



Journal of Affective Disorders xx (2005) xxx–xxx

JOURNAL OF
**AFFECTIVE
 DISORDERS**

www.elsevier.com/locate/jad

Brief report

Diurnal patterns of salivary cortisol and cortisone output in chronic fatigue syndrome

W.K. Jerjes^{a,*}, A.J. Cleare^{b,c,d}, S. Wessely^{b,d}, P.J. Wood^e, N.F. Taylor^a

^aDepartment of Clinical Biochemistry, King's College Hospital, Denmark Hill, London SE5 9RX, United Kingdom

^bDepartment of Psychological Medicine, Institute of Psychiatry, King's College London, London SE5 8AZ, United Kingdom

^cNational Affective Disorders Unit, Bethlem Royal and Maudsley Hospitals, London SE5 8AF, United Kingdom

^dChronic Fatigue Syndrome Unit, King's College Hospital, London SE5, United Kingdom

^eRegional Endocrine Unit, Southampton General Hospital, Southampton SO16 6YD, United Kingdom

Received 4 August 2004; received in revised form 21 March 2005; accepted 22 March 2005

Abstract

Background: The aim of the present study was to obtain a naturalistic measure of diurnal hypothalamic–pituitary–adrenal (HPA) axis output in CFS patients unaffected by medication or comorbid psychiatric disorder likely to influence the axis.

Method: Cortisol and cortisone levels were measured in saliva samples collected from 0600 h to 2100 h at 3-h intervals in CFS patients and healthy controls.

Results: Mean cortisol and cortisone concentrations were significantly lower in patients than controls across the whole day, as were levels at each individual time point except 2100 h. Cosinor analysis showed a significant diurnal rhythm of cortisol and cortisone that was not phase-shifted in CFS compared to controls. However, there was a lower rhythm-adjusted mean and a lower amplitude in CFS patients. The cortisol/cortisone ratio showed no diurnal rhythm and did not differ between CFS subjects and controls.

Limitations: The sample size was relatively small, and drawn from specialist referral patients who had been ill for some time; generalisation of these results to other populations is therefore unwarranted.

Conclusion: The main findings of this study are to provide further evidence for reduced basal HPA axis function in at least some patients with CFS and to show for the first time that salivary cortisone is also reduced in CFS and has a diurnal rhythm similar to that of cortisol. We have also demonstrated that the cortisol/cortisone ratio remains unchanged in

* Corresponding author. Tel.: +44 207 346 4131; fax: +44 207 737 7434.

E-mail address: w_jerjes@yahoo.co.uk (W.K. Jerjes).

CFS, suggesting that increased conversion of cortisol to cortisone cannot account for the observed lowering of salivary cortisol.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Chronic fatigue syndrome (CFS); Salivary cortisol; Salivary cortisone; Diurnal rhythm; Circadian rhythm; Hypothalamo–pituitary–adrenal axis

1. Introduction

Chronic fatigue syndrome (CFS) is characterised by persistent debilitating fatigue and exhaustion, together with a number of other characteristic symptoms, unexplained by identifiable organic disease (Fukuda et al., 1994). One line of research has suggested that hypocortisolism may be a relevant aetiological factor; this is supported by findings of low 24 h urinary free cortisol in CFS, although studies measuring serum or saliva cortisol at defined time points have been less consistent (Cleare, 2003; Parker et al., 2001). Another line of research has noted the occurrence of CFS-like symptoms in conditions in which the circadian clock is phase-shifted such as seasonal affective disorder (Avery et al., 1997; Koorengel et al., 2002). These observations have led to the suggestion that there may be an alteration of diurnal patterns of cortisol release in CFS. From the few published studies available, some show an attenuated diurnal fluctuation of cortisol and others no change (Cleare, 2003).

