ELSEVIER

Journal of Psychosomatic Research 60 (2006) 145–153

Journal of Psychosomatic Research

Diurnal excretion of urinary cortisol, cortisone, and cortisol metabolites in chronic fatigue syndrome

Walid K. Jerjes[a,*], Timothy J. Peters[a], Norman F. Taylor[a], Peter J. Wood[b], Simon Wessely[c], Anthony J. Cleare[d,e]

[a]*Department of Clinical Biochemistry, Guy's, King's and St Thomas' School of Medicine, Bessemer Road, SE5 9RS London, UK*
[b]*Regional Endocrine Unit, Southampton General Hospital, SO16 6YD Southampton, UK*
[c]*Section of General Hospital Psychiatry, Division of Psychological Medicine, Institute of Psychiatry, King's College London, De Crespigny Park, SE5 8AF London, UK*
[d]*Section of Neurobiology of Mood Disorders, Division of Psychological Medicine, Institute of Psychiatry, King's College London, De Crespigny Park, SE5 8AF London, UK*
[e]*National Affective Disorders Unit, Bethlem Royal and Maudsley Hospitals, Monk's Orchard Road, Beckenham, BR3 3BS Kent, UK*

Received 3 February 2005; received in revised form 5 July 2005; accepted 19 July 2005

Abstract

Objective: The aim of this study was to obtain comprehensive information on basal hypothalamic–pituitary–adrenal (HPA) axis activity in chronic fatigue syndrome (CFS) patients who were not affected by medication or comorbid psychiatric disorder likely to influence the HPA axis. **Method:** Steroid analysis of urine collections from 0600 to 2100 h at 3-h intervals in CFS patients and in controls. **Results:** Urinary free cortisol and cortisone concentrations showed a significant normal diurnal rhythm, but levels were lower across the cycle in CFS. In contrast, while urinary cortisol metabolites also showed a normal diurnal rhythm, levels were not significantly different between the CFS and controls at any time. Derived metabolite ratios were similar in both groups. **Conclusion:** This study provides further evidence for reduced basal HPA axis function in patients with CFS, based on lower free cortisol and cortisone levels, but this is not corroborated by cortisol metabolite data. The difference between these measures cannot be explained by an altered timing of the diurnal rhythm.

Keywords: Chronic fatigue syndrome (CFS); Diurnal rhythm; Metabolism; Urinary cortisol; Urinary cortisone

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 [1]. The aetiology of CFS remains unclear, although there is evidence that biological, psychological, and social factors all play a part [2]. Many of the symptoms of CFS can also be associated with glucocorticoid deficiency states, and low serum cortisol was reported in early studies of patients with CFS and other fatigue states [3,4]. These results–from the evening and morning, respectively–could reflect a general hypocortisolaemia in CFS. However, other studies have shown no differences in serum cortisol levels [5,6]. Thus, there remains some inconsistency in research to date using serum cortisol to measure basal hypothalamo–pituitary–adrenal (HPA) axis function in CFS.

Assessment of HPA axis function by urine analysis offers advantages. Samples are obtained by a noninvasive, stress-free procedure and are easier to collect than blood [7,8]. Urinary free cortisol is considered to reflect the integrated, unbound plasma cortisol levels and was originally used in examining the

* Corresponding author. Tel.: +44 020 7346 4131; fax: +44 0207 737 7434.
E-mail address: jerjes@kcl.ac.uk (W.K. Jerjes).

0022-3999/06/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.jpsychores.2005.07.008

hypercortisolism of Cushing's disease [7,8]. Urinary free cortisol levels have generally shown good agreement with plasma cortisol levels in hypercortisolaemic states [7], and elevated levels in depression are reduced following treatment, mirroring clinical improvements [9]. However, observations in CFS have again reached variable conclusions. Four studies have found a low basal output of urinary free cortisol over 24 h [3,10–12] and two found no change [13,14]. It is possible that this variability might be explained by changes in steroid metabolism, rather than changes in circulating cortisol levels per se because free cortisol in urine represents only 2–3% of the urinary cortisol metabolites [15].

To overcome the problem outlined above, we first examined the 24-h urinary excretion of total cortisol metabolites (TCM) in patients with CFS and found them to be unchanged in comparison with that of healthy controls (Jerjes et al., unpublished data). This method, which is based on gas chromatography, and quantifies >95% of cortisol production per day, provides a sensitive means of detecting changes in rates of cortisol secretion, as has been demonstrated in asthmatics treated with inhaled glucocorticoids [16]. It also permits examination of changes in cortisol metabolism such as cortisol–cortisone interconversion. Cortisol converts reversibly into cortisone in blood, under the control of the 11-β-hydroxysteroid dehydrogenase (11-β-HSD Types 1 and 2) enzyme. Alteration in this equilibrium has been reported in various diseases [17,18].

The apparent difference of cortisol production in CFS based on serum point estimation and integrated values in urine might be explained by changed timing of the diurnal rhythm. This is supported by observations that CFS-like symptoms can also be seen in some conditions in which the circadian clock is phase shifted, such as seasonal affective disorder and major depression [19–21]. Few studies have attempted to measure the diurnal or circadian rhythm of cortisol in CFS. MacHale et al. [22] demonstrated a significantly lower diurnal change of serum cortisol in CFS based on evening and morning sampling over two consecutive days. Additionally, there was a significant positive relationship between the degree of diurnal variation in cortisol and measures of functional capacity. In two studies based on 4-h blood sampling, Hamilos et al. [14] reported a flattened diurnal rhythm of plasma cortisol in patients with CFS, whereas Racciatti et al. [5] did not find a significant change in cortisol rhythm in CFS. We therefore aimed to recruit a new group of well-characterised CFS patients free from medication or comorbid psychiatric disorders that might confound assessment of the HPA axis and measure the levels of urinary free cortisol, cortisone, and their metabolites across a diurnal cycle to provide further information regarding the status of the HPA axis in CFS. We hypothesised that we would find a reduction in free cortisol output throughout the day, and that this would also be accompanied by a change in measures of the diurnal rhythm of cortisol and its metabolites.

