was defined as a post-DST total cortisol level $>5 \mu\text{g}/\text{dl}$ (Carroll, 1982) or a free cortisol level $>0.15 \mu\text{g}/\text{dl}$ (Ansseau *et al.*, 1984; Charles *et al.*, 1986).

Cortisol Assay

Plasma total cortisol was measured by direct radioimmunoassay (RIA) from sample of 25 μl , 40-fold diluted, and heated at 60°C for 30 min. RIA used ^{125}I -cortisol (Farnos Diagnostica, Finland) and anticortisol antiserum (made against the 3-CMO-BSA conjugate), as described previously (Sulon *et al.*, 1978). Free cortisol was measured by the equilibrium dialysis method. A total of 1 ml of diluted serum (1/10) was dialysed for 24 hr at 37°C against 1 ml of 0.05 M phosphate buffer (pH 7.4) containing tritiated cortisol. Before dialysis, another 1 ml of diluted serum was treated at 60°C for 20 min in order to denature CBG (a thermolabile alpha-2-globulin), then dialysed as described above to determine the albumin-bound cortisol fraction (Demey-Ponsart *et al.*, 1977). All samples were processed in duplicate within the same assay, with a maximal intra-assay coefficient of variation of 4.3% and a detection limit of 1.0 $\mu\text{g}/\text{dl}$.

Data Analysis

Subjects with and without oral contraceptives were compared using group *t*-tests. The relationship between total and free cortisol was assessed by the Pearson product moment correlation coefficient. As cortisol concentration tended to be log-normally distributed, the data were analyzed by using a natural log-transformation.

RESULTS

The distributions of total and free cortisol levels at 0800h before DST and at 1600h after DST are displayed in Figs. 1 and 2. At 0800h before DST, mean total cortisol was significantly higher among the women taking contraceptives than in the control group ($39.8 \pm 12.3 \mu\text{g}/\text{dl}$ vs. $16.8 \pm 8.6 \mu\text{g}/\text{dl}$, $t = 7.0$, $p = 0.0001$), whereas mean free cortisol did not significantly differ ($0.71 \pm 0.21 \mu\text{g}/\text{dl}$ vs. $0.74 \pm 0.68 \mu\text{g}/\text{dl}$, $t = 1.8$, $p = \text{NS}$).

At 1600h after DST, no significant differences were present between the two groups: for total cortisol, $2.5 \pm 2.7 \mu\text{g}/\text{dl}$ in women taking contraceptives versus $1.5 \pm 1.5 \mu\text{g}/\text{dl}$ in the control group, $t = 0.5$, $p = \text{NS}$; and for free cortisol, respectively, $0.04 \pm 0.04 \mu\text{g}/\text{dl}$ versus $0.05 \pm 0.06 \mu\text{g}/\text{dl}$, $t = 1.5$, $p = \text{NS}$.

Using total cortisol, three subjects (11%) could be considered to be DST nonsuppressors: two subjects taking contraceptives (14%) and one subject in the control group (7%). Using free cortisol, only one subject (in the control group) was a DST nonsuppressor.

In the whole sample, the relationship between total and free cortisol reached 0.65 ($df = 26$, $p = 0.0003$) before DST and 0.82 ($df = 26$, $p = 0.0001$) after DST. Corresponding figures in the women taking contraceptives were, respectively, 0.52 ($df = 12$, $p = \text{NS}$) and 0.86 ($df = 12$, $p = 0.0001$) and in the control group, 0.71 ($df = 12$, $p = 0.004$) and 0.94 ($df = 12$, $p = 0.0001$).

