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# Combined dexamethasone/corticotropin-releasing factor test in chronic fatigue syndrome

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**Background.** Studies of hypothalamic–pituitary–adrenal (HPA) axis function in chronic fatigue syndrome (CFS) point to hypofunction, although there are negative reports. Suggested mechanisms include a reduced hypothalamic or supra-hypothalamic stimulus to the HPA axis and enhanced sensitivity to the negative feedback of glucocorticoids. The aim of the current study was to investigate HPA axis function in CFS with the dexamethasone/corticotropin-releasing factor (Dex/CRF) test, in analogy with research in affective disorders.

**Method.** Thirty-four well-characterized female CFS patients and 25 healthy control subjects participated in the low-dose Dex/CRF test. Current major depressive episode was an exclusion criterion. History of early-life stress (ELS) was assessed with the Structured Trauma Interview.

**Results.** Salivary cortisol responses after 0.5 mg Dex were lower in CFS patients than in controls (before 100 µg CRF,  $p=0.038$ ; after 100 µg CRF,  $p=0.015$ ). A secondary analysis revealed an influence of early-life stress and of oestrogen intake. After removal of the 10 participants who were taking an oral oestrogen, patients without a history of ELS showed lower cortisol responses than patients with ELS and controls (before CRF,  $p=0.005$ ; after CRF,  $p=0.008$ ).

**Conclusions.** CFS is globally associated with reduced cortisol responses in the combined low-dose Dex/CRF test, but this effect is only clearly present in CFS patients without a history of ELS. This study provides further support for an enhanced glucocorticoid negative feedback and/or a reduced central HPA axis drive in CFS. Furthermore, it demonstrates that ELS is an important variable to consider in CFS research.

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**Key words:** Child abuse, cortisol, CRF, somatoform disorder, stress.

## Introduction

Chronic fatigue syndrome (CFS) is characterized by unexplained, profound disabling and long-lasting fatigue that is of new or definite onset, that is not the result of ongoing exertion and that is not substantially alleviated by rest. The fatigue must be accompanied by at least 4 or more of the following case-defining symptoms during at least 6 months of consecutive illness: sore throat, tender cervical or axillary lymph nodes, muscle pain, multi-joint pain, post-exertional malaise, unrefreshing sleep, headaches and impaired memory or concentration (Fukuda *et al.* 1994). The suggestion that CFS may be related to a dysfunction

of the hypothalamic–pituitary–adrenal (HPA) axis derives from the similarity in symptoms between CFS patients and patients with Addison's disease or after bilateral adrenalectomy (Cleare, 2003). Moreover, the HPA axis offers an interesting physiological basis for the link between CFS, immunological disturbances and physical or psychological stress (Gold & Chrousos, 2002; Gerrity *et al.* 2004; Van Houdenhove & Egle, 2004). In the past, stress has generally been associated with HPA axis hyperactivity, resulting in hypercortisolism. However, chronic stress can also lead to hypocortisolism, as is the case in several stress-related disorders (Heim *et al.* 2000a; Gold & Chrousos, 2002; Fries *et al.* 2005). In CFS, the overall balance of evidence points to reduced cortisol output in at least a proportion of the patients, despite the negative reports and the poor quality of many studies (Cleare, 2003).

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In addition to measurements of basal hormone concentrations, dynamic challenges of the HPA axis have been undertaken for several reasons: to investigate at which level there may be abnormalities in the control of the HPA axis, to detect more subtle disturbances of the axis and to provide a more reliable and standardized indicator of the HPA axis disturbance (Cleare, 2003). Blunted responses of cortisol and/or adrenocorticotrophic hormone (ACTH) to awakening and to several challenge tests [corticotropin-releasing factor (CRF), insulin tolerance test, naloxone, social stress and exercise] have been reported in CFS (Demitrack *et al.* 1991; Scott *et al.* 1998*a,b*; Gaab *et al.* 2002*a*; Roberts *et al.* 2004). Suggested mechanisms for the findings in these challenge studies in CFS include a deficiency of hypothalamic CRF or vasopressin and a reduced supra-hypothalamic stimulus to the HPA axis, resulting in hypocortisolism (Demitrack *et al.* 1991; Demitrack & Crofford, 1998; Scott *et al.* 1998*a*, 1999; Gaab *et al.* 2002*a*). Another possible mechanism of hypocortisolism is enhanced sensitivity to the negative feedback of glucocorticoids, also referred to as dexamethasone (Dex) hypersuppression (Heim *et al.* 2000*a*; Fries *et al.* 2005). As to this mechanism, Gaab *et al.* (2002*b*) have demonstrated that patients with CFS show an enhanced and prolonged suppression of salivary free cortisol after the administration of a low dose of Dex (0.5 mg). Further support for enhanced sensitivity for glucocorticoids in CFS comes from *in vitro* studies, showing that lower concentrations of Dex were needed to inhibit interleukin production and proliferation of peripheral blood mononuclear cells from subjects with CFS (Visser *et al.* 1998, 2001*a,b*; Gaab *et al.* 2003*a*).

