BIOL PSYCHIATRY 549
1986;21:549–552

Free Cortisol and the Dexamethasone Suppression Test

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Introduction

The overnight Dexamethasone Suppression Test (DST), currently the most widely used biological marker of endogenous depression, is generally performed with measurement of total plasma cortisol. In plasma, cortisol is reversibly bound to two plasma proteins, transcortin and albumin. Only the unbound (free) fraction 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 was undertaken to assess the relationship between plasma total and plasma free cortisol in the DST for endogenous depression, to define the best cut-off level for free cortisol, and to compare the diagnostic relevance of the two assays.

Methods

Subjects

Eighty-nine major depressive patients consecutively admitted to the Biological Psychiatry and Psychopharmacology Unit of the University Hospital of Liège or to the department of psychiatry of the Civil Hospital of Charleroi (Belgium) were included in the study. Patients had a score of at least 17 on the 24-item Hamilton Depression Scale. The patients were classified according to Research Diagnostic Criteria as 33 endogenous and 56 nonendogenous depressives. The sample included 38 male and 53 female patients, with 15 male and 18 female subjects in the endogenous group, and 23 male and 33 female subjects in the nonendogenous group. Ages ranged from 20 to 65 years, with a mean (SD) of 46.5 years (16.5). All patients were free of medical illness. They had also been without medication for at least 2 weeks at the time of the study and had given fully informed consent.

DST Procedure

The DST was performed according to the simplified procedure standardized by Carroll et al. (1981). Oral dexamethasone (1 mg) was administered by a nurse at 11:00 PM, and a post-dexamethasone sample was collected at 4:00 PM on day 2. Blood was immediately centrifuged, and serum was stored at −20°C until analysis.

Cortisol Assays

Total plasma cortisol was measured by direct radioimmunoassay (RIA) from samples of 25 µl, which were diluted 40-fold, and heated at 60°C for 30 min. RIA used ^{125}I-cortisol (Farmos

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Received August 10, 1985; revised October 29, 1985.

Diagnostica, Finland) and anticortisol antiserum (made against the 3-CMO-BSA conjugate), as previously described (Sulon et al. 1978). Free cortisol was measured by the equilibrium dialysis method. One milliliter of diluted serum (1/10) was dialyzed 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 transcortin (a thermolabile alpha$_2$-globulin), then dialyzed as described above to determine the albumin-bound cortisol fraction (Demey-Ponsart et al. 1977).

All assays were processed in duplicate, with maximal intra- and interassay coefficients of variation being 4.3% and 8.3%, respectively, based on multiple pool replicates.

Data Analysis

The relationship between total and free plasma cortisol was assessed by the Pearson product-moment correlation coefficient (one-tailed test). Group *t*-tests were used to analyze the differences between endogenous and nonendogenous depressives. Finally, a discriminant analysis was performed to assess the contribution of total and free cortisol to the diagnostic dichotomy. As cortisol concentrations tended to be log-normally distributed, the data were analyzed by using a natural log transformation.

Results

The relationship between total and free cortisol was very significant for the whole sample ($r = 0.90$, $p < 0.001$), as well as in the endogenous and in the nonendogenous groups taken separately ($r = 0.94$ and 0.87, $p < 0.001$, respectively). This correlation was also significant among the 50 DST suppressor depressives (total cortisol lower than 5 μg/dl) ($r = 0.73$, $p < 0.001$), as well as among the 39 DST nonsuppressor depressives (total cortisol higher than 5 μg/dl) ($r = 0.83$, $p < 0.001$).

The distribution of total and free plasma cortisol among endogenous and nonendogenous depressive patients is displayed in Figure 1. Endogenous depressives exhibited significantly higher cortisol levels than nonendogenous depressives: for total cortisol, 8.6 ± 5.8 μg/dl versus 5.1 ± 5.9 μg/dl, $t = 3.0$, $p < 0.01$, and for free cortisol, 0.46 ± 0.41 μg/dl versus 0.18 ± 0.33 μg/dl, $t = 3.5$, $p < 0.001$.