DISCUSSION

The results of the present study confirm the influence of estrogen-containing oral contraceptives on basal total cortisol levels. Even at very low doses (30 or 50 $\mu\text{g}/\text{day}$ of ethinyl estradiol), estrogens more than double basal total cortisol levels. These effects result from an increased CBG synthesis (Demey-Ponsart *et al.*, 1977; Stahl and Dörner, 1982; Rosner, 1990). CBG binds cortisol with a high affinity and in basal

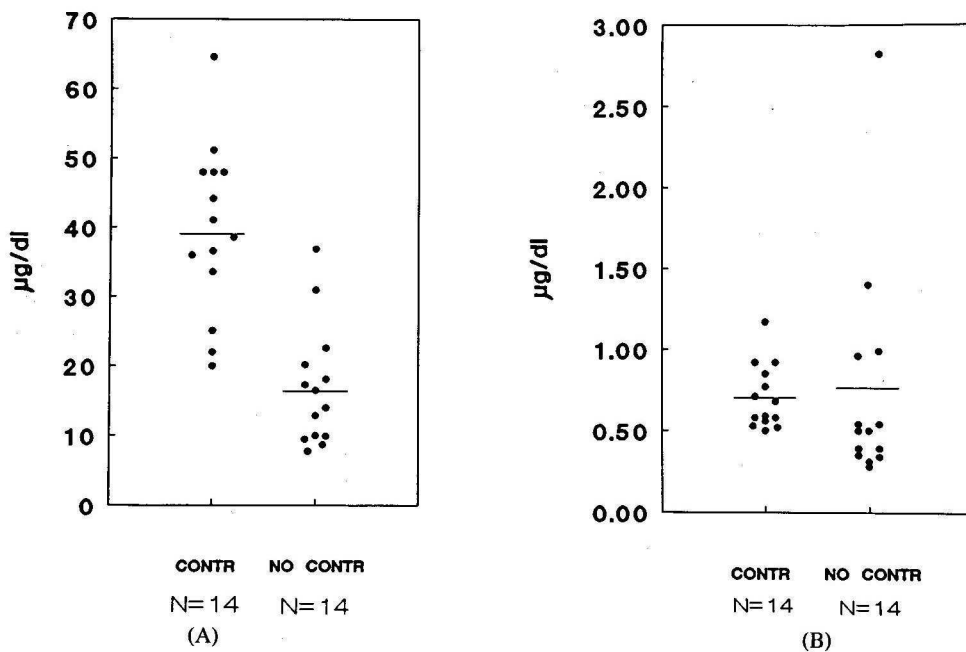


FIG. 1: Distribution of 0800h (A) total and (B) free cortisol levels before DST in 14 women taking oral contraceptives (CONTR) and in 14 age-matched women without contraceptives (NO CONTR). The horizontal bar in each case represents the mean.

conditions, nearly all the binding sites for cortisol are occupied. Only the unbound (free) fraction of cortisol is biologically active, controlling the metabolism of target tissues and exerting an inhibitory action on the secretion of corticotropin-releasing factor (CRF) and adrenocorticotrophic hormone (ACTH). Our study confirms the lack of significant influence of oral contraceptives on basal free cortisol levels, not only by the lack of significant differences between women taking oral contraceptives and the control group but also by the lack of a significant relationship between total and free cortisol in the women taking contraceptives, in contrast with the control group. Therefore, free cortisol should be preferred as a reliable index of basal cortisol secretion in all women taking oral contraceptives.

Following DST, the difference previously noted in total cortisol level between women taking contraceptives and control subjects totally disappears. This finding suggests that dexamethasone is able to influence both bound and unbound cortisol levels. The diagnostic performance of the DST in this group of healthy female subjects was therefore not modified by the intake of oral contraceptives. These results confirm a previous study in a small group of healthy controls and presurgical subjects where oral contraceptives did not seem to impair DST results (D'Haenen *et al.*, 1987).