Surprisingly, there are no published reports on investigations with the combined Dex/CRF test in CFS. This is in contrast with research in major depressive disorder (MDD), a syndrome that is – inversely to CFS – characterized by hyperfunction of the HPA axis, reduced glucocorticoid negative feedback (Dex hyposuppression) and hypercortisolism (Plotsky *et al.* 1998; Claes, 2004). More precisely, the combined (high-dose) Dex/CRF test has been shown to be the most sensitive HPA axis function test in MDD (Heuser *et al.* 1994*a*). In this test, the CRF-induced ACTH and cortisol secretion is significantly higher in most depressed patients in comparison to healthy controls if a high-dose Dex (1.5 mg) pretreatment is administered in the evening preceding the CRF challenge (Heuser *et al.* 1994*a*). Both reduced glucocorticoid negative feedback and increased central drive of the HPA axis have been proposed as underlying mechanisms in MDD (Cole *et al.* 2000; Holsboer, 2000).

The aim of this study was to compare the cortisol responses in the low-dose (0.5 mg) combined Dex/CRF test between well-characterized patients with CFS and healthy controls without CFS. It was expected that CFS would be associated with a reduced cortisol response in this test, due to enhanced negative feedback and/or reduced central drive of the HPA axis.

## Method

### Subjects

Patients attending the CFS clinic of the Department of Internal Medicine of Antwerp University Hospital were invited to participate in the study by letter. As described in the literature (Jason *et al.* 1999), the majority of the patients were women. Since gender is an important variable in HPA axis research (Heuser *et al.* 1994*b*; Kirschbaum *et al.* 1999; Künzel *et al.* 2003) and since only a few men responded to the invitation, only female patients were included. CFS was diagnosed according to the Center for Disease Control Criteria (Fukuda *et al.* 1994) by an experienced internist (G.M.). All patients underwent serial physical examinations and laboratory evaluations; no alternative medical diagnosis could be established. Initially, 35 patients were included. One patient was excluded due to a panic attack during the Dex/CRF test, leaving 34 patients. Twenty-five healthy females without a past history of CFS were recruited by an advertisement in Antwerp University Hospital. They were matched for age and educational level. Five levels of education were considered in accordance with the Belgian education system. The patient group was larger in order to allow a secondary analysis of potential influencing clinical variables within this group. The data were collected between October 2003 and November 2004. Pregnancy, a lifetime history of organic brain disease, current medical condition or drugs that might influence neuroendocrine testing (with the exception of psychotropic drugs and oral contraceptives) were exclusion criteria. All participants were Caucasian and had grandparents of European origin. The Medical Ethical Committee of the University Hospital of Antwerp approved the study. All participants were aged >18 years and signed informed consent.

### Interviews and questionnaires

All participants were interviewed with the Dutch version of SCID-I/P version 2.0 (First *et al.* 1999) by a trained psychiatrist (F.V.D.E.). The CDC psychiatric exclusion criteria for CFS were adopted, with three exceptions. First, subjects who met the criteria for

a current major depressive episode according to DSM-IV (APA, 1994) were excluded, in order to avoid a state effect of MDD on the outcome in the Dex/CRF test. Second, a history of melancholic depression was not assessed systematically, because the specification of melancholic features in the past is not provided in SCID-I/P and because it is difficult to assess this item retrospectively by means of an interview. Finally, one control subject had suffered from anorexia nervosa in the past, but was in complete remission for more than 5 years. The Checklist Individual Strength was used to measure patient's perceived fatigue concentration, motivation and physical activity. This 20-item self-rating scale is well validated and reliable (Vercoulen *et al.* 1999).

One of the factors potentially determining heterogeneity and influencing the HPA axis in CFS is early-life stress (ELS), consisting of sexual, physical and/or emotional maltreatment during childhood (Van Den Eede *et al.* 2007). Preclinical animal laboratory studies have provided evidence that ELS (maternal separation in rats or adverse rearing conditions in non-human primates) produces long-lived hyperactivity of CRF neuronal systems, as well as greater reactivity of the HPA axis (Plotsky & Meaney, 1993; Coplan *et al.* 1996; Ladd *et al.* 1996; Heim *et al.* 2004). In accordance with these findings, higher pituitary reactivity to CRF and to psychological stress has been described in adult women with a history of childhood abuse (Heim *et al.* 2000b, 2001). In addition, Rinne *et al.* (2002) demonstrated that ELS was associated with an enhanced ACTH and cortisol response in the high-dose Dex/CRF test in women with borderline personality disorder, pointing to an enhanced central CRF drive and a reduced glucocorticoid negative feedback. This is in contrast with the proposed central CRF deficiency and enhanced glucocorticoid negative feedback in CFS. As a consequence, it may be that CFS patients with a history of ELS constitute a subgroup with a different neuroendocrine function (Van Den Eede *et al.* 2007). For the assessment of ELS, the Dutch version of the Structured Trauma Interview was used (Draijer, 1989). This instrument is described in detail elsewhere (Draijer & Langeland, 1999). There have been reports of significant associations between childhood abuse scores in the Structured Trauma Interview and both responses to a challenge of the serotonin system (Rinne *et al.* 2000) and to cortisol responses in the Dex/CRF test (Rinne *et al.* 2002). All interviews were conducted by a psychiatrist (F.V.D.E.) at the end of the clinical and psychiatric investigation in a context of absolute confidentiality and mutual trust. The focus of the Structured Trauma Interview is on childhood sexual and physical abuse. Physical abuse is defined as intentional physical aggression by a parent or

