24-Hour Pituitary and Adrenal Hormone Profiles in Chronic Fatigue Syndrome

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Objectives: Disturbances of neuroendocrine function, particularly the hypothalamo–pituitary–adrenal (HPA) axis, have been implicated in the pathophysiology of chronic fatigue syndrome (CFS). However, few studies have attempted to measure blood levels of pituitary or adrenal hormones across a whole 24-hour period in CFS, and those that did so have used infrequent sampling periods. Our aim was to assess 24-hour pituitary and adrenal function using frequent blood sampling. **Methods:** We recruited 15 medication-free patients with CFS without comorbid psychiatric disorder and 10 healthy control subjects. Blood samples were collected over 24 hours and assayed for cortisol, corticotropin (ACTH), growth hormone (GH), and prolactin (PRL) levels on an hourly basis during daytime hours (10 AM to 10 PM) and every 15 minutes thereafter (10 PM to 10 AM). **Results:** Repeated-measures analyses of variance were undertaken using hormone levels averaged over 2-hour blocks to smooth curves by reducing the influence of sample timing relative to secretory burst. For ACTH, there was both a main effect of group, suggesting reduced mean ACTH secretion in patients with CFS over the whole monitoring period, and a group-by-time interaction, suggesting a differential pattern of ACTH release. Post hoc analysis showed reduced ACTH levels in CFS during the 8 AM to 10 AM period. In contrast, there were no significant abnormalities in the levels of cortisol, GH, and PRL in patients with CFS over the full cycle compared with control subjects. Cosinor analysis found no differences in the cortisol circadian rhythm parameters, but the ACTH rhythm did differ, patients with CFS showing an earlier acrophase. **Conclusions:** Patients with CFS demonstrated subtle alterations in HPA axis activity characterized by reduced ACTH over a full circadian cycle and reduced levels during the usual morning physiological peak ACTH secretion. This provides further evidence of subtle dysregulation of the HPA axis in CFS. Whether this dysregulation is a primary feature of the illness or instead represents a biologic effect secondary to having the illness itself remains unclear. **Key words:** chronic fatigue syndrome, neuroendocrinology, cortisol, hypothalamo–pituitary–adrenal axis, ACTH, circadian rhythm.

PRL = prolactin; **GH** = growth hormone; **ACTH** = corticotrophin; **CFS** = chronic fatigue syndrome; **HPA** = hypothalamo–pituitary–adrenal; **BMI** = body mass index; **CDC** = Centers for Disease Control and Prevention; **DSM-IV** = Diagnostic and Statistical Manual, Fourth Edition; **RIA** = radioimmunoassay; **IRMA** = immunoradiometric assay; **NETRIA** = North East Thames radioimmunoassay; **ANOVA** = analysis of variance; **CV** = coefficient of variance; **MESOR** = midline estimate statistic of rhythm; **CRH** = corticotrophin-releasing hormone; **CI** = confidence interval; **SD** = standard deviation.

INTRODUCTION

Chronic fatigue syndrome (CFS) is a disabling condition characterized by severe fatigue of at least 6 months' duration and accompanied by other characteristic symptoms such as sleep disturbance, muscle pain, and impaired concentration (1). Although its etiology remains by definition unexplained, emerging findings suggest that it is best understood as a multifactorial condition with a biopsychosocial underpinning.

Attempts to understand the means by which CFS is triggered and perpetuated are aided by experimental models in which symptoms can be provoked in healthy individuals. It is therefore of great interest that CFS-like symptoms, including fatigue, poor concentration, and sleep abnormalities, develop if circadian rhythms are disrupted experimentally or naturalistically by shift-working or jet-lag (2–7). Similar symptoms are also prominent in seasonal affective disorder in which the circadian clock may be phase-shifted (8–10). This observation has led to the possibility that CFS may be a condition associated with disturbances in endogenous circadian rhythms.

There is some support for this in CFS itself; most studied has been the hypothalamo–pituitary–adrenal (HPA) axis. MacHale et al. (11) demonstrated a significantly attenuated diurnal variation of serum cortisol in CFS, although the absolute concentrations at each time point were not significantly different compared with controls. Additionally, they found a significant relationship between the degree of diurnal variation in cortisol and measures of functional capacity. A similar finding of reduced diurnal variation seemed apparent in the study by Hamilos et al. (12), primarily related to a significantly reduced peak cortisol value. Further support for this comes from the demonstration of a significant decrease in the early morning surge of cortisol in a small group of patients with CFS (13). However, several other studies have not found significant changes in diurnal variation in the circadian rhythm of cortisol (14–18). Other circadian rhythms such as temperature seem to be essentially normal in CFS (19), although one study did suggest a desynchronization of the temperature and melatonin rhythms (20).

Another factor that has been widely studied in CFS is the integrity of neuroendocrine systems themselves, irrespective of alterations in circadian patterns. The balance of the evidence to date suggests that subtle neuroendocrine dysfunction may be present; more specifically, there may be disruption to the HPA axis resulting in lowered cortisol levels in at least a proportion of patients (21). Furthermore, these changes may be related to symptomatology because pharmacologic reversal of the cortisol deficit leads to an improvement in symptoms.

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Received for publication February 13, 2004; revision received November 23, 2004.

DOI: 10.1097/01.psy.0000161206.55324.8a

0033-3174/05/6703-0433

However, we have recently highlighted the lack of studies that take unstimulated, serial measures of HPA axis hormones in CFS; indeed, the most detailed examination of plasma cortisol levels involved just 7 samples over a 24-hour period (21).

Evidence relating to other hormonal systems is even less available. There is some evidence that basal release of growth hormone is reduced nocturnally in patients with CFS (22) but no assessments of growth hormone (GH) throughout a 24-hour cycle. One study of serial prolactin taken every 4 hours over 24 hours suggested a raised level at 4 AM (17).