Materials and methods

Participants

Fifteen patients with CFS (7 males and 8 females) were recruited via the CFS clinic at King's College Hospital (KCH), which sees secondary and tertiary care referrals from the south of the United Kingdom. Participants were interviewed by experienced psychiatrists who used the semi-structured interview for CFS of Sharpe et al. [23] and DSM-IV to determine the presence of any psychiatric diagnoses. Participants were eligible for inclusion if they fulfilled the 1994 Center for Disease Control (CDC) criteria for CFS [1] without any exclusionary psychiatric disorder as per these criteria. Further inclusion criteria stipulated an age range of 25–60 years and the absence of any history of neurological, endocrine, or cardiovascular disorder. To obtain as pure a measure of the HPA axis as possible, we tested only patients who had never taken any psychotropic medication or had been abstinent from such medication for at least 2 months. Furthermore, although the modification of the original CDC diagnostic criteria in 1994 permitted the inclusion of patients with comorbid major depression or anxiety disorders, patients with a current major depressive episode or anxiety disorder as defined by DSM-IV criteria were excluded from this study because of their potential impact on the HPA axis. Patients were recruited consecutively over about 6 months. None of them had taken part in any of our previously published studies.

Twenty healthy volunteers (10 males and 10 females) were recruited among the staff and student body at KCH and were well matched for age, sex, and BMI with the CFS patients. They were all in good health, without any serious medical illness or history of psychiatric disorder. Participants were all studied during winter, between October 2002 and March 2003. All participants had normal dietary habits, taking breakfast, lunch, and dinner at about the same time. All participants habitually went to bed between 2300 and 0100 h and got up between 0600 and 0800 h. All participants were asked to limit their intake of caffeine and alcohol during the collection period. While these agents may have effects on the HPA axis, it was considered that short-term avoidance of habitual intake would result in more disturbance of the axis. All participants were instructed to carry out sample collections at weekends to avoid possible increase of cortisol levels that might result from stress on working days. No female participants were on oral contraceptive or were pregnant. All participants gave written, informed consent and ethical approval for the study was obtained from our local committee.

Questionnaires

All participants completed the Hospital Anxiety and Depression scale (HADS) [24] for symptoms of anxiety and depression and the Pittsburgh Sleep Quality Index (PSQI; [25]) for sleep disturbance. Patients completed further questionnaires to characterise their illness: the Chalder

Table 1
Demographic and clinical characteristics for CFS patients and controls

	Patients with CFS (n=15)	Controls (n=20)	t Test
Age (years)	35±7.9	33±11.3	P=.4
BMI	24.4±50	24.2±4.6	P=.6
HADS questionnaire scores			
Depression	8.0±3.9	3.0±2.7	P<.01
Anxiety	7.3±5.6	3.1±2.6	P<.01
PSQI global scores	9.8±3.3	2.2±1.3	P<.01
Duration of illness (years)	2.7±0.6	N/A	N/A
Scores on the 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 are presented as mean±S.D.

Fatigue Scale [26] for fatigue severity and the Work and Social Adjustment Scale [27] for disability.

Urine collections

Participants were provided with standard containers without additives for 3-h urine collections and given clear instructions on how to complete the collection. They were told to collect urine for five 3-h blocks over the course of a day, starting at 0600 h, for a total of 15 h. They were instructed to empty their bladder normally at 0600 h and to collect all passed urine (if any) into the first container (for period 0600–0900 h). At 0900 h, they were to empty their bladder into the container and end that 3-h collection. All urine thereafter was to go into the 0900–1200 h container, and the bladder emptied at 1200 h to end that collection, and so forth, until 2100 h.

Upon receipt of the specimen at the laboratory, the exact volume was noted, and after vigorous shaking, two 20-ml aliquots were taken for freezing at −40 °C prior to subsequent analysis.

Urinary free cortisol and cortisone measurements

Urine cortisol and cortisone were extracted into dichloromethane, and dried extracts were analysed by radioimmunoassay using Guildhay sheep anticortisol antiserum (HPS 631-1G) and cortisol-3CMO-histamine-[125-I] as tracer [28]. Urine cortisone was extracted into chloroform and analysed by radioimmunoassay using rabbit anticortisone antiserum (N-137) and 21-acetyl-cortisone-3CMO-histamine-[125-I] as tracer [29]. Interassay CV% was less than 12% for cortisol concentrations over 50nmol/l and less than 10% for urine cortisone concentrations over 10 nmol/l.

Cortisol metabolite measurements

Urinary steroid profile analysis was carried out by high-resolution gas chromatography of methyloxime-trimethylsilyl ether (MO-TMS) derivatives as previously described [30]. The intra- and interassay CVs were between 7.1% and 21.1%, and 11.2% and 21.9%, respectively, for different metabolites. Cortisol metabolites were calculated as μg/3-h period. Derived sums were as previously reported [31]. TCM were determined from the sum of tetrahydrocortisone (THE), tetrahydrocortisol (THF), allo-THF (5αTHF), α-Cortolone, β-Cortolone, α-Cortol, and β-Cortol. 11-Hydroxy-cortisol metabolites (11OH) were derived from the sum of THF, 5αTHF, α-cortol, and [(β-Cortolone+ β-Cortol)×0.5]. 11-Oxo-cortisol metabolites (11OXO) were