caregiver towards a child (<16 years), which may cause injuries. Physical aggression was coded as 'severe' if it happened more than once a month and if it sometimes led to injuries. When individuals were hit hard more than once a month without experiencing injuries, or when aggression took place less frequently but with injuries, it was scored as 'moderate'. 'Mild' aggression had a lower frequency and was not accompanied with injuries. Sexual abuse is defined as sexual contacts with a child (<16 years), which took place against her/his will or when she/he had the feeling that the sexual contacts could not be refused due to physical or relational preponderance, emotional pressure, force or violence. This item was coded as severe when it appeared chronically or more than once a month during a period in childhood, when it consisted of any form of penetration, when it was associated with violence or when it appeared in the primary support group. ELS was defined as moderate or severe physical abuse and/or severe sexual abuse.

#### *Combined low-dose Dex/CRF test*

The combined low-dose Dex/CRF test was performed at Antwerp University Hospital following the protocol described by Heuser *et al.* (1994a). However, instead of measuring plasma cortisol, we measured salivary cortisol concentration by means of a cotton-wool Salivette (Sarstedt, Nümbrecht, Germany). Salivary cortisol is considered as an easy-to-assess correlate of the unbound free hormone fraction (Kirschbaum & Hellhammer, 1994; Baghai *et al.* 2002). Participants took 0.5 mg Dex orally at 23:00 hours on the evening before neuroendocrine testing. At 13:30 hours they attended the research unit where an infusion (NaCl 0.9%) was placed in the forearm. Participants then fastened, remained semi-supine or supine under resting conditions, and were not allowed to sleep. Human CRF (100 µg) was administered and flushed through the infusion at 15:00 hours. Free salivary concentration was assessed at seven time-points (14:00, 14:30, 15:00, 15:30, 15:45, 16:00 and 16:15 hours). All saliva samples were stored at -20 °C and were later delivered to the Laboratory of Physiology of Reproduction of the University of Liège. A radioimmunoassay was performed on 100 µl of salivary free cortisol samples in competition, with a HPLC preparation of cortisol-3CMO coupled with 2-[<sup>125</sup>I]iodohistamine as tracer, for specific antibodies raised against cortisol-3CMO-BSA. The incubation conditions were at 4 °C overnight. The lower detection limit of the assay was 0.1 nmol/l, the intra-assay coefficient of variation (CV) was 4.30% (*n*=10) whereas the inter-assay CV for low and high cortisol levels were 12.26% and 9.38% (*n*=30) respectively.

### Statistical analysis

The following three measures were computed: Basal,  $AUC_{tot}$  and  $AUC_{net}$ . Basal represents the mean free cortisol concentration (in ng/100 ml) over the three time-points on the day after Dex administration (Dex suppression), and before the infusion of CRF. The area under the curve (AUC) was calculated to incorporate the cortisol concentrations at the four time-points after CRF, according to the trapezoid method (Pruessner *et al.* 2003).  $AUC_{tot}$  is the total area under the curve and represents the total cortisol output.  $AUC_{net}$  is identical to  $AUC_{tot}$ , except for the removal of the area between the ground and Basal. This measure reflects the absolute increase of cortisol after CRF. For statistical analysis, we used SPSS for Windows, version 12.0 (SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was adopted to examine the distribution of the data. In view of deviations from normal distribution and the relatively small sample size of the subgroups, neuroendocrine variables were assessed non-parametrically. The Mann–Whitney test was used for two independent samples and the Kruskal–Wallis test was used for three independent samples, followed by the Dunn test for *post-hoc* analysis. One-sided testing was applied when comparing patients and controls, since it was expected that CFS would be associated with an enhanced suppression of the HPA axis (Gaab *et al.* 2002b) and thus a lower cortisol response in the low-dose combined Dex/CRF test. The following clinical variables were noted in the patient group: ELS, past history of MDD, use of benzodiazepines, use of antidepressants and use of oral oestrogens (two-sided testing). For the analysis of the sample characteristics,  $\chi^2$  analysis was applied to compare frequencies. Fisher's exact test was used when necessary. If quantitative data failed to satisfy the assumptions for parametrical statistical analyses, they were analysed non-parametrically. For all analyses, a *p* value <0.05 was adopted as the level of significance.

