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Salivary Cortisol Profiles in Chronic Fatigue Syndrome

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Key Words
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Abstract
Salivary cortisol profiles (hourly sampling over a 16-hour period) of 10 patients with chronic fatigue syndrome (CFS) but without concurrent depressive disorder were compared with those of 10 healthy volunteers matched for age, sex and menstrual cycle. The mean saliva cortisol concentration over the 16-hour period was slightly but significantly greater in the patients than the controls ($p < 0.05$). These findings are at variance with earlier reports that CFS is a hypocortisolaemic state and suggest that in CFS the symptom of fatigue is not caused by hypocortisolaemia.

Introduction

Chronic fatigue syndrome (CFS) is a controversial condition only relatively recently defined by consensus criteria [1, 2]. It is characterised by the principal complaints of abnormal fatigue and fatiguability after minimal exertion (both mental and physical), associated with functional impairment and other somatic symptoms, but without any conventional biomedical explanation. The illness is a symptom complex, and the diagnosis is essentially one of exclusion.

The aetiological considerations are complex. Despite much research, no clear physical causes have been identified to account for the symptoms of CFS. Earlier studies have investigated hypotheses of neuromuscular dysfunction and persistent viral infection to account for the abnormal fatiguability. However, neuromuscular function has been shown to be largely normal [3–5] and the pattern of fatigue appears to be of central rather than peripheral origin [6]. Similarly, despite findings of subtle immunological abnormalities in CFS and precipitation of the illness in many cases by an infective episode, no particular infectious agent has been identified as a tenable explanation for most cases [7–10].

Consequently, attention has focused on the role of the central nervous system, and in particular neuroendocrine function, in CFS. The hypothalamic-pituitary-adrenal (HPA) axis has received attention for several reasons. A similar clinical profile to CFS is known to occur in glucocorticoid insufficiency (Addison's disease). HPA activation is involved in the physiological response to stress and exercise, so that dysfunction may mediate the fatiguability associated with CFS. The association of HPA dysregulation with depressive illness, the commonest psychiatric disorder associated with CFS [11], is well known [12].

Several authors have tested components of the HPA axis in CFS and postulated that hypoactivity in this axis may underlie some of the symptoms of CFS. Demitrack et al. [13] found reduced basal evening cortisol levels and 24-hour urinary free cortisol (UFC) in CSF patients when compared to healthy controls and argued that associated findings of elevated basal evening adrenocorticotrophic hormone (ACTH) levels, enhanced cortisol responses to exogenous ACTH and reduced ACTH responses to corticotrophin-releasing hormone point to a possible central adrenal insufficiency in CFS. Bearn et al. [14] investigated ACTH and cortisol responses to the insulin tolerance and *d*-fenfluramine tests in non-depressed CFS patients and normal controls and suggested the possibility of primary adrenal cortical impairment in CFS. Cleare et al. [15] compared cortisol responses to *d*-fenfluramine in patients with CFS, depression, and healthy controls and found baseline cortisol levels highest in depressed subjects, lowest in CFS subjects with intermediate levels in the normal controls, also supporting possible reduced HPA axis activity and hypocortisolism in CFS.

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Further indication of HPA axis dysfunction in CFS may be drawn from neuroendocrine findings in fibromyalgia (FM). FM is characterised by many of the same symptoms as CFS but with diffuse myalgia and soft-tissue tenderness at specific points to the fore. Most investigators consider the condition to overlap, if not be synonymous, with CFS [16, 17]. Two recent studies of HPA axis function in FM have suggested relative adrenal hyporesponsiveness compatible either with primary adrenal insufficiency or atrophy from chronic understimulation [18, 19].

Thus, several studies to date indicate the possibility of hypocortisolaemia in CFS which may mediate the fatigue associated with this condition. However, previous studies have assessed basal cortisol secretion from 24-hour urine collection or single plasma samples. Twenty-four-hour UFC excretion has been criticised as an unreliable indicator of basal HPA activity [20]. The reliability of single plasma cortisol levels as indicators of basal cortisol secretion is limited both by the diurnal variation in cortisol secretion and the effects of stress on cortisol secretion. We are the first authors to report on the use of salivary cortisol profiles to investigate basal HPA activity in CFS. This method has distinct advantages in the assessment of basal cortisol secretion [21] – a profile of hourly salivary cortisol levels allowing an estimation of basal secretion less effected by the circadian variation in cortisol secretion, obtained in a relatively naturalistic setting and avoiding the effects of venepuncture on the secretion of this stress-sensitive hormone. The saliva concentrations of cortisol have been shown to be a reliable indicator of both total plasma cortisol [22, 23] and particularly of plasma free cortisol [24, 25].