In summary, although there is some evidence to suggest that some symptoms associated with CFS can be provoked by altering circadian rhythms, and preliminary suggestions of altered circadian rhythmicity in CFS, there has not yet been a sufficiently detailed study of HPA axis circadian rhythms in CFS. Similarly, there have been few studies adequately assessing unstimulated, basal HPA axis function in CFS across the circadian cycle. Finally, there have been suggestions of nocturnal disruption of GH and prolactin (PRL) release, a finding requiring independent replication. Therefore, the aim of this study was to improve on the existing knowledge base in CFS by undertaking a detailed assessment of basal levels of pituitary and adrenal hormones over a full circadian cycle. To this end, we measured cortisol, corticotropin (ACTH), GH, and PRL over 24 hours with frequent sampling intervals. To control for the potential confounding effect of disorders such as depression and anxiety, which may also have neuroendocrine effects, we included only subjects who had CFS but were also free of psychiatric comorbidity. We also studied only subjects who were drug-free, given potential effects of medication on neuroendocrine systems.

Based on the previous data, we hypothesized that we would find evidence in CFS of lowered circulating cortisol and reduced ACTH output, and that these changes would be most prominent during the nocturnal and early morning hours, when physiological levels of the hormones are at their highest. Similarly, we expected to find reduced levels of GH nocturnally and increased PRL in patients with CFS. However, all of these hypotheses were necessarily tentative because of the scarcity of well-collected data.

MATERIALS AND METHODS

Subjects

Twenty-five subjects entered the study: 15 patients (six male and nine female) who met Centers for Disease Control and Prevention (CDC) (1) criteria for CFS together with 10 healthy control subjects (three male and seven female) closely matched for age, weight, body mass index (BMI), and menstrual cycle. Patients with CFS were drawn from those referred to a tertiary referral outpatient center for CFS at King's College Hospital in London. Control subjects were recruited from volunteers and staff members in our institution.

Patients were interviewed using a semistructured interview for CFS and psychologic disorders (23). Subjects with CFS with an axis I Diagnostic and Statistical Manual, Fourth Edition (DSM-IV) psychiatric disorder occurring after the time of CFS onset were not included in this study. Additionally, patients who also fulfilled criteria for fibromyalgia as defined by the American College of Rheumatology were excluded (24). All participants had been through a thorough clinical evaluation, including a minimum of: full medical history, physical examination, and laboratory screening tests (full blood count, urea, electrolytes, liver function tests, erythrocyte sedimentation rate, and urinalysis, plus any other tests indicated by history and examination) to rule out an organic cause for their fatigue. At the time of the neuroendocrine assessment, subjects with CFS had been off all medications for at least 1 month; this was an inclusion criterion at the time of assessment, and no subjects were specifically withdrawn from medication for the study. All females were tested during the follicular phase of their menstrual cycle (days 1–7); none were using oral contraceptives or estrogen replacement therapy. None of the control subjects had a history of significant medical problems, CFS, DSM-IV psychiatric disorder, or was taking medications, as assessed by a psychiatrist on semistructured interview.

The study was approved by the local institutional ethics committees and both patients and control subjects gave their written, informed consent before participation.

Neuroendocrine Procedures

Subjects were admitted to the clinical research centre at 8 AM after an overnight fast from midnight. A cannula was inserted into a forearm vein and after a 2-hour adaptation period, blood samples were obtained every 15 minutes for a 24-hour period running from 10 AM to 10 AM. One milliliter of heparinized saline flash (Hepsal, heparin saline 10 U/mL) was administered after each sampling to keep the cannula patent during the monitoring period; the first 1.5 mL of each sample was discarded and 4 mL taken into EDTA-treated tubes. Samples were immediately spun in a refrigerated centrifuge, and the plasma immediately separated and frozen at −40°C until assayed. Assays took place in batches within 3 months of freezing, all specimens from the same subject being assayed at the same time. The total blood removed for each individual was approximately 400 mL.

Subjects received standardized meals during the study (lunch at 12 PM and dinner at 6 PM on day one; breakfast at 10 AM on day two) and spent the day relaxed and semirecumbent with normal bathroom privileges. During the night (11 PM until 7 AM), a small-bore extension line (183-cm arterial line tubing) was used to permit sampling from outside the subject's bed curtains.

Lights were off from 11 PM to 7 AM (complete darkness). The sleep/wake status of each subject was recorded every 15 minutes using an observational chart. Patients were all woken at 7 AM. Sleeping during the daytime period was discouraged. Subjects were all studied during wintertime hours, between October 1998 and April 1999.

Questionnaires

All subjects completed the Chalder Fatigue Scale (25) to assess mental and physical fatigue, the Medical Outcomes Survey Short Form-36 (SF-36) (26) and the Work and Social Adjustment Scale (27) to assess functional and social disability, the General Health Questionnaire (GHQ-12) (28) to assess psychiatric symptoms, and the Somatic Symptom Checklist of 40 items (29) to assess somatic symptoms.

Hormone Assays

Hormone concentrations were measured blind to subject status in the Department of Medicine, King's College Hospital. We assayed hourly samples from 10 AM to 10 PM and every 15 minutes from 10 PM to 10 AM.

ACTH was measured using an immunoradiometric assay from DSL (TX). The sensitivity of the assay was 0.8 pg/mL. The ACTH interassay coefficients of variance (CVs) were 9.6% at 35 pg/mL, 4.0% at 72 pg/mL, and 7.3% at 160 pg/mL. The intraassay CVs were 6.9% at 35 pg/mL, 5.9% at 71 pg/mL, and 5.3% at 152 pg/mL.

GH was estimated using the North East Thames radioimmunoassay (NETRIA) two-site immunoradiometric assay (IRMA) with a ^{125}I label. Sensitivity was 0.2 mU/L. The GH intraassay CVs were 2.7% at 0.8 mU/L, 2.4% at 4.5 mU/L, and 2.6% at 86.5 mU/L. The interassay CVs were 3.3% at 7.7 mU/L, 5.2% at 21.7 mU/L, and 5.5% at 45.8 mU/L.