## Results

### Characteristics of study groups

Characteristics of the study groups are presented in Table 1. All patients (with one exception) were unable to work because of chronic fatigue, whereas all control subjects had a working employment status. As expected, the scores on the different items of the Checklist Individual Strength were significantly higher in the patient group (Vercoulen *et al.* 1999). None of the participants met the criteria for a current diagnosis of post-traumatic stress disorder (PTSD), although this was not an exclusion criterion. As

expected, there was a higher frequency of other current anxiety disorders [specific phobia ( $n=1$ ), social phobia ( $n=1$ ), generalized anxiety disorder ( $n=3$ ), panic disorder with or without agoraphobia ( $n=3$ ), anxiety not otherwise specified ( $n=1$ )] and a higher frequency of a history of one or more major depressive episodes in the patient group (Afari & Buchwald, 2003). None of the controls were diagnosed with generalized anxiety disorder or panic disorder and none of the participants had obsessive–compulsive disorder. The severity of the current anxiety disorders in both samples was mild or moderate; in three participants the anxiety disorder was in partial remission. The frequency of ELS was not different between the patients and the controls.

In relation to medication, there were differences in the frequency of individuals who were taking oral oestrogens (all but one as oral contraceptive), antidepressants and benzodiazepines. Patients took the following antidepressants: amitriptyline ( $n=3$ , 25–75 mg/day); paroxetine ( $n=3$ , 20–30 mg/day); citalopram ( $n=2$ , 20 mg/day); escitalopram ( $n=1$ , 10 mg/day); fluoxetine ( $n=1$ , 20 mg/day); mirtazapine ( $n=1$ , 30 mg/day); melitracen/flupentixol ( $n=1$ , 10/0.5 mg/day) and trazodone ( $n=1$ , 50 mg/day). In addition, four patients were treated with trazodone as a second antidepressant for sleep disturbances (50–150 mg/day). Furthermore, 10 patients took  $\leq 1$  mg/day clonazepam and one patient took 2 mg/day. The other benzodiazepines were: tetrazepam ( $n=3$ ), lorazepam ( $n=2$ ), lormetazepam ( $n=2$ ), alprazolam ( $n=2$ ) and prazepam ( $n=1$ ) in patients, and lormetazepam ( $n=1$ ) and tetrazepam ( $n=1$ ) in controls.

### Combined low-dose Dex/CRF test

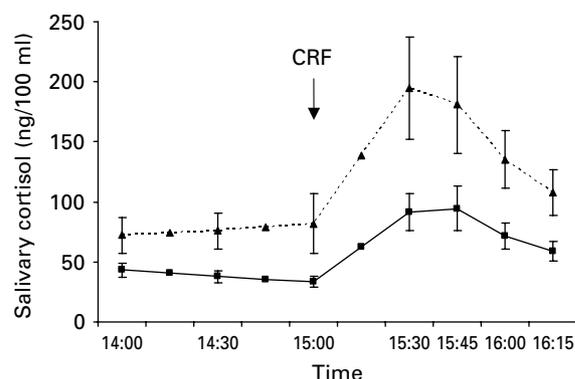
Fig. 1 presents the mean free salivary cortisol responses in CFS patients and controls in the combined Dex/CRF test. The mean cortisol responses before and after CRF were lower in the patient sample. Table 2 lists the mean values of the three Dex/CRF variables (Basal,  $AUC_{tot}$  and  $AUC_{net}$ ) with the standard deviations. None of these variables showed a normal distribution in either sample (Kolmogorov–Smirnov,  $p < 0.020$ ). In the non-parametrical testing (Mann–Whitney, one-sided), patients showed significantly lower values of Basal and  $AUC_{tot}$  and a trend towards lower values of  $AUC_{net}$ .

When comparing CFS patients with ( $n=12$ ) and without ELS ( $n=22$ ) (ELS+/ELS–), the cortisol responses before and after CRF were significantly lower in the ELS– group (Mann–Whitney, two-sided: Basal,  $p=0.016$ ;  $AUC_{tot}$ ,  $p=0.008$ ;  $AUC_{net}$ ,  $p=0.012$ ). Regarding the patient characteristics, there was a higher