We compared salivary cortisol profiles, obtained by hourly saliva sampling over a 16-hour period, of CFS patients and matched controls. We also compared the mean of the cortisol samples from 13.00 to 16.00 h and 16.00–19.00 h, as several workers have shown the mean 13.00- to 16.00-hour and 16.00- to 19.00-hour plasma cortisol concentrations to be reliable indicators of the basal HPA activity [20, 26, 27].

Methods

Subjects

Ten patients, referred to the CFS clinic at King's College Hospital (KCH), volunteered to participate in the study. All these volunteers fulfilled the recent British and American operational criteria for the diagnosis of CFS [1, 2]. Patients were recruited if aged 18–60 years, without a history of neurological, cardiovascular, or endocrine disease; with normal haematological and biochemical screening profiles including thyroid function and no abnormality on physical examination. None were currently taking antidepressant medication.

Ten normal subjects were recruited from the staff and student body at KCH, and were matched for age, sex, and menstrual cycle with the study sample of CFS subjects. They were all in good health without any serious medical illness or history of psychiatric disorder and were medication-free.

All subjects were interviewed by a psychiatrist (B.W., S.W.) to exclude current depressive illness according to DSM-III-R criteria [28]. All subjects completed a Beck Depression Inventory (BDI) [29] on the day of salivary cortisol testing to provide a measure of subclinical depressive symptomatology. They were woken at 6.00 h on the day of the study, and saliva samples were collected at hourly intervals from 7.00 h to 22.00 h. During the study subjects relaxed in their own rooms and were provided with three standard meals.

All subjects provided written informed consent before participating in the study, and Ethical Committee approval for the study was obtained.

Salivary Cortisol Measurements

Specimen Collection. Hourly saliva specimens were collected in salivettes (Sarstede, Leicester, UK) which containted an untreated cotton swab. They were kept at 4°C overnight and centrifuged the following morning. The clear fluid was stored at –20°C until analysis. There was no significant absorption of cortisol onto the cotton swab at the concentration of 4 nm/l.

Measurement. Salivary cortisol was quantified with the 'Magic Cortisol' RIA kit (Ciba Corning, Halstead, UK) using the method described by Kirschbaum and Hellhammer [21] but with the following modifications: (1) lowest and highest standards were 0.7 and 41.4 nm/l, respectively; (2) incubation time shortened to 1.5 h and the precipitate was washed (1 ×) with phosphate-buffered saline (0.3 ml) containing 1% Tween 20. (3) magnetic separations were for 15 min, and (4) internal walls of the RIA tubes were carefully wiped with a wet tissue before counting.

All aliquots of standards and specimens were dispensed to the RIA tubes with the Microlab 'M' (Hamilton, UK) semi-automatic micropipette taking care to eliminate 'carry-over' problems. The interassay precision (CV) was about 5% at the concentration range of 4–27 nm/l and the intra-assay precision about 4% at the same concentration range.

Results

Demographic Characteristics

The clinical characteristics of the subjects are shown in table 1. The study was conducted on 10 patients with CFS and 10 matched normal controls, comprising 6 women and 4 men in each group. There was no difference between the mean ages of each group (CFS: mean = 34.9 years, SD 6.1; controls: 34.2 years, SD 5.4). The BDI scores of the study group were significantly greater than the control group (Wilcoxon test, p = 0.003), with 5 patients scoring over 12 (range 15–19) suggesting some depressive symptomatology, despite not fulfilling DSM-III-R criteria.

Salivary Cortisol Assay Results

The mean and SD of the salivary cortisol concentrations of the patient and control subjects between 7.00 and

22.00 h are shown in figure 1. Analysis of variance of repeated measures over the whole time interval showed a trend towards higher cortisol levels in the CFS patient group, which just failed to reach significance ($F = 4.84$, $p = 0.055$). The mean cortisol levels (7.00–22.00 h) of the patients (mean 9.47; SD 1.57) were also slightly higher than those of matched controls (mean 8.38; SD 2.07; paired t test; $p = 0.05$; power = 0.7 at $a = 0.05$).

There was a significant interaction with time, cortisol levels declining during the day in both groups ($F = 35.60$, $p = <0.001$), with no difference in time/cortisol-level interaction between patients and controls.

Comparison of different 3-hour segments (13.00–16.00 h, 16.00–19.00 h) of the afternoon salivary cortisol curve showed no differences in cortisol levels between patient and control groups (paired t test). Nor were there any differences between the saliva cortisol values at 20.00 h at which time Demitrack et al. [13] had reported lower plasma cortisol levels in CFS patients. A subanalysis of the CFS group comparing the 5 patients with BDI scores of 9 or less to the 5 patients with scores of 15–19 revealed no significant differences between the cortisol levels of the two groups during either of the 3-hour afternoon periods studied.