In fact, these results were predictable from what is known about cortisol dynamics and the effects of dexamethasone. Dexamethasone completely suppresses ACTH-

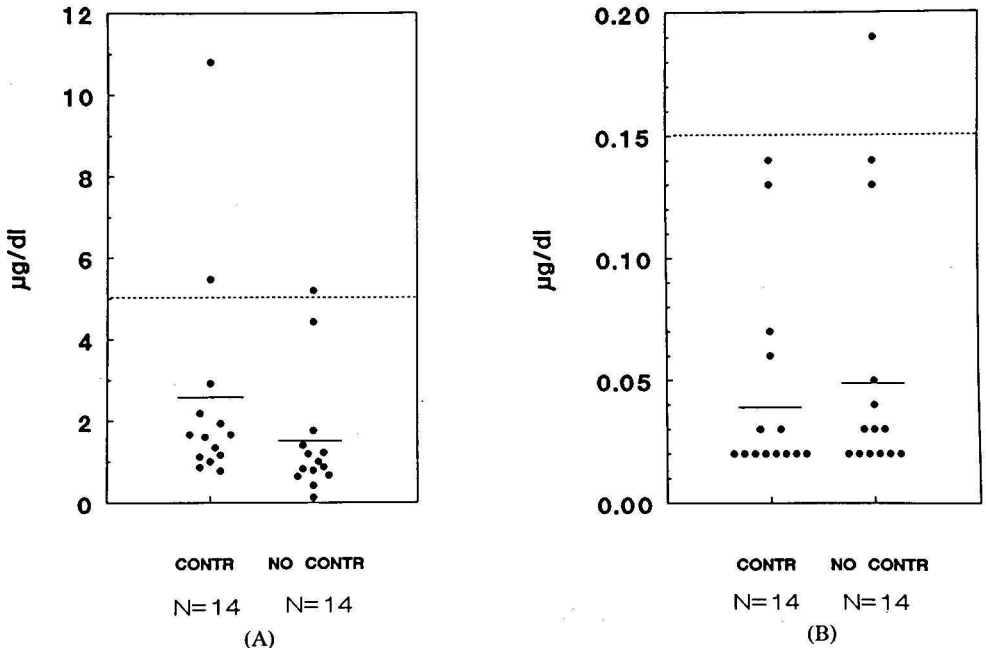


FIG. 2: Distribution of 1600h (A) total and (B) free cortisol levels after DST in 14 women taking oral contraceptives (CONTR) and in 14 age-matched women without contraceptives (NO CONTR). The horizontal bar in each case represents the mean.

adrenal function (Liddle, 1960; Saito *et al.*, 1979; Holsboer *et al.*, 1984; Pfohl *et al.*, 1985). The binding kinetics of cortisol to CBG and the half-life of cortisol (60–90 min) are such that by 8 hr after dexamethasone, all cortisol has been unbound and metabolized in either normal and estrogen-treated subjects (Sandberg *et al.*, 1964; Krieger and Aschoff, 1979; Hammond, 1990; Rosner, 1990). This is the same mechanism that accounts for the low nadir cortisol values observed during each circadian cycle (Krieger and Allen, 1975).

The use of our whole sample of healthy subjects as control group in DST studies would yield an overall specificity of 89%. This figure is very close to the mean specificity of the DST in major depressives versus normal controls (93%) calculated in a survey of 30 studies involving a total of 1130 subjects (Arana *et al.*, 1985). The figures for nonsuppression of cortisol after 1 mg of dexamethasone in normal control subjects however varied widely: 4% (Carroll *et al.*, 1981), 4.3% (Rush *et al.*, 1982), 10.3% (Stokes *et al.*, 1983), 11% (Coppen *et al.*, 1983), 15.1% (Amsterdam *et al.*, 1982), and 19% (Hällström *et al.*, 1983).

Besides the measurement of plasma free cortisol itself (Charles *et al.*, 1986), other possible ways to circumvent the influence of estrogens as confounding factors in the assessment of cortisol secretion include the assay of urinary cortisol (Charles *et al.*, 1981) or saliva cortisol (Poland and Rubin, 1982; Anseau *et al.*, 1984; Copolov *et al.*, 1985), directly related to plasma free cortisol.

In conclusion, this study demonstrates the powerful influence of estrogen- containing contraceptives on basal total cortisol levels but suggests a lack of significant influence on DST results. The recourse to the assay of free cortisol therefore does not seem necessary in the interpretation of DST results, at least if the daily estrogen content does not exceed 50 μg .

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