Cortisol levels were obtained using the solid-phase radioimmunoassay (RIA) from DPC Coat-a-count, CA, USA. Sensitivity was 5.5 nmol/L. The cortisol intraassay CVs were 4.8% at 85 nmol/L, 4.7% at 273 nmol/L, and

3.0% at 551 nmol/L. The cortisol interassay CVs were 5.2% at 91 nmol/L, 4.0% at 579 nmol/L, and 6.4% at 993 nmol/L.

PRL was measured using a solid-phase IRMA from NETRIA. Sensitivity was 10 mU/L. The prolactin intraassay CVs were 2.5% at 165 mU/L, 1.4% at 562 mU/L, and 2.6% at 1159 mU/L. The prolactin interassay CVs were 8.3% at 173 mU/L, 5.1% at 506 mU/L, 7.9% at 1103 mU/L.

Statistical Analyses

All analyses were undertaken using the Statistical Package for Social Sciences (SPSS) version 10.0. Exploratory analysis showed that ACTH, cortisol, and PRL values were normally distributed. Therefore, we compared patients with CFS and controls using parametric tests for these hormones. The GH values were not normally distributed; therefore, we applied a log transformation before analysis, setting the undetectable values at 0.1 mU/L, representing 50% of the lower level of detection of the assay. For clarity, we present the raw values for GH in the Results section.

We analyzed data in accordance with previous work that used a similar design (30). When necessary, missing values were dealt with by linear interpolation. For outliers, data were visually inspected for each subject, and unexpected peaks were crosschecked against the data logs regarding any unexpected events such as recannulation. If there were explanatory events, these values were excluded; if there were no explanatory events, data were included as likely to represent part of the normal fluctuation in the HPA axis in response to day-to-day events. We undertook repeated-measures analyses of variance (ANOVA) for each hormone to assess hormone release across the entire period. To smooth curves and reduce the influence of sample timing relative to secretory burst, we used hormone levels averaged over 2-hour blocks in analyses. Additionally, we performed post hoc *t* tests looking for any difference in each of the 2-hour blocks to determine if there were any differences in hormone levels at a particular time of the day, as well as comparing mean values for the overnight period (10 AM to 6 AM) and the whole 24-hour period. Correlational analysis was performed using Pearson's product-moment correlation coefficients when relevant.

Finally, to determine the circadian rhythm parameters, we also undertook individual and population mean cosinor analysis using all cortisol and ACTH raw data. To keep the data interval uniform, we used hourly data for the full 24-hour circadian analysis and the 15-minute interval data for a hemicircadian analysis from 10 PM to 10 AM. We used TSA-Seriel Cosinor software (Expert Soft Technologie, Laboratoire d'Informatique BioMédicale, France) for analysis of biologic 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, so the formula (value in degrees/360° * 24 hours) can be used to establish the time of peak relative to the starting time.

RESULTS

Patients and control subjects did not differ significantly in age, weight, sex ratio, or BMI. Demographic and clinical details of all subjects are presented in Table 1. Sleep charts taken at the time showed that no subjects were observed to have significant difficulties sleeping (defined as a more than 50% reduction in their normal time asleep).

Individual Hormone Levels

Corticotrophin

The repeated-measures ANOVA showed significant main effects of group (F [1,20] = 4.4, p = .04) and time (Hoteling's trace = 6.90, F [11,10] = 6.28, p = .002) and a significant group-by-time interaction (Hoteling's trace = 3.45, F [11,10] = 3.14, p = .04) in subjects with CFS. Thus, ACTH levels were lower in patients with CFS across the period (main effect

TABLE 1. Demographic and Clinical Characteristics for Patients With Chronic Fatigue Syndrome and Control Subjects

	Patients With Chronic Fatigue Syndrome (n = 15)	Control Subjects (n = 10)	t Test
Age (y)	38.7 ± 14.4	38.3 ± 12.5	NS
Gender (no. and % female)	9 (60%)	7 (70%)	NS[a]
Weight (kg)	69.9 ± 11.0	72.2 ± 11.2	NS
Body mass index (kg/m^2)	23.5 ± 3.8	25.4 ± 3.0	NS
GHQ-12	18.5 ± 14.6	9.2 ± 3.6	NS
SCL	14.8 ± 5.7	2.3 ± 4.6	$p < .01$
Chalder Fatigue Scale			
Total fatigue	22.7 ± 6.4	9.5 ± 2.5	$p < .01$
Mental fatigue	7.6 ± 3.0	2.7 ± 1.5	$p < .01$
Physical fatigue	14.9 ± 4.0	6.8 ± 1.5	$p < .01$
WSAS	24.6 ± 7.5	NA	NA
SF-36			
Physical functioning	43.6 ± 23.6	100	$p < .01$
Social functioning	48.4 ± 23.3	100	$p < .01$
Emotional role limitation	62.2 ± 43.4	100	$p < .01$
Physical role limitation	10.0 ± 18.4	100	$p < .01$
General health	33.8 ± 16.8	95.2 ± 4.6	$p < .01$
Mental health	62.4 ± 22.1	93.4 ± 9.9	$p < .01$
Fatigue	30.6 ± 23.1	90.5 ± 10.1	$p < .01$
Bodily pain	52.0 ± 29.1	90 ± 31.6	$p < .01$

WSAS = Work and Social Adjustment Scale (maximum = 40, higher = more disabled); SF-36 = Medical Outcome Survey Short Form 36; (100 = full functional capacity, 0 = no functional capacity); SCL = Somatic Symptom Check List (number of symptoms, maximum score 40); GHQ-12 = 12-item General Health Questionnaire (maximum score 36); NS = not significant; NA = not applicable.

[a]Chi-squared test.