**Table 1.** Characteristics of study groups

Variable	Patients (n=34)	Controls (n=25)	Test statistic	p value
Mean age (years)	42.91	42.72	$t=0.97$	0.923
Education (mean rank)	29.53	30.64	$U=409$	0.796
Having child(ren)	25 (74%)	19 (76%)	$\chi^2=0.046$	0.829
Living alone	6 (18%)	6 (24%)	$\chi^2=0.359$	0.549
Employment status: working	1 (2,9%)	25 (100%)	$\chi^2=55.062$	<b>&lt;0.001</b>
Mean body mass index	24.57	24.75	$U=398$	0.679
Mean time diagnosis (months)	31.91	-	-	-
<b>Checklist Individual Strength</b>				
Fatigue (mean)	46.03	21.28	$U=36$	<b>&lt;0.001</b>
Concentration (mean)	28.94	11.36	$U=29.5$	<b>&lt;0.001</b>
Motivation (mean)	15.73	8.64	$U=109.5$	<b>&lt;0.001</b>
Physical activity (mean)	14.77	5.24	$U=59.5$	<b>&lt;0.001</b>
Total score (mean)	105.5	46.52	$U=14$	<b>&lt;0.001</b>
<b>Current psychiatric disorder</b>				
Somatization disorder	8 (24%)	0	Fisher's exact	<b>0.008</b>
PTSD	0	0	-	-
Other anxiety disorder	9 (26%)	3 (12%)	$\chi^2=1.862$	0.172
MDD (past)	16 (47%)	7 (28%)	$\chi^2=2.053$	0.152
Early-life stress	12 (35%)	10 (40%)	$\chi^2=0.136$	0.712
Oral estrogen	8 (24%)	2 (8%)	Fisher's exact	0.166
<b>Psychotropic medication</b>				
Antidepressant	13 (38%)	0	$\chi^2=12.260$	<b>&lt;0.001</b>
Benzodiazepine	21 (62%)	2 (8%)	$\chi^2=17.508$	<b>&lt;0.001</b>

PTSD, Post-traumatic stress disorder; MDD, major depressive disorder. Significant differences are indicated in bold.



**Fig. 1.** Mean salivary (free) cortisol values (ng/100 ml)  $\pm$  S.E.M. at seven time-points during the combined low-dose dexamethasone/corticotropin-releasing factor (CRF) test in CFS patients (■, n=34) and in control subjects (▲, n=25). Participants took 0.5 mg dexamethasone orally at 23:00 hours on the evening before neuroendocrine testing. Human CRF (100  $\mu$ g) was administered 16 h later at 15:00 hours.

frequency of patients who experienced one or more major depressive episodes in the past in the patient group with ELS (75%) compared to patients without

ELS (37%) ( $p=0.038$ ), which is consistent with findings in other studies (Chapman *et al.* 2004). With the exception of a higher level of subjective complaints of concentration (as measured with Checklist Individual Strength) in the ELS+ group, there were no other significant differences in characteristics between both patient groups. Within the control group, subjects with ELS ( $n=10$ ) showed lower cortisol responses before and after CRF than subjects without ELS ( $n=15$ ), inversely to the findings in the patient group. However, the differences between the two groups were not statistically significant (Mann-Whitney, two-sided: Basal,  $p=0.375$ ;  $AUC_{tot}$ ,  $p=0.405$ ;  $AUC_{net}$ ,  $p=1$ ).

There were no significant differences in the distributions of any of the three Dex/CRF variables between patients with and without a history of one or more major depressive episodes in the combined Dex/CRF test ( $p>0.45$ ). ELS and MDD were correlated, but within the CFS group with a history of one or more major depressive episodes ( $n=16$ ), cortisol responses remained significantly different between ELS- ( $n=7$ ) and the ELS+ ( $n=9$ ) patients (Mann-Whitney, two-sided: Basal,  $p=0.017$ ;  $AUC_{tot}$

**Table 2.** Mean values of the three computed variables in the combined low-dose dexamethasone/corticotropin-releasing factor test in patients and controls

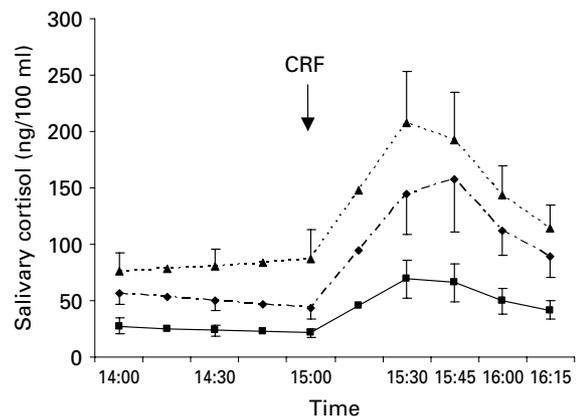
Variable	Patients	Controls	U statistic	p value
Basal	38.01 (30.11)	76.63 (75.69)	309	0.038
AUC <sub>tot</sub>	5557.47 (5168.48)	11076.49 (10904.67)	283	0.015
AUC <sub>net</sub>	2706.42 (3940.16)	5329.18 (7187.03)	342	0.102

Basal, mean salivary cortisol concentration (ng/100 ml) over three time-points after dexamethasone but before CRF; AUC<sub>tot</sub> is the total area under the curve and represents the total cortisol output after CRF; AUC<sub>net</sub> is identical to AUC<sub>tot</sub> except for removal of the area between ground and Basal.