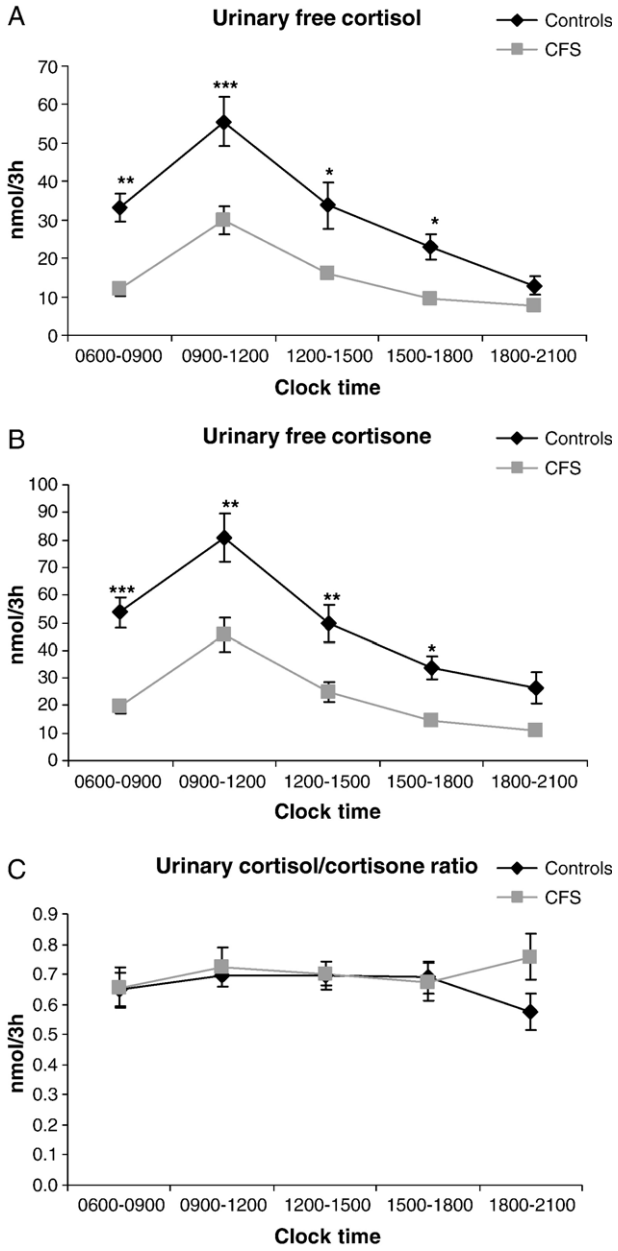

Fig. 1. Mean values and standard errors of (A) urinary cortisol, (B) urinary cortisone, and (C) urinary cortisol/cortisone ratio in CFS patients (n=15) and healthy controls (n=20) at a 3-h interval for 15 h. *P<.05, **P<.01, and ***P<.001.

derived from the sum of THE, α-Cortolone, and [(β-Cortolone+β-Cortol)×0.5]. 20-Hydroxy metabolites of cortisol were determined from the sum of α-Cortolone, β-Cortolone, α-Cortol, and β-Cortol, and 20-oxo metabolites of cortisol were determined from the sum of THE, THF, and 5αTHF. The ratios of 11OH/11OXO metabolites and THFs/THE ((THF+5αTHF)/THE) were calculated as indices of total net 11-β-HSD activity. The ratio 5α/5βTHF (5αTHF/THF) was calculated as an index of 5α versus 5β reduction and 20OH/20OXO metabolites as an index of net 20-HSD activity.

Circadian rhythm analysis

To determine the circadian rhythm parameters of each variable, individual and population mean cosinor analysis was performed using TSA-Seriel Cosinor software (Expert Soft Technologie, Laboratoire d'Informatique BioMédicale, France), for analysis of biological time series by least squares estimation. Population-mean cosinor analysis is based on the means of parameter estimates obtained from individuals in the study sample to derive the following parameters: (1) the goodness of fit of a cosinor curve fitted to the data; (2) midline estimate statistic of rhythm (MESOR), defined as the rhythm adjusted mean; (3) amplitude, defined as half the extent of rhythmic change in a cycle approximated by a fitted curve (difference between nadir and peak); and (4) acrophase, defined as the time of peak in the cosinor curve fitted to the data. The acrophase is expressed as a phase angle in degrees; thus, the formula ((value in degrees/360°)×24 h) can be used to establish the time of peak.

Statistical analyses

Urinary free cortisol and cortisone values were calculated as nmol/3 h, while urinary cortisol metabolites were calculated as μg/3 h. Group comparisons were made by the independent *t* test (using SPSS for windows V 11) because males and females did not show significant deviations from a normal distribution. For comparison of hormone levels across the entire period, we used a repeated measures analyses of variance (ANOVA) with planned post hoc *t* tests on values for each of the 3-h blocks over 24 h to determine if there were any differences in cortisol levels at a particular time of the day.

Results

The demographic and clinical details of the CFS participants are presented in Table 1. There was no difference between the mean age and BMI of each group.

Urinary free cortisol and cortisone

The levels of urinary free cortisol and cortisone are shown in Fig. 1A, B. Using ANOVA, levels of urinary free cortisol and cortisone showed significant main effects of group [$F(1,33)=27.6$ and $F(1,33)=30.2$, respectively, both $P<.0001$] and time [Hotelling's Trace=2.9, $F(2,76)=22.8$, $P<.0001$; 2.2, $F(2,80)=16.8$, $P<.0001$, respectively], but no difference in the group-by-time interaction [Hotelling's Trace=0.10, $F(2,76)=1.2$, $P=.22$; 0.18, $F(2,80)=1.4$, $P=.27$, respectively]. Thus, cortisol and cortisone levels were significantly lower in CFS across the period (main effect of group), there was a diurnal fluctuation in cortisol and cortisone levels (main effect of time), and the diurnal pattern was not significantly different between CFS and controls (group-by-time interaction). Post hoc *t* tests showed significantly lower levels of cortisol and cortisone at all time points except for 1800–2100 h. Mean differences (with 95% confidence intervals in parentheses) between the patients and controls for free cortisol were as follows (nmol/3 h): (a) 0600–0900 h: 24.5 (6–43), $P<.01$; (b) 0900–1200 h: 27 (9–46), $P<.001$; (c) 1200–1500 h: 20 (2–39), $P<.05$; and (d) 1500–1800 h: 15 (3–31), $P<.05$. The same values for cortisone were (a) 0600–0900 h: 36 (11–62), $P<.001$; (b) 0900–1200 h: 35 (9–60), $P<.01$; (c) 1200–1500 h: 25 (6–43), $P<.01$; and (d) 1500–1800 h: 19 (0.4–37), $P<.05$.