Circulating cortisol converts reversibly into cortisone. 11- β -Hydroxysteroid dehydrogenase (11- β -HSD) type 2 converts cortisol to cortisone (Agarwall et al., 1994; Mazzocchi et al., 1998; Stewart et al., 1995; Roland and Funder, 1996) while 11- β -HSD type 1 converts cortisone to cortisol (Walker et al., 1992). One potential explanation for lowered free cortisol levels is of increased cortisol conversion to cortisone rather than a reduction in cortisol output. Alteration in this equilibrium has been reported in various illnesses (Morineau et al., 1997; Normura et al., 1996; Raven and Taylor, 1998; Poor et al., 2004), but we are not aware of any prior studies measuring salivary cortisone and cortisol together in CFS.

The aim of this study was to recruit CFS patients free from medication or comorbid psychiatric disorders that might confound assessment of the HPA axis,

and measure salivary free cortisol, cortisone and their ratio across a diurnal cycle. We hypothesised that there would be lowered levels of free cortisol, and no alteration in the interconversion shuttle between cortisol and cortisone, compared to controls.

2. Materials and methods

2.1. Subjects

Fifteen CFS patients (7 males and 8 females) were recruited via the CFS clinic at King's College Hospital (KCH). Subjects were interviewed using the semi-structured format of Sharpe et al. (1997) and DSM-IV to assess psychiatric diagnoses. Subjects were eligible for inclusion if they fulfilled the Centres for Disease Control (CDC) criteria for CFS (Fukuda et al., 1994), were aged 25–60 years and had no history of major medical illness. In order to obtain as pure a measure of the HPA axis as possible, we tested only patients who were not taking any psychotropic medication or other medication that might affect the HPA axis, had been abstinent from such medication for at least 2 months, and had no current DSM-IV major depressive episode or anxiety disorder.

Twenty healthy subjects (10 males and 10 females) were recruited from staff and students at KCH and were well matched for age, sex and BMI with patients. They were all in good health without any serious medical illness or history of psychiatric disorder.

Subjects were all studied during wintertime hours, between October 2002 and March 2003. All subjects had normal dietary habits, taking breakfast, lunch and dinner at about the same time. All subjects habitually went to bed between 2300 and 0100 and got up at 0600. All subjects were asked to limit their intake of caffeine and alcohol during the collection period. All subjects gave written,

Table 1
Demographic and clinical characteristics for CFS patients and controls

	Patients with CFS	Controls	t-test
Age (years)	35 ± 7.9	33 ± 11.3	p=0.4
BMI	24.4 ± 5.0	24.2 ± 4.6	p=0.6
HADS questionnaire scores			
For depression	8.0 ± 3.9	3.0 ± 2.7	p<0.01
For anxiety	7.3 ± 5.6	3.1 ± 2.6	p<0.01
PSQI global scores	9.8 ± 3.3	2.2 ± 1.3	p<0.01
Duration of illness (years)	2.7 ± 0.6	N/A	N/A
Scores on Chalder Fatigue Questionnaire (maximum=33)	25.1 ± 3.0	N/A	N/A
Disability from illness on the Work and Social Adjustment Scale (maximum=40)	22.5 ± 4.7	N/A	N/A

Data presented as mean ± S.D.

PSQI: The Pittsburgh Sleep Quality Index.

HADS: The Hospital Anxiety and Depression scale.

informed consent. The local ethical committee approved the study.

2.2. Questionnaires

All subjects completed the Hospital Anxiety and Depression scale (HADS) (Keedwell and Snaith, 1996) and the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989). Patients completed further questionnaires to characterise their illness: the Chalder Fatigue Scale (Chalder et al., 1993); and the Work and Social Adjustment Scale (Marks, 1986).

2.3. Saliva collections

Subjects were provided with plain Salivettes (Sarstedt, Leicester, UK) to collect samples at home at three-hourly intervals between 0600 h and 2100 h. The protocol for avoiding confounding variables has been published in full elsewhere (Roberts et al., 2004). Subjects kept the samples in their refrigerator overnight and returned them to the laboratory the next day. Upon receipt at the laboratory, saliva samples were centrifuged and the clear fluid stored at -20 °C until analysis. Salivary cortisol and cortisone were analysed by direct immunofluorimetric assay using

the “DELFLIA” system as previously described (Wood et al., 1997).

