Attenuated Morning Salivary Cortisol Concentrations in a Population-Based Study of Persons with Chronic Fatigue Syndrome and Well Controls

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Context: A substantial body of research on the pathophysiology of chronic fatigue syndrome (CFS) has focused on hypothalamic-pituitary-adrenal axis dysregulation. The cortisol awakening response has received particular attention as a marker of hypothalamic-pituitary-adrenal axis dysregulation.

Objective: The objective of the current study was to evaluate morning salivary cortisol profiles in persons with CFS and well controls identified from the general population.

Design and Setting: We conducted a case-control study at an outpatient research clinic.

Cases and Other Participants: We screened a sample of 19,381 residents of Georgia and identified those with CFS and a matched sample of well controls. Seventy-five medication-free CFS cases and 110 medication-free well controls provided complete sets of saliva samples.

Main Outcome Measures: We assessed free cortisol concentrations in saliva collected on a regular workday immediately upon awakening and 30 and 60 min after awakening.

Results: There was a significant interaction effect, indicating different profiles of cortisol concentrations over time between groups, with the CFS group showing an attenuated morning cortisol profile. Notably, we observed a sex difference in this effect. Women with CFS exhibited significantly attenuated morning cortisol profiles compared with well women. In contrast, cortisol profiles were similar in men with CFS and male controls.

Conclusions: CFS was associated with an attenuated morning cortisol response, but the effect was limited to women. Our results suggest that a sex difference in hypocortisolism may contribute to increased risk of CFS in women. **(*J Clin Endocrinol Metab* 93: 703–709, 2008)**

Chronic fatigue syndrome (CFS) is an important public health problem with unique diagnostic and management challenges. Population-based studies have estimated the prevalence of CFS in adults between 0.24 and 2.54% (1), with a clear preponderance in women. CFS is defined by 1) clinically unexplained, persistent, or relapsing fatigue of at least 6 months' duration; and 2) concurrent occurrence of at least four accompanying symptoms, such as significant impairment in memory/concentration or muscle pain (2). The pathophysiology of CFS remains inchoate, and as yet, there is no definitive treatment (3).

Better understanding of the pathophysiology would improve diagnostic precision and clinical management of CFS. Research increasingly indicates that CFS reflects hypothalamic-pituitary-adrenal (HPA) axis dysregulation. For example, glucocorticoid

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Abbreviations: BMI, Body mass index; CFS, chronic fatigue syndrome; CI, confidence interval; FDR, false discovery rate; HPA, hypothalamic-pituitary-adrenal; MFI, Multidimensional Fatigue Inventory; SDS, Self-Rating Depression Scale; SF-36, Short-Form Health Survey; SI, Symptom Inventory.

deficiency may be associated with fatigue, malaise, somnolence, myalgia, and arthralgia, core CFS symptoms (4). Cortisol secretion follows a circadian pattern with low levels during night, steadily increasing levels toward the morning hours, and a steep increase within the first 30 min after awakening (5). The cortisol awakening response (time between awakening and the peak concentration about 30 min later) (6, 7) reflects basal HPA axis activity and provides an indicator of HPA axis dysregulation (8). Elevated cortisol awakening response has been associated with chronic work overload (9), social stress (10), depression (11), and neuroticism (12). An attenuated response occurs in persisting pain (13) and burnout (14), in people reporting general health problems (15), associated with noise stress (16), in posttraumatic stress disorder patients (17), and in people with early loss experience (18).

In a previous study of a population-based sample of CFS subjects in Wichita, Kansas, we observed a flattened diurnal salivary cortisol curve; those with CFS had lower morning concentrations and higher evening concentrations than matched nonfatigued controls (19). Few other studies have evaluated morning salivary cortisol profiles, and the results have been contradictory (20, 21). Two studies have reported on 24-h serum cortisol in patients with CFS or fibromyalgia and healthy controls and found no substantial differences within the awakening time frame (22, 23).

Several reasons might account for these disparate findings. Importantly, these studies involved patients referred from primary or specialty clinics and may suffer from referral bias; around 85% of CFS cases in the population have not sought medical care, leaving a significant amount of CFS cases not examined in scientific studies (24, 25). Second, because there are no known diagnostic clinical signs or laboratory abnormalities associated with CFS, cases are identified by self-reported symptoms and ruling out certain medical and psychiatric conditions. Although in most published studies CFS was diagnosed according to criteria of the 1994 international research case definition (2), the definition was not applied uniformly, and it remained unclear how the different components of the definition were measured (26).

The objectives of the current study were 1) to evaluate morning salivary cortisol profiles (measured as both overall secretory activity in the period after awakening and dynamics of the response); and 2) to evaluate associations between morning cortisol profiles and functional status.

Subjects and Methods

This study adhered to U.S. Department of Health and Human Services human experimentation guidelines and received Institutional Review Board approval from the Centers for Disease Control and Prevention (CDC) and collaborating institutions. All participants gave informed consent.