The ratio of urinary free cortisol to cortisone across the day is shown in Fig. 1C. Using ANOVA, there was no significant main effect of group [$F(1,33)=0.67$, $P=.42$], time [Hotelling's Trace=0.095, $F(3,97)=0.7$, $P=.60$], or group-by-time interaction [Hotelling's Trace=0.16, $F(3,97)=1.2$, $P=.33$].

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

	Controls (n=20)			Patients with CFS (n=15)		
	Urinary cortisol	Urinary cortisone	Cortisol/ cortisone ratio	Urinary cortisol	Urinary cortisone	Cortisol/ cortisone ratio
% Rhythm (goodness of fit)	77 $P<.0005$	78 $P<.0005$	58 $P=.1$	70 $P<.0005$	71 $P<.0005$	58 $P=.9$
MESOR (nmol/3 h)	31 (23–37)**	47 (36–52)**	0.67 (0.59–0.80)	14 (8.0–13.0)	22 (14–24)	0.70 (0.63–0.78)
Amplitude (nmol/3 h)	21 (13–28)*	25 (15–32)*	0.0088 (0.003–0.02)	12 (8–15)	16 (13–19)	0.009 (0.002–0.01)
Acrophase	−175 (−184 to −143)	−176 (−191 to −168)	−171 (−179 to −145)	−177 (−183 to −154)	−175 (−186 to −145)	−190 (−200 to −176)

Values are expressed as means (95% confidence intervals). Acrophase is presented as phase angle in degrees, where 3600=24 h.
* For controls vs. CFS: $P<.05$.
** For controls vs. CFS: $P<.0005$.

Thus, there was no overall difference in the ratio between patients and controls.

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 cortisol (70%) and cortisone (71%, both $P<.0005$), similar to the patterns seen in controls. There was no difference in the acrophase between the CFS and controls. However, MESOR and the amplitude of both urinary cortisol and urinary cortisone were significantly lower in the CFS compared with the controls. The mean difference (95% CI) for urinary free cortisol MESOR was 17 nmol/3 h (25 to 8, $P<.0005$) and 9 nmol/3 h for amplitude (18 to 2, $P<.05$). The values for urinary free cortisone showed a mean difference in the MESOR of 25 nmol/3 h (35 to 15, $P<.0005$) and a mean difference in amplitude of 9 nmol/3 h (19 to 2, $P<.05$). No significant rhythm of the urinary free cortisol/urinary cortisone ratio was noted for either CFS or controls (58%, $P=.1$; 58%, $P=.09$, respectively).

Urinary cortisol metabolites

The levels of urinary cortisol metabolites and cortisol metabolite ratios are shown in Fig. 2A–D. On ANOVA, there was a significant main effect of time on TCM [Hotelling's Trace=2.15, $F(2,67)=16.1$, $P\leq.0001$] and a significant group-by-time interaction [Hotelling's Trace=0.37, $F(2,67)=2.8$, $P=.045$], demonstrating that the diurnal pattern of cortisol metabolites in CFS is significantly different than that in controls, but no group difference [$F(1,33)=2.4$, $P=.13$], confirming that patients and controls had similar overall urinary cortisol metabolite levels. Post hoc t tests showed that urinary cortisol metabolite excretion was not significantly different between CFS and controls at any of the individual time points.

The ratio 11OH/11OXO in CFS showed no effect of group [$F(1,33)=0.13$, $P=.7$] or time [Hotelling's Trace=0.17, $F(3,91)=1.3$, $P=.20$] and no group-by-time interaction [Hotelling's Trace=0.32, $F(3,91)=2.4$, $P=.67$]. Findings were similar for THFs/THE.

The ratios of 5α/5βTHF and 20OH/20OXO in CFS showed significant effects of time [Hotelling's Trace=0.44, $F(3,84)=3.0$, $P=.02$; and 0.41, $F(3,100)=3.0$, $P=.03$, respectively], confirming the presence of a diurnal change. However, there was no group difference [$F(1,33)=1.15$, $P=.2$, and $F(1,33)=1.20$, $P=.3$, respectively] or group-by-time interaction [Hotelling's Trace=0.13, $F(3,84)=1.02$, $P=.4$, and 0.027 $F(3,100)=0.2$, $P=.9$, respectively]. Considering the differences in 5α/5βTHF and 20OH/20OXO ratios at each 3-h period, post hoc t tests showed that these ratios were not significantly different between CFS and controls at any of the individual time points (Fig. 2C and D).