2.4. Circadian rhythm analysis

Individual and population mean cosinor analysis was performed using TSA-Seriel Cosinor software (Expert Soft Technologie, Laboratoire d'Informatique BioMédicale, France). We derived: (1) the ‘goodness of fit’ of a cosinor curve fitted to the data; (2) midline

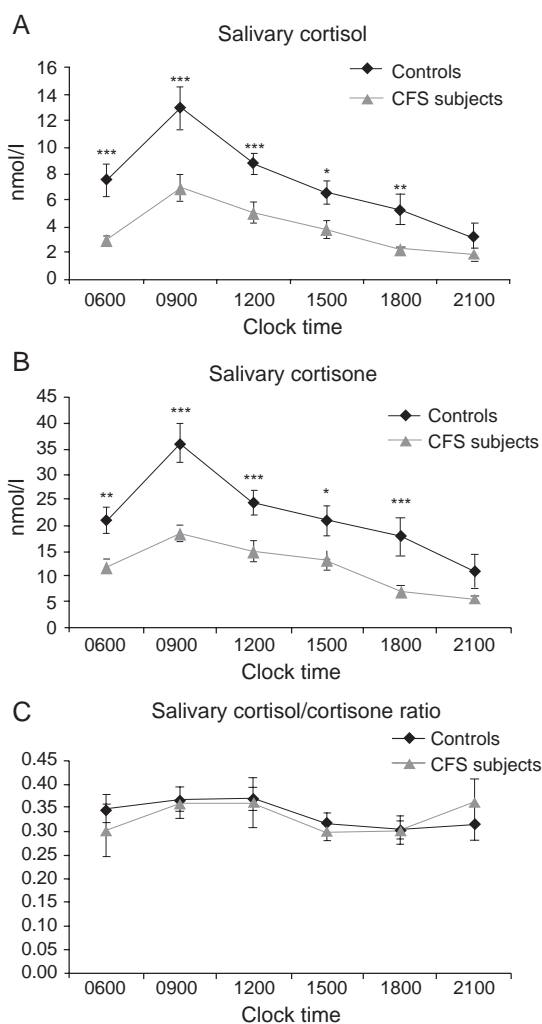


Fig. 1. Graph showing the mean values and standard errors of: (A) salivary cortisol, (B) salivary cortisone, and (C) cortisol/cortisone ratio in CFS patients ($n=15$) and healthy controls ($n=20$) at 3-h interval for 15 h. * $p<0.01$, ** $p<0.001$, *** $p<0.0001$.

estimate statistic of rhythm (MESOR), defined as the rhythm adjusted mean; (3) amplitude (defined as half the difference between nadir and peak); (4) acrophase, defined as the time of peak in the cosinor curve and expressed as a phase angle in degrees, so the formula ((Value in degrees/360°)*24 h) can be used to establish the time of peak relative to the starting point.

2.5. Statistical analyses

Group comparisons were made using independent *t*-tests (SPSSv11 for windows), since data were normally distributed. For comparison of hormone levels we used repeated measures analyses of variance (ANOVA) with planned post hoc *t*-tests for each of the 3 h blocks. The coefficient of correlation between questionnaire measures and MESOR of both cortisol and cortisone was calculated by the general linear regression method.

3. Results

There was no difference between the mean age and BMI of each group. Demographic and clinical details of the CFS subjects are presented in Table 1. There were no gender differences in salivary cortisol and cortisone for either group. The data were therefore combined.