Values are mean ± standard deviation.

of group); there was a diurnal fluctuation in ACTH levels (main effect of time); and the diurnal pattern was significantly different between patients with CFS and control subjects (group-by-time interaction). The ACTH values are shown in Figure 1. Post hoc *t* tests showed significantly lower ACTH at 8 to 10 AM (mean difference = 34.4 pg/mL, 95% CI: −65.8 to −3.0, $p = .03$), but not at other time points (Table 2). In summary, there was evidence of an overall reduction in ACTH release in patients and an altered circadian pattern characterized by a reduction in the physiological morning peak ACTH release.

Cortisol

Patients and controls had similar cortisol levels (Fig. 2). On ANOVA, there was a significant main effect of time (Hoteling's trace = 13.79, F [11,8] = 0.30, $p = .02$), confirming the circadian pattern, but no group difference (F [1,18] = 0.36, $p = .55$) or group-by-time interaction (Hoteling's trace = 0.41, F [11,8] = 10.03, $p = .96$). No differences were observed on post hoc *t* tests between patients and control subjects on the 2-hourly values or in mean values (24 hours or overnight) (Table 2). The cortisol rhythm is shown in Figure 2. In summary, there was a circadian change in cortisol levels but no difference in mean cortisol levels or in the cortisol rhythm between patients and control subjects.

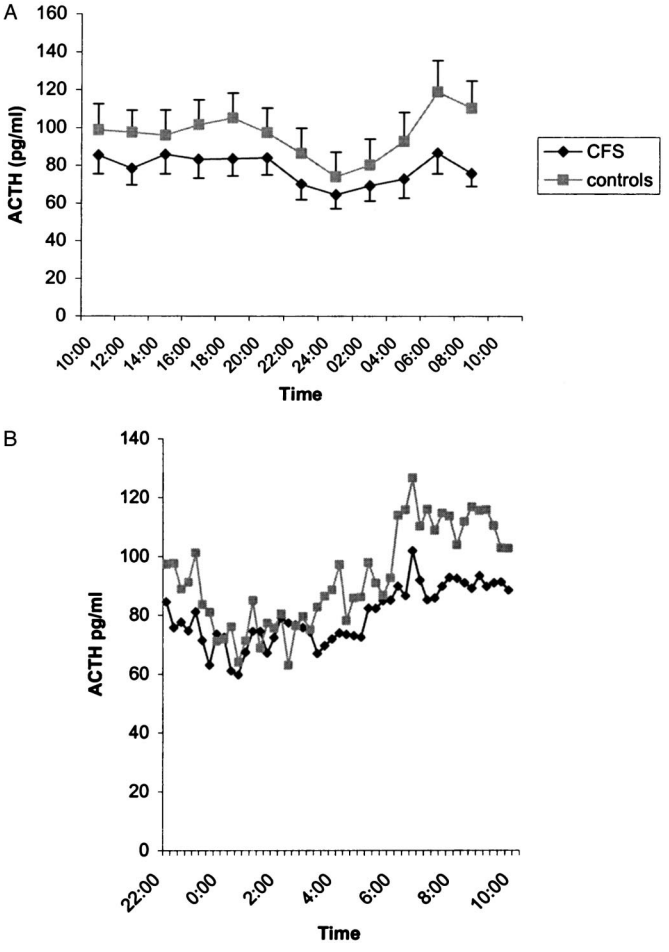

Figure 1. Graph showing the mean values of corticotrophin (ACTH) in patients with chronic fatigue syndrome ($n = 15$) and healthy control subjects ($n = 10$). (**A**) The whole 24-hour period, with each point representing the mean of values and standard errors over a 2-hour period. (**B**) The values at each 15-minute sampling point during the overnight (10 PM to 10 AM) period.

Prolactin

There was no effect of group (F [1,18] = 1.97, $p = .17$) or time (Hoteling's trace = 2.09, F [11,8] = 1.52, $p = .28$) and no group-by-time interaction (Hoteling's trace = 0.90, F [11,8] = 0.65, $p = .74$) on PRL values on the ANOVA. There were no significant differences in mean values at *t* test between subjects with CFS and control subjects (Table 2). In summary, we did not detect a diurnal change in prolactin levels, and the levels were not different between patients and control subjects.

Growth Hormone

There was a significant effect of time on GH responses (Hoteling's trace = 4.67, F [11,10] = 4.24, $p = .015$), confirming a diurnal pattern. The group effect (F [1,20] = 0.62, $p = .44$) and the group-by-time interaction (Hoteling's trace = 1.67, F [11,10] = 1.51, $p = .26$) were not significant. There were no differences in mean values between subjects with CFS and control subjects (Table 2). In summary, there were no differences in GH levels, or in the diurnal pattern of release, between patients and control subjects.

Cosinor Analysis

Cortisol data from both patients (40%) and control subjects (46%) showed a significant fit to a cosinor curve over the 24-hour circadian period (Table 3). These values represent a moderately good fit of the data and are similar to previous data in similar-sized groups of normal controls and patients with major depression (31). The parameters of the fitted cortisol circadian rhythms are shown in Table 3; there were no differences evident between CFS and control subjects. Similarly, there was a significant fit of cosinor 24-hour circadian rhythms to the ACTH data for patients and control subjects (Table 3). However, parameters of the fitted rhythms showed an earlier mean acrophase (circadian peak) in patients with CFS, with a phase advance of 38° (equivalent to 2.53 hours).

We were also able to fit a weaker but significant hemicircadian cosinor pattern to the ACTH data between 10 PM and 10 AM (Table 4). The hemicircadian pattern of nocturnal ACTH release showed reduced amplitude in patients with CFS, consistent with the earlier findings of a reduced morning surge.

We also looked for correlations between the acrophase time of ACTH and cortisol to see if there was evidence of a desynchronization of the two rhythms in CFS. However, there was not a significant correlation between ACTH and cortisol acrophase either in control subjects ($r = -0.27$, $p = .46$) or patients with CFS ($r = -0.15$, $p = .60$).