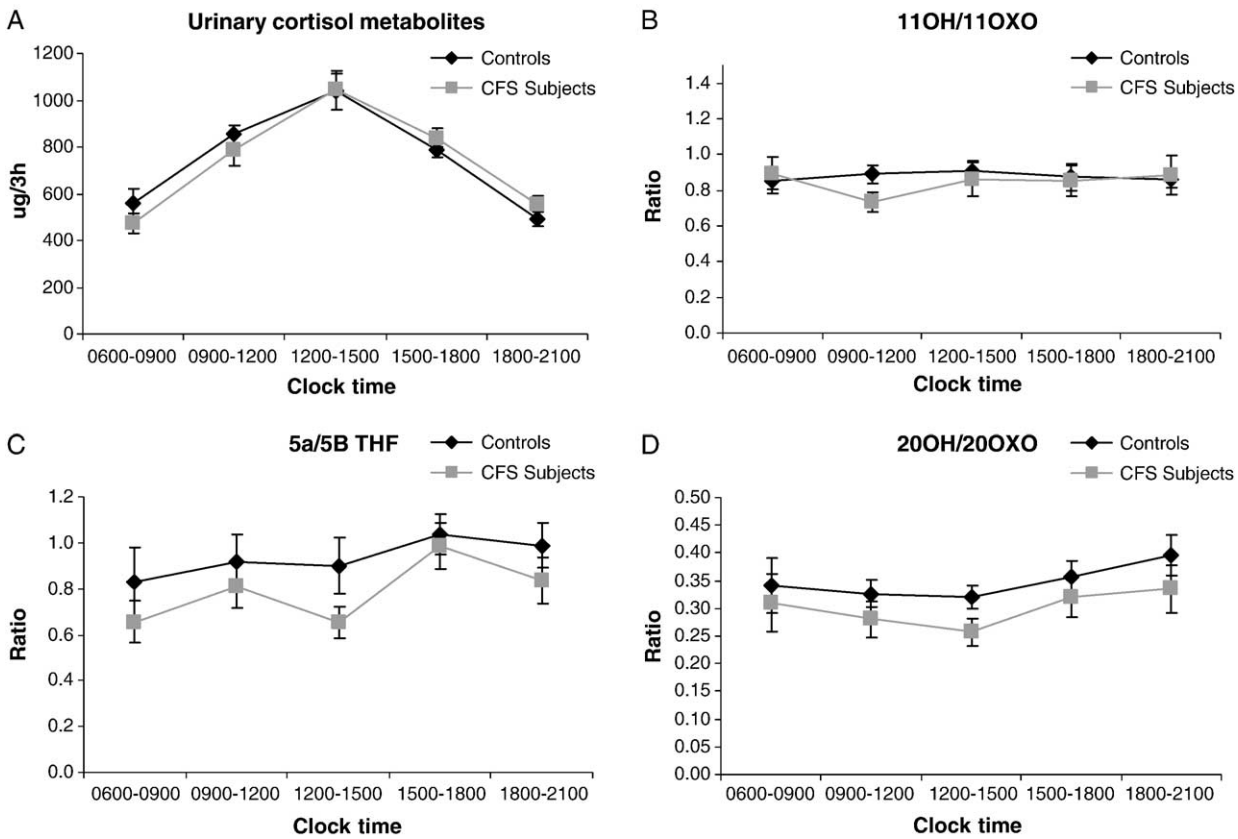

Fig. 2. Mean values and standard errors of (A) urinary cortisol metabolites, (B) 11OH/11OXO ratio, (C) 5α/5β THF ratio, and (D) 20OH/20OXO ratio in CFS patients ($n=15$) and healthy controls ($n=20$) at a 3-h interval for 15 h.

Table 3
Comparison of circadian rhythm parameters of total urinary cortisol metabolites and their metabolite ratios in patients with CFS and controls over 15 h

	Controls (n=20)				Patients with CFS (n=15)			
	Urinary cortisol metabolites	11-OH/11-Oxo	5±/5±THF	20OH/20OXO	Urinary cortisol metabolites	11-OH/11-Oxo	5±/5±THF	20OH/20OXO
% Rhythm (goodness of fit)	74 P<.0005	45 P=.2	69 P<.005	60 P<.005	72 P<.0005	51 P=.1	66 P<.05	60 P<.05
MESOR (±g/3 h)	747 (570–881)	0.87 (0.76–0.94)	0.89 (0.76–1.0)	0.33 (0.26–0.36)	741 (557–862)	0.84 (0.70–1.0)	0.80 (0.60–0.90)	0.29 (0.25–0.33)
Amplitude (±g/3 h)	371 (241–500)	0.075 (0.01–0.23)	0.14 (0.043–0.23)	0.0042 (0.002–0.007)	346 (259–433)	0.089 (0.067–0.25)	0.14 (0.060–0.24)	0.0048 (0.002–0.009)
Acrophase	−207 (−257 to −171)	−278 (−289 to −258)	−265 (−300 to −229)	−289 (−323 to −256)	−201 (−265 to − 181)	−267 (−285 to −257)	−256 (−274 to −239)	−301 (−320 to −253)

Values are expressed as means (95% CI). Acrophase is presented as phase angle in degrees, where 3600=24 h.
Controls vs. CFS: The P values of all the above parameters were greater than .05, taken as nonsignificant, using independent t test.

Cosinor analysis-derived population-mean circadian parameter estimates for controls and patients with CFS are detailed in Table 3. Patients with CFS showed a significant rhythm of total urinary cortisol metabolites (72%, P<.0005). The amplitude and MESOR were not different in the CFS compared with the controls (mean difference=−25, 95% CI: −193 to 143, P=.7; −11, 95% CI: −170 to 148, P=.8, respectively), with no significant changes in the acrophase. The ratio of 5α/5βTHF and 20OH/20OXO showed a significant circadian rhythm in CFS (66% and 60%, both P<.05, respectively), whereas there was no significant rhythm of 11OH/11OXO ratio in either CFS or controls (51%, P=.1, and 45%, P=.2, respectively).

Discussion

Diurnal levels of urinary cortisol

In this study, we found lower levels of urinary free cortisol throughout the course of the day in patients with CFS, but with unchanged diurnal rhythm. These findings are consistent with and add weight to the previous literature showing reduced urinary free cortisol in CFS [3,10–12]. Furthermore, our findings have shown this reduction in urinary free cortisol at nearly all times of the day, something not previously reported; previous studies all used single 24-h collections. However, two other studies in the literature found no change in urinary cortisol in CFS [13,14]. Murphy [32] noted that all studies of UFC in CFS had been obtained using radioimmunoassays without chromatography, which gives values considerably higher than those reported in studies that used prior chromatography, and suggested that the higher levels in normals than in CFS might thus be due to a higher cross-reactivity of cortisol metabolites. However, we have found no difference in cortisol metabolites in CFS that might account for a changed level of cross reactivity (Jerjes et al., unpublished data). While the studies based on high-resolution chromatography by Schoneshofer et al. [33] might be expected to give values least distorted by interference, values based on GC-MS are higher and closer to those of radioimmunoassays [32]; hence, doubt remains about the accuracy of these alternative approaches.