With regard to total cortisol, the best cut-off was 5 μg/dl, yielding a sensitivity of 76%, a specificity of 77%, and a diagnostic confidence of 66%. A free cortisol cut-off of 0.15 μg/dl resulted in a similar diagnostic performance (Figure 2). Discriminant analysis showed that free cortisol was able to correctly classify 76.4% of the sample, as compared to 70.7% for total cortisol, whereas the use of both free and total cortisol results slightly improved diagnostic performance to 77.5%.

Discussion

The results of the present study show a high correlation between plasma total and free cortisol in depressive patients following DST. This indicates that one can use free cortisol in the DST for diagnostic confirmation of endogenous depression with results at least equivalent to total cortisol. The suggested cut-off is 0.15 μg/dl.

These findings carry certain theoretical implications. The biological activity of steroids parallels the "free" hormone concentration. Transcortin binds cortisol with a high affinity, and in basal conditions, nearly all the binding sites for cortisol are occupied. However, for a given transcortin concentration the number of binding sites available for cortisol can be modified by the level of other steroids competing for the same sites (progesterone, 17-hydroxyprogesterone). Moreover, transcortin displays a circadian rhythm that influences the number of its binding sites (Sandberg et al. 1964). In contrast, the binding with albumin is characterized by a high capacity and a low affinity. Therefore, free cortisol levels can be appropriately used instead of total cortisol in all conditions where total cortisol assay yields invalid results due to changes in protein levels, as in pregnancy or after intake of oral contraceptives.

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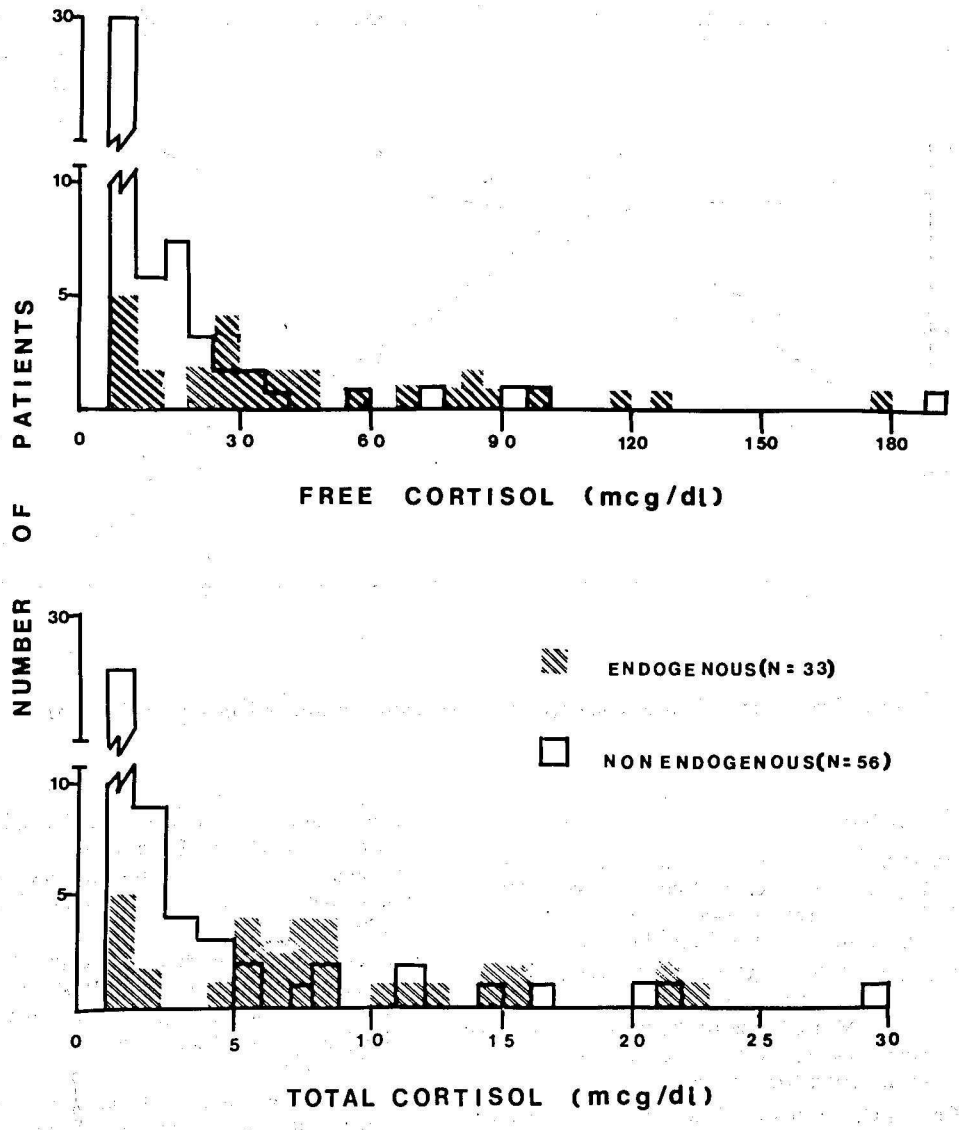