DISCUSSION

In this study, we undertook frequent blood sampling over the course of 24 hours to assess several pituitary and adrenal

TABLE 2. Summary Mean Hormone Levels for Patients With Chronic Fatigue Syndrome and Control Subjects

	Patients With Chronic Fatigue Syndrome		Control Subjects	
	24 Hr	Overnight	24 Hr	Overnight
Corticotropin (pg/mL)	79.0 ± 32.4	69.1 ± 30.6	96.5 ± 40.7	83.3 ± 42.8
Cortisol (nmol/L)	243.3 ± 81.3	173.9 ± 79.6	219.4 ± 79.5	158.6 ± 74.1
Prolactin (IU/L)	181.6 ± 70.3	201.3 ± 88.8	257 ± 191.3	315.2 ± 248.7
Growth hormone (IU/L)	2.3 ± 2.0	2.9 ± 1.9	1.4 ± 1.0	2.2 ± 2.6

Values are mean ± standard deviation.

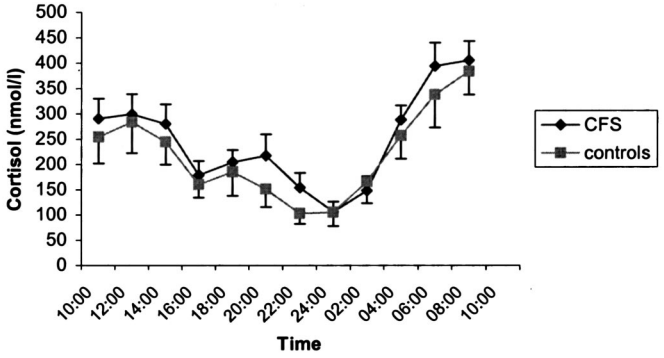

Figure 2. Graph showing the mean values and standard errors of cortisol in patients with chronic fatigue syndrome (n = 15) and healthy control subjects (n = 10) over the whole 24-hour period, with each point representing the mean of values over a 2-hour period.

hormones. The main findings from this study are that, in comparison to control subjects, patients with CFS showed reduced ACTH levels throughout the 24-hour monitoring period but no differences in cortisol, PRL, and GH. The rhythm of ACTH secretion was also altered, with a reduced morning secretory burst and a phase advance.

Hypothalamo–Pituitary–Adrenal Axis

There were clear differences in ACTH release seen in this study. First, the magnitude of ACTH release was significantly decreased throughout the 24-hour monitoring period in patients with CFS in comparison to control subjects. Second, there was evidence of a different pattern of ACTH release, and the most prominent change was of a blunting of the usual morning increase in ACTH release between 8 AM and 10 AM in patients with CFS. This latter finding mirrors to some extent the results of a recent study of 15 patients with CFS and 15 control subjects (32) that also measured ACTH across a 24-hour circadian cycle and found numerically (but not statistically) lower ACTH values in the early morning in CFS. A similar finding, of decreased rhythm amplitude of the ACTH peak in the early morning in CFS, was found in a further study (14,17). Little other data exist regarding serial ACTH plasma levels in CFS.

However, the significance of the low baseline ACTH in our CFS population is unclear, particularly as it is in the context of normal cortisol levels in this sample. In an interesting parallel, a recent study in psychotic depression found evidence for increased ACTH release across the circadian cycle, but also normal cortisol levels (33); thus, like in previous studies, the findings in severe depressive illness are in the opposite direction as that seen in CFS (34). Of interest, the same study (33) also looked at nonpsychotic major depression in comparison to control subjects and found no significant change in mean cortisol or ACTH levels but a reduced cortisol amplitude. One explanation for the low ACTH but normal cortisol levels in CFS, hypothesized by Demitrack and colleagues (35), is that blunted ACTH is in part the result of an impaired central nervous system drive inducing a reduction of hypothalamic output of corticotrophin-releasing hormone (CRH) and/or other secretagogues. This consequently leads to reduced ACTH levels and a compensatory upregulation of the adrenal cortex responsivity to ACTH, something previously found by some (36) but not others (37–39).

Furthermore, indirect support for a reduction in the central drive to the HPA axis comes from Altemus et al. (40), who found a reduced ACTH response to arginine vasopressin (AVP) infusion in CFS. Because the ACTH response to AVP is critically dependent on central levels of CRH, this suggests that there is low ambient hypothalamic CRH tone in CFS. However, some authors argue that blunted ACTH response to AVP may be also the result of abnormalities in AVP release or receptors (41,42).

Another explanation of low ACTH levels could be of an impaired pituitary responsiveness to CRH. Three studies (36,41,43) have reported attenuated ACTH response in CFS, whereas two others (44,45) demonstrated no difference in ACTH response between subjects with CFS and control subjects. However, significantly reduced release of ACTH has also been found in CFS with several other challenge tests, including naloxone, psychosocial stress, exercise, and hypoglycemia (21). These reductions in ACTH responses are frequently accompanied by normal cortisol responses, a similar pattern as our unstimulated responses in this article. These results suggest that patients are capable of mounting a sufficient cortisol response under different types of stress, but that on a central level, subtle dysregulation of the HPA axis may exist. Scott et al. (43) suggested that an initial stress may cause an elevation in CRH with consequent downregulation of CRH receptors on the pituitary corticotrophs. They hypothesized that this downregulation in CFS fails to normalize after the alleviation of stress, or the subsequent reduction in CRH levels, and this represents an example of abnormal plasticity of the CRH receptor.