Patient selection criteria may be another confounder contributing to inconsistent findings in the literature. In the study of Hamilos et al. [14], 2/7 patients had major depression and 1/7 panic disorder. In the study of Young et al. [13], patients with major depression and anxiety were excluded, but 10/22 with CFS had previous depressive disorder. Furthermore, testing in both those two studies was not carried out in a naturalistic setting, as the participants all came into a laboratory for the duration of the sampling. There may have been an additional effect of stress that might have obscured any underlying changes in basal cortisol levels.

Notwithstanding these potential explanations, we have previously commented on the lack of consensus of studies of basal HPA axis function in CFS [34]. Some of the differences can be ascribed to differences in the populations studied (including psychiatric comorbidity, duration of illness, symptom profile, and severity of illness) and quality of methodology. However, approximately half of the best-designed studies do show low cortisol levels. Our study adds further to this literature by using a more frequent and noninvasive sampling protocol over the course of 15 h in a naturalistic setting and finding lowered levels of cortisol in urine in CFS.

Diurnal levels of urinary cortisone

We found that the urinary cortisone profile in CFS shows a diurnal rhythm similar to that of cortisol, with highest values in the morning at between 0900 and 1200 h and a nadir in the evening. The reduced urinary cortisone MESOR and reduced cortisone values at individual time points parallels the decreased urine cortisol in CFS patients compared with controls. A similar covariance of serum cortisol and cortisone has been seen in patients with major depression, although in the direction of increased levels of both [35]. This is, to our knowledge, the first study to demonstrate a reduced spontaneous urinary cortisone concentration in patients with CFS.

Diurnal levels of urinary cortisol metabolites

To the best of our knowledge, the diurnal rhythm of urinary cortisol metabolites has not been investigated to date in patients with CFS. We found that, as with cortisol itself, the cortisol metabolite rhythm in urine was not phase shifted. The amplitude and MESOR of the cortisol metabolite rhythm were not significantly different in CFS compared with controls. These latter findings were consistent with the overall unchanged daily mean levels of cortisol metabolites as well as the unchanged values at most individual time points over 15 h on ANOVA. Our study of 24-h excretion of cortisol metabolites in CFS in a separate group of patients also showed no differences compared with healthy controls (Jerjes et al., unpublished data).

Cortisol to cortisone interconversion

In humans, cortisone derives mainly from the oxidation of the 11-hydroxyl function of cortisol to the 11-keto form by 11-β-HSD Type 2. 11-HSD Type 2 is mainly located in mineralcorticoid target tissues, principally kidney, colon [36,37], and the parotid gland [38,39]. Inversely, the conversion of cortisone to cortisol takes place mainly in the liver [40], under the action of 11-β-HSD Type 1. The ratios of urinary free cortisol/cortisone and 11OH/11OXO represent an index of 11-β-HSD activity. Weber et al. [35] reported no alteration in serum cortisol/cortisone in patients with severe major depression. In contrast, Raven and Taylor [41] reported an increased of 11OH/11OXO ratio, suggesting an alteration of 11-β-HSD activity in depressed women, although the phenomenon could not be demonstrated in depressed men. Further support for this comes from a recent study demonstrating an increase of ratio in both depressed men and women [42]. The increase of net cortisol to cortisone interconversion is coupled with enhancement of cortisol metabolic clearance and vice versa [43].

To the best of our knowledge, the ratio of free urinary cortisol/cortisone and their metabolites have not been previously measured in CFS patients. We found no significant diurnal rhythm in the urinary free cortisol/cortisone and 11OH/ 11OXO ratios in either patients or controls. There were no significant differences in the ratio at each time point between patients and controls. This underscores the tight relationship between cortisol and cortisone and points to an unchanged activity of the 11-β-HSD enzymes. These data parallel our observations of no change in the salivary cortisol/cortisone ratio in the same CFS group [44].

Other pathways of cortisol metabolism

We also demonstrated significant diurnal rhythms of 5α/5β THF and 20OH/20OXO ratios in CFS, which were not different from those of the controls. In contrast, an increase of 5α/5β THF ratio was noted in patients with major depression [45,46].

Implications

We have thus found that urine free cortisol and free cortisone are lower in CFS, but urinary excretion of cortisol metabolites is unchanged and indices of cortisol metabolism show no differences. This combination of findings cannot be explained by an altered diurnal rhythm in CFS, but could be explained by an enhanced cortisol metabolic clearance rate. No direct measures of cortisol clearance have been undertaken in CFS to date. An increase in metabolic clearance rate of cortisol can result from changed cortisol metabolism: We have proposed that a reduced 11OH/11OXO ratio in patients with polycystic ovary was indicative of an elevated cortisol oxidation, which resulted in the enhancement of the cortisol metabolic clearance rate [47]. This study provides no support for such changes in CFS, but because urinary free cortisol represents only 2–3% of total cortisol production, a relatively small increase in the rate of cortisol metabolism could conceivably lead to a reduction in detectable free steroid without being reflected in our measures of metabolites. Similarly, the possibility of lower free steroid levels in CFS being due to lower cross-reaction in the assay cannot be excluded. However, the metabolite analysis has shown no differences that might suggest a candidate cross-reactant. The methodology we used, based on immunoassay of methylene chloride extracted material, has been questioned [32], but we are not convinced that any method can yet be claimed to guarantee a "true" value. Further work needs to explore the use of other measures to assess cortisol synthesis in CFS, such as infusion of isotopically labelled cortisol to measure production rate, with the ultimate aim of determining the role of cortisol in the pathogenesis CFS.

The following limitations should be noted. Patient selection criteria were rigorous; thus, our results may not be representative of CFS patients as a whole. The consumption of alcohol and caffeine was not recorded. We cannot therefore exclude an effect of differences in this on our results, although all participants were instructed to limit to their intake during the collection period. We did not record menstrual cycle in female participants because we have previously found no changes of the studied steroid metabolites over the menstrual cycle (NFT, unpublished observation) and no relationship of menstrual cycle and cortisol circadian rhythm has been found using saliva sampling [48]. Furthermore, a recent study found no differences in plasma cortisol between follicular and luteal phases in either CFS patients or healthy controls [49].