Mean levels of salivary free cortisol and cortisone are shown in Fig. 1A and B. Using ANOVA, levels of salivary free cortisol and cortisone showed significant

main effects of group ($F(1,33)=25.2$; $F(1,33)=40.4$, respectively, both $p<0.0001$) and time (Hotelling's Trace=5.4, $F(3,98)=31.5$; 3.7, $F(4,90)=21.7$, respectively, both $p<0.0001$), but no group-by-time interaction (Hotelling's Trace=0.10, $F(3,98)=2.5$, $p=0.07$; 0.18, $F(4,90)=1.9$, $p=0.09$, respectively). Post hoc *t*-tests showed significantly lower levels of salivary cortisol and cortisone at all time points except for 2100 h (salivary cortisol: $p<0.0001$ for 0600 h, 0900 h and 1200 h, $p<0.001$ for 1800 h and $p<0.01$ for 1500 h; and salivary cortisone: $p<0.0001$ for 0900 h, 1200 h and 1800 h, $p<0.001$ for 0600 h and $p<0.01$ for 1500 h).

The ratio of salivary free cortisol to cortisone across the day is shown in Fig. 1c. Using ANOVA, there was no significant main effect of group ($F(1,33)=0.3$, $p=0.8$), time (Hotelling's Trace=0.28, $F(4,98)=0.7$, $p=0.1$), or group-by-time interaction (Hotelling's Trace=0.07, $F(4,98)=0.39$, $p=0.4$).

Cosinor analysis-derived population-mean circadian parameter estimates for controls and patients with CFS are detailed in Table 2. Patients with CFS showed a significant rhythm of both cortisol and cortisone. The amplitude and MESOR of both salivary cortisol and salivary cortisone were significantly lower in CFS compared to controls with no significant changes in the acrophase. There was no significant rhythm of the salivary cortisol/salivary cortisone ratio for either CFS or controls.

No correlation was noted between both MESOR of cortisol and cortisone and HADS questionnaires (for depression: $r=0.045$, $p=0.9$; -0.102 , $p=0.8$, respec-

Table 2
Circadian rhythm parameters of salivary cortisol, cortisone and their ratio in healthy controls and patients with CFS over 15 h

	Controls ($n=20$)			Patients with CFS ($n=15$)		
	Salivary cortisol	Salivary cortisone	Cortisol/cortisone ratio	Salivary cortisol	Salivary cortisone	Cortisol/cortisone ratio
% Rhythm (goodness of fit)	85%, $p<0.0001$	84%, $p<0.001$	56% $p=0.1$	70%, $p<0.0001$	77%, $p<0.0001$	55%, $p=0.9$
MESOR (nmol/l)	6.77 (5.90–7.63)*	21.0 (18.1–22.1)*	0.033 (0.030–0.035)	3.27 (2.60–3.93)	11.5 (8.85–12.2)	0.033 (0.283–0.378)
Amplitude (nmol/l)	3.79 (2.74–4.84)**	9.41 (5.44–13.4)**	0.0031 (0.0011–0.0072)	2.28 (1.84–2.72)	6.25 (3.79–8.71)	0.0057 (0.0052–0.0064)
Acrophase	–145 (–165 to –125)	–150 (–174 to –126)	–120 (–110 to –125)	–150 (–174 to –143)	–156 (–168 to –143)	–117 (–109 to –123)

Values are expressed as means (95% confidence intervals), Acrophase is presented as phase angle in degrees where $360^\circ=24$ h. For controls vs. CFS: * $p<0.0001$. ** $p<0.01$.

tively and for anxiety: $r=0.17$, $p=0.6$; $r=0.092$, $p=0.7$, respectively). We also found no correlation between MESOR of both cortisol and cortisone and the PSQI global scores ($r=0.47$, $p=0.08$; $r=0.48$, $p=0.07$, respectively).

4. Discussion

We have found further evidence of reduced salivary cortisol levels in a new sample of patients with CFS, selected to be free of the confounding influence of medication and psychiatric co-morbidity. Our findings are consistent with two previous studies that found lower morning levels of salivary cortisol (Strickland et al., 1998; Roberts et al., 2004) but contrast with others that did not find a lowering across a diurnal cycle (Young et al., 1998; Gaab et al., 2002; Wood et al., 1998). We also found a reduced diurnal fluctuation in cortisol levels, thus replicating with an increased sample frequency previous findings in saliva (MacHale et al., 1998) and blood (Hamilos et al., 1998).