TABLE 3. Circadian Rhythm Parameters of Serum Cortisol and Corticotrophin in Patients With Chronic Fatigue Syndrome and Control Subjects Using Cosinor Analysis Over 24 Hr

	Cortisol			Corticotrophin		
	Chronic Fatigue Syndrome	Control Subjects	*t* Test	Chronic Fatigue Syndrome	Control Subjects	*t* Test
Percent rhythm (goodness of fit, r^2)	40%, $p = .01$ ($r = 0.63$)	46%, $p = .01$ ($r = 0.68$)	NA	43%, $p = .001$ ($r = 0.66$)	47%, $p = .001$ ($r = 0.69$)	NA
Midline estimate statistic of rhythm (cortisol: nmol/L, corticotrophin: pg/mL)	244 (205–283)	220 (170–269)	$p = .4$	85 (68–102)	97.9 (74.6–121)	$p = .3$
Amplitude (cortisol: nmol/L, corticotrophin: pg/mL)	114 (53–175)	113 (50–176)	$p = .9$	5.3 (4.4–7.1)	7.5 (5.7–12.0)	$p = .1$
Acrophase	−343 (−363 to −323)	−343 (−388 to −299)	$p = .9$	−62 (−87 to −36)	−24 (40 to −8)	$p = .02$
Equivalent time	11.06 h	11.06 h		05.54 h	08.24 h	

NA = not applicable.
Values are expressed as means (95% confidence intervals). Acrophase is presented as phase angle in degrees in which 360° = 24 hr and the phase is relative to the starting hour (10 am). Equivalent times are given for convenience.

Nevertheless, our finding of normal serum cortisol levels throughout a 24-hour period runs against several previous studies finding a reduction in serial serum cortisol levels but is consistent with several studies that found normal cortisol levels (21). Of note is that the only other study that took frequent cortisol blood samples over 24 hours also found no significant change in cortisol levels either (although the authors do report a numerically lower early-morning cortisol) (32). In an attempt to understand the implications of this variation in findings from recent studies, we have argued that there is unlikely to be a single, uniform change to the HPA axis in CFS (21). Instead, it is likely that there are several factors influencing the HPA axis in CFS such as inactivity, sleep disturbance, medication, depression, early life trauma, and ongoing stress, some of which may be consequences of illness (46). It has also been suggested that hippocampal atrophy (47), possibly in response to stress, and accelerated changes relating to aging (32) could also contribute to HPA axis change in CFS. Given the likely heterogeneous nature of CFS, it is also likely that the predominance of these different factors varies between the patient samples in different studies. This may explain, at least in part, some of the inconsistencies in results seen in the different studies. Nevertheless, a common theme to studies has been that of blunting of either the cortisol or ACTH release; the finding in the present study of consistently reduced ACTH release over a 24-hour period provides further evidence in support of this.

Other Pituitary Hormones

Our results do not suggest that there is generalized hypothalamo–pituitary dysfunction, because cortisol, PRL, and GH mean values were similar in both groups at almost every sampling point. Furthermore, we have failed to find support for the suggestion by Moorkens and colleagues that there is a deficient nocturnal GH release in CFS (22). This is in keeping with our own data on a separate group of patients with CFS finding no alteration in GH function using several basal and dynamic measures (44).

We have also found no alterations in PRL levels over 24 hours. This result differs from that of Racciatti and colleagues who found higher PRL levels in the early morning (4 AM) in patients with CFS compared with control subjects (17). The pathophysiological significance of any alterations in prolactin release that may be present remains unclear.

Circadian Rhythm

Detailed examination over a full circadian cycle suggests that subjects with CFS have no disturbances in the regulation of circadian rhythmicity. In particular, the cortisol rhythm, which is the strongest indicator of the circadian pattern, was strikingly similar in subjects with CFS and healthy control subjects on all parameters. However, we were able to detect a significant phase advance in the ACTH rhythm in patients compared with control subjects, with an earlier acrophase in the modeled cosinor curve. The hemicircadian ACTH rhythm modeled on the overnight sampling (10 PM to 10 AM) found a

TABLE 4. Hemicircadian Rhythm Parameters of Corticotrophin Rhythm Over 12 Hr (10 PM to 10 AM)

	Corticotrophin		
	Chronic Fatigue Syndrome	Control Subjects	*t* Test
Percent rhythm (goodness of fit, r^2)	31%, $p = .02$ ($r = 0.56$)	38%, $p = .01$ ($r = 0.62$)	NA
Midline estimate statistic of rhythm (pg/mL)	86 (69–102)	105 (79–130)	$p = .1$
Amplitude (pg/mL)	14 (10–19)	26 (20–28)	$p = .001$
Acrophase	−220 (−253 to −187)	−225 (−260 to −190)	$p = .8$
Equivalent time	07.20 h	07.00 h	

NA = not applicable.
Values are expressed as means (95% confidence intervals). Acrophase is presented as phase angle in degrees in which 360° = 24 hr and the phase is relative to the starting hour (10 pm). Equivalent times are given for convenience.

lower amplitude, consistent with the reduced ACTH output levels found on ANOVA. The significance of the phase advance in the ACTH rhythm is unclear (and it is not present on the hemicircadian analysis), but it is notable that the cortisol rhythm was not phase-shifted. Thus, on a group basis, there is a degree of desynchronization of the ACTH and cortisol rhythms in this group of patients with CFS. However, individually, we could not find correlations between the ACTH and cortisol phase in either control subjects or patients. Nevertheless, possible desynchronization in circadian rhythms is compatible with the previous study that found temperature and melatonin rhythms to be desynchronized (20) and may point to further evidence that this desynchronization is a factor in the maintenance of CFS symptoms. However, given the presence of altered patterns of sleep and activity in CFS, it is perhaps more likely that such desynchronization is at least partly secondary to these changes rather than a primary pathophysiological cause.

Limitations

The main limitation of the present study is the relatively restricted sample size of 15 patients with CFS and 10 control subjects. However, we attempted to obtain as homogeneous a sample as possible by using restricted entry criteria, testing only medication-free subjects and excluding those with comorbid DSM-IV psychiatric disorders. Furthermore, we obtained carefully collected data over 24 hours. We were not able to obtain concurrent results in these patients from other endocrine measures such as dynamic tests. Clearly, such methods would be needed to confirm or refute the hypotheses that impaired central drive could explain the blunted ACTH in our CFS population. Another limitation is that the study was carried out in an experimental setting rather than the subjects' natural surroundings. Thus, within our results may be the effect of a potentially stressful change of environment. We did not observe a large sleep disruption effect, but our assessment of this was crude (50% reduction in sleep) and smaller degrees of sleep disturbance may have been missed. There are also the possible stress-related effects of removing around 400 mL of blood. It is not known whether these potential stressors may differentially affect those with CFS and control subjects.