Conclusion

The main findings of this study are that we found further evidence of reduced urinary cortisol levels in a new sample of patients with CFS, selected to be free of the confounding influence of medication and psychiatric comorbidity. We have demonstrated that the reduction in urinary free cortisol is present throughout the day, and we have also shown for the first time that urinary cortisone has a similar circadian

variation to urinary cortisol, with reduced levels of cortisone also present in CFS patients. We have also demonstrated in CFS a significant diurnal rhythm of urinary cortisol metabolites, which is not different from that of controls, and with unchanged levels. Finally, we have demonstrated similar ratios of urinary free cortisol/cortisone and 11OH/11OXO, which remain almost constant throughout the day, suggesting that the activity of 11-β-HSD 1 relative to 11-β-HSD 2 has not changed in CFS patients. Changes in this metabolic pathway are not likely to underlie previous findings of low urinary free cortisol levels in CFS.

Acknowledgments

We thank Dorothy Blair for her excellent help in patient recruitment and sample collection.

References

[1] Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study International Chronic Fatigue Syndrome Study Group. Ann Intern Med 1994;121:953–9.
[2] Wessely S, Hotopf M, Sharpe M. Chronic fatigue and its syndromes. Oxford: Oxford Univ Press, 1998.
[3] Demitrack MA, Dale JK, Straus SE, Laue L, Listwak SJ, Kruesi MJ, Chrousos GP, Gold PW. Evidence for impaired activation of the hypothalamic–pituitary–adrenal axis in patients with chronic fatigue syndrome. J Clin Endocrinol Metab 1991;73:1224–34.
[4] Cleare AJ, Bearn J, Allain T, McGregor A, Wessely S, Murray RM, O'Keane V. Contrasting neuroendocrine responses in depression and chronic fatigue syndrome. J Affect Disord 1995;34:283–9.
[5] Racciatti D, Sensi S, DeRemigis P, Barberio A, Sciascio T, Pizzigallo E. Neuroendocrine aspects of chronic fatigue syndrome. Am J Med 1998;104:1S–3S.
[6] Altemus M, Dale JK, Michelson D, Demitrack MA, Gold PW, Straus SE. Abnormalities in response to vasopressin infusion in chronic fatigue syndrome. Psychoneuroendocrinology 2001;26:175–88.
[7] Murphy BE. Clinical evaluation of urinary cortisol determinations by competitive protein-binding radioassay. J Clin Endocrinol Metab 1968;28:343–8.
[8] Murphy BE. Steroids and depression. J Steroid Biochem Mol Biol 1991;38:537–59.
[9] Kling MA, Geracioti TD, Licinio J, Michelson D, Oldfield EH, Gold PW. Effects of electroconvulsive therapy on the CRH–ACTH–cortisol system in melancholic depression: preliminary findings. Psychopharmacol Bull 1994;30:489–94.
[10] Cleare AJ, Miell J, Heap E, Sookdeo S, Young L, Malhi GS, O'Keane V. Hypothalamo–pituitary–adrenal axis dysfunction in chronic fatigue syndrome, and the effects of low-dose hydrocortisone therapy. J Clin Endocrinol Metab 2001;86:3545–54.
[11] Cleare AJ, Blair D, Chambers S, Wessely S. Urinary free cortisol in chronic fatigue syndrome. Am J Psychiatry 2001;158:641–3.
[12] Scott LV, Dinan TG. Urinary free cortisol excretion in chronic fatigue syndrome, major depression and in healthy volunteers. J Affect Disord 1998;47:49–54.
[13] Young AH, Sharpe M, Clements A, Dowling B, Hawton KE, Cowen PJ. Basal activity of the hypothalamic–pituitary–adrenal axis in patients with the chronic fatigue syndrome (Neurasthenia). Biol Psychiatry 1998;43:236–7.
[14] Hamilos DL, Nutter D, Gershtenson J, Redmond DP, Clementi JD, Schmaling KB, Make BJ, Jones JF. Core body temperature is normal in chronic fatigue syndrome. Biol Psychiatry 1998;43:293–302.
[15] Raven PW, Taylor NF. Sex differences in the human metabolism of cortisol. Endocr Res 1996;22:751–5.
[16] Fink RS, Pierre LN, Daley-Yates PT, Richards DH, Gibson A, Honour JW. Hypothalamic–pituitary–adrenal axis function after inhaled corticosteroids: unreliability of urinary free cortisol estimation. J Clin Endocrinol Metab 2002;87:4541–6.
[17] Morineau G, Boudi A, Barka A, Gourmelen M, Degeilh F, Hardy N, Al-Halnak A, Soliman H, Gosling JP, Julien R, Brerault JL, Boudou P, Aubert P, Villette JM, Pruna A, Galons H, Fiet J. Radioimmunoassay of cortisone in serum, urine, and saliva to assess the status of the cortisol–cortisone shuttle. Clin Chem 1997;43:1397–407.
[18] Nomura S, Fujitaka M, Sakura N, Ueda K. Circadian rhythms in plasma cortisone and cortisol and the cortisone/cortisol ratio. Clin Chim Acta 1997;266:83–91.
[19] Avery DH, Dahl K, Savage MV, Brengelmann GL, Larsen LH, Kenny MA, Eder DN, Vitiello MV, Prinz PN. Circadian temperature and cortisol rhythms during a constant routine are phase-delayed in hypersomnic winter depression. Biol Psychiatry 1997;41:1109–23.
[20] Lewy AJ, Bauer VK, Cutler NL, Sack RL. Melatonin treatment of winter depression: a pilot study. Psychiatry Res 1998;77:57–61.
[21] Koorengevel KM, Beersma DG, den Boer JA, Van den Hoofdakker RH. A forced desynchrony study of circadian pacemaker characteristics in seasonal affective disorder. J Biol Rhythms 2002;17:463–75.
[22] MacHale SM, Cavanagh JT, Bennie J, Carroll S, Goodwin GM, Lawrie SM. Diurnal variation of adrenocortical activity in chronic fatigue syndrome. Neuropsychobiology 1998;38:213–7.
[23] Sharpe M, Chalder T, Palmer I, Wessely S. Chronic fatigue syndrome: a practical guide to assessment and management. Gen Hosp Psychiatry 1997;19:185–99.
[24] Snaith RP, Zigmond AS. The Hospital Anxiety and Depression Scale. Acta Psychiatr Scand 1983;67:361–70.
[25] Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res 1989;28:193–213.
[26] Chalder T, Berelowitz G, Pawlikowska T, Watts L, Wessely S, Wright D, Wallace EP. Development of a fatigue scale. J Psychosom Res 1993;37:147–53.
[27] Marks I. Behavioural psychotherapy: Maudsley pocket book of clinical management. Bristol: Wright, 1986.
[28] Moore A, Aitken R, Burke C, Gaskell S, Groom G, Holder G, et al. Cortisol assays: guidelines for the provision of a clinical biochemistry service. Ann Clin Biochem 1985;22:435–54.
[29] Wood PJ, Donovan D, Glenn C. New serum and urine radioimmunoassays for cortisone. Proceedings of XVI International Congress of Clinical Chemistry, London, 1996. pp. 478.
[30] Raven PW, Checkley SA, Taylor NF. Extra-adrenal effects of metyrapone include inhibition of the 11-oxoreductase activity of 11 beta-hydroxysteroid dehydrogenase: a model for 11-HSD I deficiency. Clin Endocrinol 1995;43:637–44.
[31] Trainer PJ, Drake WM, Perry LA, Taylor NF, Besser GM, Monson JP. Modulation of cortisol metabolism by the growth hormone receptor antagonist pegvisomant in patients with acromegaly. J Clin Endocrinol Metab 2001;86:2989–92.
[32] Murphy BEP. Urinary free cortisol determinations: what they measure. Endocrinologist 2002;12:143–50.
[33] Schoneshofer M, Fenner A, Altinok G, Dulce HJ. Specific and practicable assessment of urinary free cortisol by combination of automatic high-pressure liquid chromatography and radioimmunoassay. Clin Chim Acta 1980;106:63–73.
[34] Cleare AJ. The neuroendocrinology of chronic fatigue syndrome. Endocr Rev 2003;24:236–52.
[35] Weber B, Lewicka S, Deuschle M, Colla M, Vecsei P, Heuser I. Increased diurnal plasma concentrations of cortisone in depressed patients. J Clin Endocrinol Metab 2000;85:1133–6.