Standard deviations are presented within parentheses. In the non-parametrical testing (Mann-Whitney, one sided), patients ( $n=34$ ) showed significantly lower values of Basal and AUC<sub>tot</sub> and a trend towards lower values of AUC<sub>net</sub> compared to controls ( $n=25$ ).

$p=0.005$ ; AUC<sub>net</sub>  $p=0.003$ ). Furthermore, there were no significant differences in the distributions of the three Dex/CRF variables when dividing the patient sample on the basis of benzodiazepine use ( $p>0.22$ , benzodiazepines higher mean rank) or antidepressants ( $p>0.38$ , antidepressants higher mean rank, except for Basal).

Patients who took an oral oestrogen ( $n=8$ ) showed significantly lower cortisol responses in the combined Dex/CRF test than patients who did not take an oral oestrogen ( $n=26$ ), as measured by AUC<sub>net</sub> ( $p=0.023$ ). The values of AUC<sub>tot</sub> were also lower in the oestrogen group, whereas the values of Basal were higher. However, the differences for these two variables were not statistically significant between the two groups (Mann-Whitney, two-sided: Basal,  $p=0.350$ ; AUC<sub>tot</sub>,  $p=0.417$ ). Since there was a higher frequency of subjects taking an oral oestrogen in the patient group compared with the control group (see Table 1), oral oestrogens could have represented a confounding factor in the previous analyses. Therefore, we performed an additional analysis after removal of the eight patients and two control subjects taking an oral oestrogen. Fig. 2 presents the mean free salivary cortisol responses in CFS patients with and without ELS and controls in the combined Dex/CRF test after removal of subjects taking an oral oestrogen. The cortisol responses before and after CRF were clearly lower in patients without ELS than in the other two groups. Table 3 lists the mean values of the three Dex/CRF variables with the standard deviations. The differences in distributions of Basal and AUC<sub>tot</sub> were significant in the (one-sided) analysis between two groups (patients and controls) and in the analysis between three groups (patients with and without ELS and controls), but not the differences in distributions of AUC<sub>net</sub>. The Dunn test for *post-hoc*



**Fig. 2.** Mean salivary (free) cortisol values (ng/100 ml)  $\pm$  S.E.M. at seven time-points during the combined low-dose dexamethasone/corticotropin-releasing factor (CRF) test in CFS patients with (ELS+,  $\blacklozenge$ ,  $n=11$ ) and without (ELS-,  $\blacksquare$ ,  $n=15$ ) early-life stress and control subjects ( $\blacktriangle$ ,  $n=23$ ) after removal of the eight patients and two controls who were taking an oral oestrogen.

analysis revealed significant differences for Basal and AUC<sub>tot</sub> between ELS- patients and controls, and between the ELS- and ELS+ patients. The differences between the ELS+ patients and controls were not significant for any of the variables. Comparable results were obtained when excluding controls with a past history of ELS: patients ( $n=26$ ) versus controls ELS- ( $n=14$ ): Mann-Whitney, two-sided: Basal,  $p=0.050$ ; AUC<sub>tot</sub>,  $p=0.033$ ; AUC<sub>net</sub>,  $p=0.427$ . Patients ELS+ ( $n=15$ ) versus patients ELS- ( $n=11$ ) versus controls ELS- ( $n=14$ ): Kruskal-Wallis: Basal,  $p=0.004$ ; AUC<sub>tot</sub>,  $p=0.008$ ; AUC<sub>net</sub>,  $p=0.223$  (Dunn: significant differences for Basal and AUC<sub>tot</sub> between ELS- patients and controls, and between ELS- and ELS+ patients).

**Table 3.** Mean values of the three computed variables in the combined low-dose dexamethasone/corticotropin-releasing factor test when differentiating patients on the basis of early-life stress (ELS) and after removal of eight patients and two control subjects who were taking an oral oestrogen

Variable	Patients total	Patients ELS–	Patients ELS+	Controls	U statistic [1]	$\chi^2$ statistic [2]	p value [1]	p value [2]
Basal	34.91 (27.23)	24.03 (20.59)	49.76 (28.98)	80.86 (77.47)	196	10.730	0.020	0.005
AUC <sub>tot</sub>	5979.71 (5608.31)	3964.22 (3557.18)	8728.11 (6820.24)	11796.56 (11084.35)	190	9.599	0.015	0.008
AUC <sub>net</sub>	3361.19 (4153.22)	2162.17 (2400.53)	4996.22 (5473.42)	5732.07 (7363.95)	252	3.128	0.173	0.209

For an explanation of the variables see Table 2. Standard deviations are presented within parentheses. When repeating the statistical analyses without the eight patients and two control subjects who were taking an oral oestrogen, the differences in distributions of Basal and AUC<sub>tot</sub> were significant in the (one-sided) analysis between patients (total sample,  $n=26$ ) and controls ( $n=23$ ) [1] and in the analysis between patients without ELS (ELS–) ( $n=15$ ) and with ELS (ELS+) ( $n=11$ ) and controls [2], but not the differences in distributions of AUC<sub>net</sub>. The Dunn test for *post-hoc* analysis revealed significant differences for Basal and AUC<sub>tot</sub> between the ELS– patients and controls and between ELS– and ELS+ patients. The differences between the ELS+ patients and controls were not significant for any of the variables.