Like with many other studies of CFS, we cannot be sure that this sample recruited from a specialist setting generalizes to CFS seen in the community. It is notable that the SF-36 measures in the sample reveal a relatively low level of physical disability in comparison to many other samples and that the fatigue scores are somewhat lower than in other studies (although still considerably raised above normal).

Although we have hypothesized that physical fitness and deconditioning is one of the factors likely to be contributing to the HPA axis changes in CFS, we have not measured this directly in this study. Therefore, we cannot test explicitly the degree to which our observed differences in the HPA axis between patients and control subjects reflect physical deconditioning. Arguing against this, Crofford and colleagues specifically recruited control subjects who were sedentary for 2 months (32) and found similar results to us, suggesting that medium-term inactivity is an insufficient explanation for HPA axis changes in CFS.

CONCLUSION

In this group of patients with CFS, we found evidence of reduced ACTH levels and a blunted morning physiological rise in ACTH, together with an earlier acrophase in the circadian rhythm of ACTH. This provides further support for the suggestion that there is a subtle central dysfunction in the HPA axis in CFS. However, from this study, we cannot draw any conclusions about the clinical relevance of these findings, or whether these changes are a primary feature of the illness or occur secondarily to other consequences of long-term illness with CFS.

We thank The Linbury Trust who supported Dr. Cleare with a research fellowship and provided the funding for this study. Dr. Hudson received partial financial support from Shire Pharmaceuticals. We also thank Jenny Jones, Sam Sookdeo, John Miell, and Alan McGregor for providing hormone assays and clinical testing facilities and Dr. Adekunle Yesufu for help in sample collections. This research was undertaken with the assistance of the Chronic Fatigue Syndrome Unit, directed by Professor Simon Wessely, and we thank him for his support.