Figure 1. Distribution of free (top) and total (bottom) cortisol levels at 4:00 PM after DST in endogenous and nonendogenous depressive patients.

Free cortisol can also be measured in urine or in saliva. However, the use of urinary cortisol in the DST is limited by the need to collect urine over a 24-hr period and does not enhance the diagnostic sensitivity of the test (Charles et al. 1981). Saliva cortisol can also be used in the DST for diagnostic confirmation of endogenous depression, with a suggested cut-off of 70 ng/ml (Ansseau et al. 1984). The measurement of free cortisol in plasma or in saliva should be further tested for its reliability as an index of non-suppression in all conditions where the total cortisol assay is devoid of diagnostic significance in the DST.

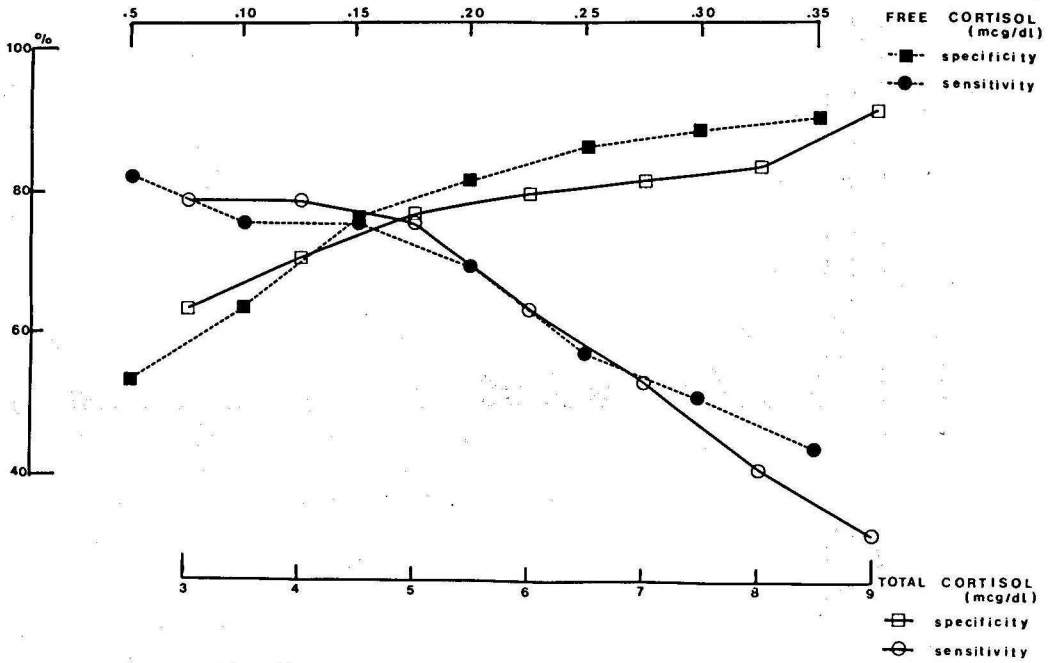

Figure 2. Diagnostic performance in endogenous depression of free and total cortisol at 4:00 PM after DST.

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