We have previously commented that some of the differences between studies of cortisol in CFS can be ascribed to differences in populations studied (including psychiatric comorbidity, duration of illness, symptom profile and severity of illness) and quality of methodology (Cleare, 2003). However, approximately half of the best-designed studies do show low cortisol levels on serial sampling of blood or saliva, or using 24-h urine (Cleare, 2003); our study adds further to this literature by using a relatively frequent, non-invasive sampling protocol over the course of 15 h in a naturalistic setting, and finding lowered levels of salivary cortisol in CFS throughout the day. However, like previous studies, we have not been able to link these changes directly to any clinical features of CFS.

This is to our knowledge the first study that has also measured cortisone levels in CFS. We have shown that the salivary cortisone profile in CFS shows a diurnal rhythm similar to that of cortisol and that cortisone levels are similarly low compared to healthy controls. We have also demonstrated that the ratio of salivary cortisol to cortisone remains almost constant throughout the day and is unchanged in CFS. This underscores the tight relationship

between cortisol and cortisone and suggests that the activity of 11- β -HSD 1 relative to 11- β -HSD 2 is unchanged in CFS. Thus, changes in this metabolic pathway are unlikely to underlie these or previous findings of low salivary cortisol levels. Finally, this finding provides another contrast between CFS and major depression, given previous studies suggesting an increased cortisol to cortisone ratio in depression (Raven and Taylor, 1998; Poor et al., 2004).

References

- Agarwall, A.K., Mune, T., Monder, C., White, P.C., 1994. NAD⁺ dependent isoform of 11- β -hydroxysteroid dehydrogenase cloning and characterization of cDNA from sheep kidney. *J. Biol. Chem.* 269, 259–262.
- Avery, D., Dahl, K., Savage, M., Brengelmann, G., Larsen, L., Kenny, M., Eder, D., Vitiello, M., Prinz, P., 1997. Circadian temperature and cortisol rhythms during a constant routine are phase-delayed in hypersomnic winter depression. *Biol. Psychiatry* 41, 1109–1123.
- Buysse, D.J., Reynolds, C.F.D., Monk, T.H., Berman, S.R., Kupfer, D.J., 1989. The Pittsburgh sleep quality index: a new instrument for psychiatric practice and research. *Psychiatry Res.* 28, 193–213.
- Chalder, T., Berelowitz, G., Pawlikowska, T., Watta, L., Wessely, S., Wright, D., Wallace, E.P., 1993. Development of a fatigue scale. *J. Psychosom. Res.* 37, 147–153.
- Cleare, A., 2003. The neuroendocrinology of chronic fatigue syndrome. *Endocr. Rev.* 24, 236–252.
- Fukuda, K., Straus, S.E., Hickie, I., Sharpe, M.C., Dobbins, J.G., Komoroff, A., 1994. The chronic fatigue syndrome. *Ann. Intern. Med.* 121, 953–959.
- Gaab, J., Huster, D., Peisen, R., Engert, V., Schad, T., Schurmeyer, T.H., Ehlert, U., 2002. The low dose dexamethasone suppression test in chronic fatigue syndrome and health. *Psychosom. Med.* 64, 311–318.
- Hamilos, D.L., Nutter, D., Gershtenson, J., Redmond, D.P., Clementi, J.D., Schmaling, K.B., Make, B.J., Jones, J.F., 1998. Core body temperature is normal in chronic fatigue syndrome. *Biol. Psychiatry* 43, 293–302.
- Keedwell, P., Snaith, R.P., 1996. What do anxiety scales measure? *Acta Psychiatr. Scand.* 93, 177–180.
- Koorengel, K., Beersma, D., Den Boer, J., Van Den Hoofdakker, R., 2002. A forced desynchrony study of circadian pacemaker characteristics in seasonal affective disorder. *J. Biol. Rhythms* 17, 463–475.
- MacHale, S.M., Cavanagh, J.T., Bennie, J., Carroll, S., Goodwin, G.M., Lawrie, S.M., 1998. Diurnal variation of adrenocortical activity in chronic fatigue syndrome. *Neuropsychobiology* 38, 213–217.
- Marks, I., 1986. Behavioural Psychotherapy: Maudsley Pocket Book of Clinical Management. Wright, Bristol.