Study design

Study participants were identified during a survey of unwellness in metropolitan, urban, and rural populations of Georgia, conducted between September 2004 and July 2005 (1). In brief, the study used random digit dialing to screen 10,837 households followed by detailed telephone interviews of 5630 people identified by the household informant as well or unwell (overall 75% response rate). Based on responses to the detailed telephone interview, 469 persons who fit criteria for CFS (CFS-like), 505 chronically unwell, and 481 who were well (matched the CFS-like subjects by age ± 3 yr, sex, race/ethnicity, and geographic stratum) were invited for a detailed clinical assessment; 292 CFS-like, 268 chronically unwell, and 163 well subjects participated.

To identify psychiatric conditions exclusionary for CFS, the research version of the Structured Clinical Interview for DSM-IV (SCID) was administered (28). The SCID is a semistructured interview that is assesses the presence or absence of each of the disorders being considered for current episode (past month) and for lifetime occurrence. To screen for medical conditions considered exclusionary for CFS, participants completed past medical history questionnaires. A standardized physical examination was performed. Blood and urine specimens were obtained for laboratory screening tests to identify possible underlying or contributing medical conditions as stipulated by the case definition. Laboratory tests included a complete blood count with differential, C-reactive protein, alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, total bilirubin, calcium, carbon dioxide, chloride, creatinine, glucose, potassium, total protein, sodium, blood urea nitrogen, antinuclear antibodies, rheumatoid factor, TSH, free T_4, and urinalysis.

Clinical assessment and classification

Case definition

We classified those who did not have an exclusionary condition as CFS (or well) according to criteria of the 1994 CFS Case Definition (2) based on standardized reproducible criteria recommended by the International CFS Study Group (26) (see also Ref. 29). We used the Short-Form Health Survey (SF-36) (30) to measure functional impairment, the Multidimensional Fatigue Inventory (MFI) (31) to assess fatigue status, and the CDC Symptom Inventory (SI) (32) to evaluate occurrence, frequency, and severity of the eight CFS defining symptoms. As in previous CDC population-based CFS studies (23, 32), CFS was defined by 1) a score of at least 13 on the general fatigue scale or at least 10 on the reduced activity scale of the MFI; 2) a score of no more than 70 in the physical function or no more than 50 role physical, or no more than 75 social function, or no more than 67 role emotional scales of the SF-36; and 3) at least four CFS defining symptoms and a score at least 25 on the SI (n = 113). Those who met none of the criteria were considered to be well (n = 124).

Demographic and clinical data

Information on age, sex, and race were obtained by a standardized interview conducted by telephone. Height and weight were measured at the clinic. Body mass index (BMI) was computed based on the ratio of weight in kilograms to height in meters squared.

Psychometric instruments

Volunteers seen in the clinic completed the Self-Rating Depression Scale (SDS) (33), a 20-item questionnaire measuring core symptoms of depression on a 4-point Likert scale. The SDS measures severity of current core depressive symptoms.

Medications

Subjects brought all their current prescription and over-the-counter medications and supplements to the clinic, and medical staff recorded the data. Nineteen CFS cases and 22 well subjects used hormonal contraceptives or hormone replacement medications ($P = 0.458$). All 20 CFS and seven well subjects taking α-adrenergic stimulants, antidepressants, benzodiazepines, β-blockers, estrogen receptor modulators, corticosteroids, or testosterone receptor blockers were excluded from the current analysis.

Salivary cortisol

Subjects were instructed to collect saliva on a regular workday within 3 d of their clinic visit by using Salivettes (Sarstedt, Newton, NC). They collected saliva immediately upon awakening (while still lying in bed) and 30 and 60 min later, recorded the collection times, and stored the salivettes in their refrigerators. They were instructed not to brush their teeth, smoke, drink, or eat during the saliva collection period. Analyses used only data from those who complied fully with the collection schedule. Subjects providing samples deviating more than 10 min from the collection times were excluded from the analyses. Salivettes were stored in the participants' refrigerators and brought to their clinic visit. All samples were stored at −20 C until assayed for free cortisol concentrations by a commercial RIA (Esoterix, Calabasas, CA). All cortisol analyses were performed by Esoterix. Interassay and intraassay coefficients of variance were 12.6 and 7.4%, respectively. Assay sensitivity was 0.05 μg/dl.