## Discussion

This is the first study to investigate HPA axis function in patients with CFS by means of the combined Dex/CRF test and also to examine the influence of a history of ELS on the outcome in this test. We intentionally administered a low dose of Dex (0.5 mg) because of the *a priori* hypothesis that CFS would be associated with an enhanced glucocorticoid feedback and/or a reduced central drive of the HPA axis. In accordance with this hypothesis, the salivary free cortisol responses in the combined Dex/CRF test were lower in CFS patients than in controls.

The total free cortisol responses before (Basal) and after CRF (AUC<sub>tot</sub>) were significantly different between the two groups. The differences in absolute free cortisol output after CRF (AUC<sub>net</sub>) did not reach statistical significance, although CFS patients showed lower values than controls. A larger sample may be required to examine AUC<sub>net</sub> in CFS.

In MDD, the high-dose (1.5 mg) Dex/CRF test has been shown to be more sensitive than the Dex suppression test (Heuser *et al.* 1994a). The results of the current study do not point to the same conclusion with regard to the low-dose Dex/CRF test in CFS. More precisely, comparable *p* values were obtained for Basal and AUC<sub>tot</sub> (see Table 3). The less invasive low-dose Dex suppression test may be sensitive enough to examine the glucocorticoid feedback function in CFS, but further studies on the validity of these tests in CFS are required.

The current findings are consistent with the findings from Gaab *et al.* (2002b). These authors demonstrated enhanced suppression of salivary free cortisol after the administration of a low-dose of Dex (0.5 mg) in CFS. Recently, Jerjes *et al.* (2007) reported a higher

level of cortisol suppression after prednisolone in CFS, providing further evidence for enhanced negative feedback sensitivity in CFS. Next to the glucocorticoid negative feedback mechanism, the outcome in the combined Dex/CRF test may also be influenced by the central drive of the HPA axis (Holsboer, 2000). Regarding to this, several studies have pointed to a reduced hypothalamic central secretion of CRF and arginine vasopressin or to a reduced supra-hypothalamic stimulus to the HPA axis in CFS (Demitrack *et al.* 1991; Scott *et al.* 1998a, 1999; Altemus *et al.* 2001; Gaab *et al.* 2002a), although there have also been negative reports (Moorkens *et al.* 2000; Cleare *et al.* 2001; Inder *et al.* 2005). Alternatively, desensitization of target receptors on anterior pituitary corticotropes may also explain the reduced cortisol output in CFS, although this is inconsistent with hypothalamic deficiency (Scott *et al.* 1998a, 1999). Furthermore, other neurobiological pathways may also be involved primarily or secondarily in HPA axis dysfunction. For instance, studies measuring cortisol and prolactin responses to a serotonin agonist have provided evidence for a disturbed relationship between the serotonergic system and the HPA axis in CFS (Parker *et al.* 2001).

Further analysis revealed an influence of ELS and oestrogen intake on the cortisol responses. Interestingly, patients without a history of ELS showed significantly lower cortisol responses than patients with ELS and controls. CFS patients with ELS did not show a significantly enhanced glucocorticoid negative feedback. This is not surprising when considering the findings of Rinne *et al.* (2002), who demonstrated that ELS was associated with an enhanced ACTH and cortisol response in the combined high-dose Dex/CRF test in women with borderline

personality disorder, pointing to an enhanced central CRF drive and/or a reduced glucocorticoid negative feedback, rather than the opposite. In contrast, there have also been reports of an enhanced glucocorticoid negative feedback in patients who experienced ELS (Stein *et al.* 1997; Newport *et al.* 2004). However, in both studies, patients with ELS showed a high co-morbidity of current PTSD (94% and 68% respectively), a syndrome that has been associated with enhanced glucocorticoid negative feedback (Yehuda *et al.* 1993). Moreover, in the previously mentioned study by Rinne *et al.* (2002), co-morbid PTSD was shown to attenuate the ACTH response in the combined Dex/CRF test. Furthermore, in the studies by Newport *et al.* (2004) and Yehuda *et al.* (2004), ELS was only associated with Dex hypersuppression in patients with co-morbid MDD or PTSD, respectively. This suggests that enhanced glucocorticoid feedback is not an invariable consequence of ELS, but is more related to the resultant psychiatric illness in traumatized individuals. In the current study, PTSD or MDD were not confounding factors, since none of the participants were diagnosed with current PTSD or MDD. Further research on the neuroendocrine characteristics of ELS is required.