- Mazzocchi, G., Rossi, G.P., Neri, G., Malendowicz, L.K., Albertin, G., Nussdorfer, G.G., 1998. 11 β -Hydroxysteroid dehydrogenase expression and activity in the human adrenal cortex. *FASEB J.* 12, 1533–1539.
- Morineau, G., Boudi, A., Barka, A., Gourmelen, M., Degeilh, F., Hardy, N., Halnak, A., Soliman, H., Gosling, J.P., Julien, R., Brerault, J.L., Boudou, P., Aubert, P., Villette, J.M., Pruna, A., Galons, H., Fiet, J., 1997. Radioimmunoassay of cortisone in serum, urine, and saliva to assess the status of the cortisol–cortisone shuttle. *Clin. Chem.* 43, 1397–1407.
- Normura, A., Fujitaka, M., Jinno, K., Sakura, N., Ueda, K., 1996. Clinical significance of cortisone and cortisone/cortisol ratio in evaluating children with adrenal diseases. *Clin. Chim. Acta* 256, 1–11.
- Parker, A.J.R., Wessely, S., Cleare, A.J., 2001. The neuroendocrinology of chronic fatigue syndrome and fibromyalgia. *Psychol. Med.* 31, 1331–1345.
- Poor, V., Juricskay, S., Gati, A., Osvath, P., Tenyi, T., 2004. Urinary steroid metabolites and 11 β -Hydroxysteroid dehydrogenase activity in patients with unipolar recurrent major depression. *J. Affect. Disord.* 81, 55–59.
- Raven, P.W., Taylor, N.F., 1998. 11 β -HSD and 17 β -HSD as biological markers of depression: sex differences and correlation with symptom severity. *Endocr. Res.* 24, 659–662.
- Roberts, A.D.L., Wessely, S., Chalder, T., Papadopoulos, A., Cleare, A.J., 2004. Salivary cortisol response to awakening in chronic fatigue syndrome. *Br. J. Psychiatry* 184, 136–141.
- Roland, B.L., Funder, J.W., 1996. Localization of 11- β -hydroxysteroid dehydrogenase type 2 in rats tissues: in situ studies. *Endocrinology* 137, 1123–1128.
- Sharpe, M., Chalder, T., Palmer, I., Wessely, S., 1997. Chronic fatigue syndrome. A practical guide to assessment and management. *Gen. Hosp. Psych.* 19, 185–199.
- Stewart, P.M., Whorwood, C.B., Mason, J.I., 1995. Type 2 11- β -hydroxysteroid dehydrogenase in foetal and adults life. *J. Steroid Biochem. Mol. Biol.* 55, 465–471.
- Strickland, P., Morriss, R., Wearden, A., Deakin, W., 1998. A comparison of salivary cortisol in chronic fatiguesyndrome, community depression and healthy controls. *J. Affect. Disord.* 47, 191–194.
- Walker, B.R., Campbell, J.C., Fraser, R., Stewart, P.M., Edwards, C.R.W., 1992. Mineralcorticoid excess and inhibition of 11- β -hydroxysteroid dehydrogenase in patients with ectopic ACTH syndrome. *Clin. Endocrinol.* 37, 483–492.
- Wood, P.J., Kilpatrick, K., Barnard, G., 1997. New direct salivary cortisol and cortisone assays using the “Delfia” system. *J. Endocrinol.* 155, 71.
- Wood, B., Wessely, S., Papadopoulos, A., Poon, L., Checkley, S., 1998. Salivary cortisol profiles in chronic fatigue syndrome. *Neuropsychobiology* 37, 1–4.
- Young, A.H., Sharpe, M., Clements, A., Dowling, B., Hawton, K.E., Cowen, P.J., 1998. Basal activity of the hypothalamic–pituitary–adrenal axis in patients with chronic fatigue syndrome. *Biol. Psychiatry* 43, 236–237.