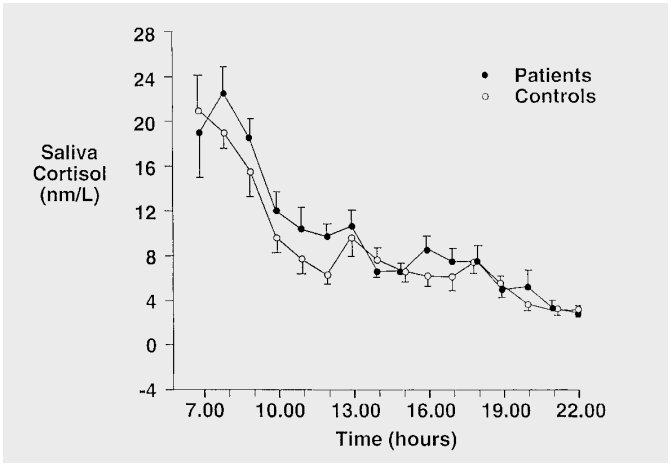

Fig. 1. Saliva cortisol (mean ± SEM) of 10 CFS patients and 10 controls, 7.00–22.00 h.

Table 1. Demographic characteristics and BDI scores of CFS and control subjects

	CFS subjects	Sex	Duration CFS months	Age years	BDI		Control subjects	Sex	Age years	BDI
1	T.J.	F	9	34	3	1	H.T.	F	34	2
2	M.H.	M	49	39	9	2	J.H.	M	40	0
3	T.T.	M	18	32	5	3	S.B.	M	33	3
4	L.D.	F	30	28	9	4	M.B.	F	28	0
5	D.K.	F	33	33	9	5	W.A.	F	34	0
6	S.A.	F	10	30	17	6	K.L.	F	27	7
7	M.G.	M	50	31	15	7	P.O.C.	M	31	0
8	A.E.	F	52	49	18	8	S.S.	F	45	2
9	C.H.	F	96	39	16	9	R.G.	F	37	8
10	M.R.	M	21	34	19	10	M.S.	M	33	4

Discussion

Using salivary cortisol profiles we have been unable to replicate previous work suggesting low baseline cortisol levels in CSF patients. Basal evaluation of the HPA axis is notoriously difficult due to the pulsatile, episodic nature of HPA function and its distinct circadian variation. Demitrack et al. [13] estimated HPA axis activity using 24-hour UFC excretion and evening (22.00 h) plasma total and free cortisol concentrations. However, Thompson et al. [20], who compared a number of basal cortisol measures in 40 depressed patients and 40 matched normal control subjects, concluded that the UFC excretion did not correlate with the mean 24-hour serum cortisol and was not a reliable indicator of basal HPA activity. The measurement of cortisol in saliva has advantages when compared with the measurement of cortisol in urine or plasma. Samples can be collected in naturalistic settings without the stress of venepuncture or cannulation. Patients and controls can conduct normal daily activities albeit in the setting of a research ward. A further advantage is that cortisol in saliva is a measure of free cortisol [25] which is the fraction of total plasma cortisol which has access to the brain. If change in cortisol causes any of the symptoms of CFS through the action of cortisol at corticosteroid receptors within the brain, then it must be the free rather than the total (free plus bound) fraction of cortisol which is relevant. Using hourly salivary cortisol measurements over a 16-hour period (7.00–22.00 h) we expected to achieve a more accurate assessment of basal cortisol levels than those reported in previous studies of CFS patients. However we found the mean cortisol levels of the CFS patients over this 16-hour period to be significantly higher than those of the control group. Separate analysis of two different 3-hour afternoon periods which have been shown to be reliable indicators of basal HPA activity [20] failed to demonstrate any significant differences between our patient and control groups. Nor were there differences between the patients and controls at 20.00 h, at which time reduced levels have been reported by others [13].

A large body of evidence indicates that major depression is associated with HPA axis overdrive and hypercortisolism in about 50% of cases. The possibility of depressive symptomatology contributing to elevation of cortisol levels in our CSF group is slight, as none met the criteria for major depression and there was no relationship between BDI scores and cortisol levels in our small sample. In addition the BDI includes symptoms that are an inherent part of the definition of CFS (e.g. fatiguability, insomnia) so patients' scores would be expected to exceed controls even in the absence of depression. Furthermore, Demitrack et al. [13] included CFS patients fulfilling DSM-III-R criteria for major depression whilst we excluded them. Of the 19 CFS patients who underwent basal HPA axis evaluation in the study of Demitrack et al., 3 had BDI scores of 19 or greater indicating moderate to severe depression, whilst only 1 of our patients scored as high as 19.

The main limitation of the present study is the small sample size of 10 CFS patients and 10 controls. However, the entry criteria were rigorous in terms of CFS criteria used and exclusion of current depressive disorder, and the measurements obtained for each subject were complex. Certainly a larger sample size would have been preferable, but no other researchers to date have used these improved methods to investigate cortisol secretion in CFS.

In conclusion, although hypocortisolaemic states can result in symptoms similar to those of CFS, the present study provides no new evidence that in CFS the symptom of fatigue results from a reduced secretion of cortisol.

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