Some limitations need to be considered when interpreting the results. All patients were female and they were all attending a tertiary care centre. Consequently, we cannot directly extrapolate these results to male CFS patients and to CFS patients in primary or secondary care. The validity of retrospective reports of ELS has been critically discussed (Widom *et al.* 2004). Problems include: accuracy, recall bias, sampling bias and examination of causal relationships. However, the conclusion of a review on this topic was that retrospective reports of ELS are sufficiently valid, if they rely on reasonable operationalization (Hardt & Rutter, 2004). Although there are no clear-cut guidelines for severity rating of child abuse, we focused on behaviors that unambiguously point to physical or sexual abuse. When applying the operational criteria, ELS could clearly be rated for each subject. Furthermore, the frequency of abuse has previously been found to be a reliable predictor of neuroendocrine sequels of ELS (Rinne *et al.* 2000, 2002). According to Hardt & Rutter (2004), retrospective reports are likely to provide underestimates of the incidence of abuse or neglect, rather than overestimates. Regarding this, the use of a structured interview is likely to be more valid than a questionnaire. The rates for ELS in the control subjects (40%) were slightly higher than the ones reported in a large study with 8667 participants (35%) (McCauley *et al.* 1997). However, the high prevalence of ELS did not affect the results of this study. More precisely,

comparable results were obtained after excluding controls with a history of ELS. In contrast with the studies by Van Houdenhove *et al.* (2001) and Heim *et al.* (2006), there was no higher rate of ELS in CFS. This may be due to the fact that in the current study, emotional neglect and adult victimization were not assessed.

Furthermore, concentrations of ACTH were not measured in the combined Dex/CRF test. Therefore, it cannot be excluded that the observed lower cortisol output in CFS patients is due to adrenal insufficiency. However, in two challenge studies with  $\geq 1 \mu\text{g}$  ACTH, there were no differences in cortisol responses between CFS patients and healthy subjects (Hudson & Cleare, 1999; Gaab *et al.* 2003b), suggesting that adrenal insufficiency is unlikely to play a significant role in CFS. Scott *et al.* (1998c) and Demitrack *et al.* (1991) reported reduced cortisol responses in the ACTH test, but taking the findings of challenge studies into account, both research groups interpreted these results as a diminished adrenocortical reserve in CFS, secondary to a reduced stimulation from pituitary ACTH. Another limitation is the absence of a baseline collection of salivary cortisol at the same time of day without Dex. Therefore, it cannot be excluded that patients were hypocortisolaemic at baseline, with a subsequent reduced response to both Dex and CRF. However, in every analysis, the values of the absolute cortisol increase after CRF ( $\text{AUC}_{\text{net}}$ ) were lower in CFS patients compared to controls (see Tables 2 and 3). Based on this finding and on the results of the previously mentioned study by Gaab *et al.* (2002b) (in which there was no significant baseline difference between patients and controls), it seems unlikely that the differences in total free salivary cortisol concentrations between CFS patients and controls after Dex and after CRF were only due to a possible baseline hypocortisolism in CFS. An additional limitation is that it cannot be excluded that there was no suppression in the control group, resulting in a CRF test in this group, rather than a Dex/CRF test. However, the values of salivary cortisol after 0.5 Dex (CFS patients: 38.01 ng/100 ml = 1.05 nmol/l; controls: 76.63 ng/100 ml = 2.11 nmol/l; see Table 2) were very low in both groups, pointing to Dex suppression. Moreover, these salivary cortisol values were comparable with the values at the 15:00 hours time-point after 0.5 mg Dex in the study by Gaab *et al.* (2002b). These authors did measure baseline salivary cortisol and reported significantly lower values after 0.5 mg Dex in both CFS patients and controls (although a stronger decrease in CFS patients). Taking this evidence into account, it is unlikely that there was no suppression in the control group in our study.

Psychotropic drugs may have an influence on HPA axis function, but there was no significant influence of antidepressants or benzodiazepines in this study (and the CRF response was even higher in patients taking those medications). In accordance with the findings of Kirschbaum *et al.* (1999), oestrogen intake affected the absolute free cortisol concentrations after CRF ( $AUC_{net}$ ). Oestrogens induce an increased production of corticosteroid-binding globulin, resulting in a reduced fraction of free cortisol (Kirschbaum *et al.* 1999). The frequency of oestrogen intake was higher in the patient group, but the main findings of this study remained significant after correcting for this confounding factor (Table 3). However, we did not control for menstrual cycle or menopause status, nor did we control for Dex plasma levels.

In conclusion, CFS was associated with reduced cortisol responses in the combined low-dose Dex/CRF test, but this effect was only significant in CFS patients without a history of ELS. This study provides further support for an enhanced glucocorticoid negative feedback and/or a reduced central HPA axis drive in CFS. Further validation of the low-dose Dex suppression test and Dex/CRF test in CFS is required. Furthermore, CFS with and without a history of ELS are possibly different from a neurobiological point of view, and future studies on the pathogenesis and treatment of the disorder should take this into account.

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### Declaration of Interest

None.

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