REFERENCES

1. Fukuda K, Straus S, Hickie I, Sharpe M, Dobbins J, 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. Akerstedt T. Psychological and psychophysiological effects of shift work. Scand J Work Environ Health 1990;16:67–73.
3. Leese G, Chattington P, Fraser W, Vora J, Edwards R, Williams G. Short-term night-shift working mimics the pituitary-adrenocortical dysfunction in chronic fatigue syndrome. J Clin Endocrinol Metab 1996;81: 1867–70.
4. Arendt J. Biological rhythms: the science of chronobiology. J R Coll Physicians Lond 1998;32:27–35.
5. Katz G, Durst R, Zislin Y, Barel Y, Knobler H. Psychiatric aspects of jet lag: review and hypothesis. Med Hypotheses 2001;56:20–3.
6. Waterhouse J, Edwards B, Nevill A, Carvalho S, Atkinson G, Buckley P, Reilly T, Godfrey R, Ramsay R. Identifying some determinants of 'jet lag' and its symptoms: a study of athletes and other travellers. Br J Sports Med 2002;36:54–60.
7. Party B. Jet lag: minimizing its effects with critically timed bright light and melatonin. J Mol Microbiol Biotechnol 2002;4:463–6.
8. Avery D, Dahl K, Savage M, Brengelmann G, Larsen L, Kenny M, Eder D, Vitiello M, Prinz P. Circadian temperature and cortisol rhythms during a constant routine are phase-delayed in hypersomnic winter depression. Biol Psychiatry 1997;41:1109–23.
9. Lewy A, Bauer V, Cutler N, Sack R. Melatonin treatment of winter depression: a pilot study. Psychiatry Res 1998;77:57–61.
10. Koorengevel K, Beersma D, den Boer J, van den Hoofdakker R. A forced desynchrony study of circadian pacemaker characteristics in seasonal affective disorder. J Biol Rhythms 2002;17:463–75.
11. MacHale S, Cavanagh J, Bennie J, Carroll S, Goodwin G, Lawrie S. Diurnal variation of adrenocortical activity in chronic fatigue syndrome. Neuropsychobiology 1998;38:213–7.
12. Hamilos D, Nutter D, Gershtenson J, Redmond D, Clementi J, Schmaling K, Make B, Jones J. Core body temperature is normal in chronic fatigue syndrome. Biol Psychiatry 1998;43:293–302.
13. Papadopoulos E, Crofford LJ, Engleberg NC, Korszun A, Brucksch C, Eisner S, Demitrack MA. Impaired HPA axis activity in chronic fatigue syndrome and fibromyalgia. Biol Psychiatry 1997;41:29S.
14. Racciatti D, Sensi S, De Remigis P, Barberio A, Sciascio T, Pizzigallo E. Neuroendocrine aspects of chronic fatigue syndrome. Am J Med 1998; 104:1S–3S
15. Wood B, Wessely S, Papadopoulos A, Poon L, Checkley S. Salivary cortisol profiles in chronic fatigue syndrome. Neuropsychobiology 1998; 37:1–4.
16. Young A, Sharpe M, Clements A, Dowling B, Hawton K, Cowen P. Basal activity of the hypothalamic–pituitary–adrenal axis in patients with the chronic fatigue syndrome (neurasthenia). Biol Psychiatry 1998;43: 236–7.
17. Racciatti D, Guagnano M, Vecchiet J, De Remigis P, Pizzigallo E, Della Vecchia R, Di Sciascio T, Merlitti D, Sensi S. Chronic fatigue syndrome: circadian rhythm and hypothalamic–pituitary–adrenal (HPA) axis impairment. Int J Immunopathol Pharmacol 2001;14:11–5.
18. Korszun A, Sackett-Lundeen L, Papadopoulos E, Brucksch C, Masterson L, Engelberg N, Haus E, Demitrack M, Crofford L. Melatonin levels in women with fibromyalgia and chronic fatigue syndrome. J Rheumatol 1999;26:2675–80.
19. Hamilos D, Nutter D, Gershtenson J, Ikle D, Hamilos S, Redmond D, Di Clementi J, Schmaling K, Jones J. Circadian rhythm of core body temperature in subjects with chronic fatigue syndrome. Clin Physiol 2001;21:184–95.
20. Williams G, Pirmohamed J, Minors D, Waterhouse J, Buchan I, Arendt J, Edwards R. Dissociation of body-temperature and melatonin secretion circadian rhythms in patients with chronic fatigue syndrome. Clin Physiol 1996;16:327–37.
21. Cleare A. The neuroendocrinology of chronic fatigue syndrome. Endocr Rev 2003;24:236–52.
22. Moorkens G, Berwaerts J, Wynants H, Abs R. Characterization of pituitary function with emphasis on GH secretion in the chronic fatigue syndrome. Clin Endocrinol 2000;53:99–106.
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. Wolfe F, Smythe H, Yunus M, Bennett R, Bombardier C, Goldenberg D, Tugwell P, Campbell S, Abeles M, Clark P, Fam A, Farber S, Feichtner J, Franklin C, Gatter R, Hamaty D, Lessard J, Lichtbroun A, Masi A, McGain G, Reynolds W, Romano T, Russell I, Sheon R. The American College of Rheumatology 1990 criteria for the classification of fibromyalgia: report of the multicenter criteria committee. Arthritis Rheum 1990;33:160–73.
25. Chalder T, Berelowitz G, Pawlikowska T, Watts L, Wessely S, Wright D, Wallace E. Development of a fatigue scale. J Psychosom Res 1993;37: 147–53.
26. Stewart A, Hays R, Ware J. The MOS Short-form General Health Survey: reliability and validity in a patient population. Med Care 1988;26: 724–32.
27. Marks I. Behavioural Psychotherapy: Maudsley Pocket Book of Clinical Management. Bristol: Wright, 1986.
28. Goldberg D. The Detection of Psychiatric Illness by Questionnaire. London: Oxford University Press, 1972.
29. Wittenborn J, Buhler R. Somatic discomforts among depressed women. Arch Gen Psychiatry 1979;36:465–71.
30. Abelson JL, Curtis GC. Hypothalamic–pituitary–adrenal axis activity in panic disorder. Arch Gen Psychiatry 1996;53:323–31.
31. Yehuda R, Teicher M, Trestman R, Levengood R, Siever L. Cortisol regulation in posttraumatic stress disorder and major depression: a chronobiological analysis. Biol Psychiatry 1996;40:79–88.
32. Crofford LJ, Young EA, Engleberg NC, Korszun A, Brucksch CB, McClure LA, Brown MB, Demitrack MA. Basal circadian and pulsatile ACTH and cortisol secretion in patients with fibromyalgia and/or chronic fatigue syndrome. Brain Behav Immun 2004;18:314–25.
33. Posener JA, DeBattista C, Williams GH, Kraemer HC, Kalehzan BM, Schatzberg AF. 24-hour monitoring of cortisol and corticotropin secretion in psychotic and nonpsychotic major depression. Arch Gen Psychiatry 2000;57:755–60.
34. 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.
35. Demitrack M, Crofford L. Evidence for and pathophysiologic implications of hypothalamic–pituitary–adrenal axis dysregulation in fibromyalgia and chronic fatigue syndrome. Ann N Y Acad Sci 1998;840:684–97.
36. Demitrack M, Dale J, Straus S, Laue L, Listwak S, Kruesi M, Chrousos G, Gold P. Evidence for impaired activation of the hypothalamic–pituitary–adrenal axis in patients with chronic fatigue syndrome. J Clin Endocrinol Metab 1991;73:1224–34.
37. Hudson M, Cleare A. The 1microg short Synacthen test in chronic fatigue syndrome. Clin Endocrinol 1999;51:625–30.
38. Gaab J, Huster D, Peisen R, Engert V, Heitz V, Schad T, Schurmeyer T, Ehlert U. Hypothalamic–pituitary–adrenal axis reactivity in chronic fatigue syndrome and health under psychological, physiological, and pharmacological stimulation. Psychosom Med 2002;64:951–62.
39. Scott L, Burnett F, Medbak S, Dinan T. Naloxone-mediated activation of the hypothalamic-pituitary-adrenal axis in chronic fatigue syndrome. Psychol Med 1998;28:285–93.
40. Altemus M, Dale J, Michelson D, Demitrack M, Gold P, Straus S. Abnormalities in response to vasopressin infusion in chronic fatigue syndrome. Psychoneuroendocrinology 2001;26:175–88.
41. Scott L, Medbak S, Dinan T. Desmopressin augments pituitary–adrenal responsivity to corticotropin-releasing hormone in subjects with chronic fatigue syndrome and in healthy volunteers. Biol Psychiatry 1999;45: 1447–54.
42. Bakheit A, Behan P, Watson W, Morton J. Abnormal arginine–vasopressin secretion and water metabolism in patients with postviral fatigue syndrome. Acta Neurol Scand 1993;87:234–8.
43. Scott L, Medbak S, Dinan T. Blunted adrenocorticotropin and cortisol responses to corticotropin-releasing hormone stimulation in chronic fatigue syndrome. Acta Psychiatr Scand 1998;97:450–7.
44. Cleare A, Miell J, Heap E, Sookdeo S, Young L, Malhi G, 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.
45. Kavelaars A, Kuis W, Knook L, Sinnema G, Heijnen C. Disturbed neuroendocrine-immune interactions in chronic fatigue syndrome. J Clin Endocrinol Metab 2000;85:692–6.
46. Cleare AJ. The HPA axis and the genesis of chronic fatigue syndrome. Trends Endocrinol Metab 2004;15:55–9.
47. Brooks J, Roberts N, Whitehouse G, Majeed T. Proton magnetic resonance spectroscopy and morphometry of the hippocampus in chronic fatigue syndrome. Br J Radiol 2000;73:1206–8.