[36] Mazzocchi G, Rossi GP, Neri G, Malendowicz LK, Albertin G, Nussdorfer GG. 11beta-hydroxysteroid dehydrogenase expression and activity in the human adrenal cortex. FASEB J 1998;12:1533–9.
[37] Agarwal AK, Mune T, Monder C, White PC. NAD(+)-dependent isoform of 11 beta-hydroxysteroid dehydrogenase. Cloning and characterization of cDNA from sheep kidney. J Biol Chem 1994;269: 25959–62.
[38] Stewart PM, Whorwood CB, Mason JI. Type 2 11 beta-hydroxysteroid dehydrogenase in foetal and adult life. J Steroid Biochem Mol Biol 1995;55:465–71.
[39] Roland BL, Funder JW. Localization of 11beta-hydroxysteroid dehydrogenase type 2 in rat tissues: in situ studies. Endocrinology 1996;137:1123–8.
[40] Walker BR, Campbell JC, Fraser R, Stewart PM, Edwards CR. Mineralocorticoid excess and inhibition of 11 beta-hydroxysteroid dehydrogenase in patients with ectopic ACTH syndrome. Clin Endocrinol 1992;37:483–92.
[41] Raven PW, Taylor NF. 11Beta-HSD and 17beta-HSD as biological markers of depression: sex differences and correlation with symptom severity. Endocr Res 1998;24:659–62.
[42] Poor V, Juricskay S, Gati A, Osvath P, Tenyi T. Urinary steroid metabolites and 11 beta-hydroxysteroid dehydrogenase activity in patients with unipolar recurrent major depression. J Affect Disord 2004;81:55–9.
[43] Rodin A, Thakkar H, Taylor N, Clayton R. Hyperandrogenism in polycystic ovary syndrome. Evidence of dysregulation of 11 beta-hydroxysteroid dehydrogenase. N Engl J Med 1994;330: 460–5.
[44] Jerjes WK, Cleare AJ, Wessely S, Wood PJ, Taylor NF. Diurnal patterns of salivary cortisol and cortisone output in chronic fatigue syndrome. J Affect Disord 2005;87(23);299–304.
[45] Raven PW, Taylor NF. Evidence for independent modulation of human 11-HSD and 5 alpha/5 beta reductase activities. Endocr Res 1996;22: 811–5.
[46] Raven PW, Taylor NF. Dissociation of human 11-HSD and 5a/5B reductase activities. J Endocrinol 1997;152(Suppl): p. 275 [abstract].
[47] Rodin DA, Thakkar H, Taylor NF, Clayton RN. Hyperandrogenism in polycystic ovary syndrome Evidence of dysregulation of 11β-hydroxysteroid dehydrogenase. N Engl J Med 1993; 330:460–5.
[48] Kudielka BM, Kirschbaum CA. Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. Psychoneuroendocrinology 2003;28:35–47.
[49] Cevik R, Gur A, Acar S, Nas K, Sarac AJ. Hypothalamic–pituitary–gonadal axis hormones and cortisol in both menstrual phases of women with chronic fatigue syndrome and effect of depressive mood on these hormones. BMC Musculoskelet Disord 2004;5:47–56.