Statistical analyses

Due to skewed distributions of salivary cortisol concentrations, this measure was logarithm-transformed. All analyses were conducted with the log-transformed values. For the sake of comparability with other studies, nontransformed values are shown when means are reported. Four indices for morning cortisol levels were computed. 1) The cortisol awakening response was computed by subtracting the awakening time point from the peak after 30 min (8). In addition, 2) area under the curve total and 3) area under the curve with respect to the first measure point (area under the curve increase) was calculated for salivary cortisol using the trapezoid formula (34). Finally, 4) a slope reflecting the morning pattern of salivary cortisol secretion was computed by estimating a simple linear regression model for each participant, where his or her cortisol values were regressed on time of collection. Data were tested for normal distribution and homogeneity of variance by means of the Kolmogorov-Smirnov and Levene's test before statistical procedures were applied. For group comparisons of psychometric data, the Wilcoxon test was used. Two-way ANOVA for repeated measures were computed to reveal possible effects of the time and group factors and their interaction. All reported ANOVA results were corrected by the Greenhouse-Geisser procedure where appropriate under the violation of sphericity assumption, reflected by the degrees of freedom with decimal values. All ANOVA analyses included adjustment for age, sex, race, BMI, and SDS scores. Pearson's product-moment correlations were used. For all analyses, significance level was $\alpha = 0.05$. In the case of multiple comparisons, significance levels (P values) were evaluated in light of the false discovery rate (FDR) using the method of Benjamini and Hochberg (35). Unless indicated, all results shown are means ± SEM.

Results

Demographic and illness variables

Seventy-five people with CFS and 110 well controls (who were not taking medications known to influence endocrine or immune function) provided complete sets of morning saliva samples and fulfilled compliance criteria as defined above. For these subjects, demographic features were similar among participants with CFS and those who were well (Table 1). CFS cases displayed

TABLE 1. Demographic and clinical features of cases with CFS and well controls

	CFS (n = 75)	Well (n = 110)	Statistics (df)	P
Mean age, yr (95% CI)	43.9 (41.7–46.1)	44.8 (42.9–46.7)	Z = −0.80	0.423
Age range, yr	21–59	18–59		
Mean BMI (95% CI)	28.1 (26.9–29.3)	26.3 (25.3–27.3)	Z = −2.35	0.019
Sex, n (%)				
Male	17 (22.7)	28 (25.5)		
Female	58 (77.3)	82 (74.5)	χ^2 (1) = 0.19	0.664
Race, n (%)				
White	60 (80.0)	84 (76.4)		
Black	11 (14.7)	25 (22.7)		
Others	4 (5.3)	1 (0.9)	χ^2 (2) = 4.8	0.091
SDS index (95% CI)	68.4 (42.9–93.9)	45.0 (27.6–62.4)	Z = −10.20	<0.001
Mean SF-36 scores (95% CI)				
General health	41.8 (37.8–45.9)	85.2 (83.1–87.4)	Z = −11.04	<0.001
Physical functioning	61.3 (56.2–66.5)	94.6 (93.4–95.7)	Z = −10.12	<0.001
Social functioning	55.5 (49.9–61.1)	99.1 (98.5–99.7)	Z = −11.75	<0.001
Mental health	58.9 (53.6–64.3)	89.0 (87.2–90.8)	Z = −9.20	<0.001
Role physical	12.7 (7.3–18.1)	95.2 (93.4–97.1)	Z = −12.01	<0.001
Role emotional	35.6 (26.3–44.8)	98.8 (97.6–100.0)	Z = −10.53	<0.001
Bodily pain	41.9 (37.6–46.2)	83.8 (81.0–86.5)	Z = −10.52	<0.001
Vitality	22.6 (19.2–26.0)	80.6 (78.1–83.1)	Z = −11.41	<0.001
Mean MFI scores (95% CI)				
General fatigue	17.0 (16.5–17.5)	6.9 (6.5–7.4)	Z = −11.54	<0.001
Physical fatigue	14.1 (13.4–14.9)	6.3 (5.9–6.6)	Z = −10.88	<0.001
Mental fatigue	13.5 (12.6–14.4)	6.5 (6.0–7.0)	Z = −9.83	<0.001
Reduced activity	11.5 (10.5–12.4)	5.8 (5.5–6.1)	Z = −9.37	<0.001
Reduced motivation	11.8 (11.1–12.5)	5.9 (5.5–6.3)	Z = −10.16	<0.001
Mean SI scores (95% CI)				
No. of symptoms	9.4 (8.8–10.0)	1.7 (1.4–2.0)	Z = −11.48	<0.001
Intensity score	37.4 (11.4–63.3)	3.0 (2.4–3.5)	Z = −11.53	<0.001
Frequency score	38.9 (12.9–64.8)	3.4 (2.8–4.1)	Z = −11.54	<0.001
Total score	85.1 (59.5–110.7)	6.7 (5.2–8.3)	Z = −11.53	<0.001
Case definition score	56.5 (30.9–82.1)	3.0 (2.2–3.8)	Z = −11.68	<0.001
Other symptoms score	27.2 (23.3–31.0)	3.7 (2.7–4.8)	Z = −10.57	<0.001

higher depressivity scores (SDS) than well controls. As expected, there were marked differences in clinical features between participants with CFS and well controls, as measured by the MFI, SF-36, and SI. The average duration of illness for persons with CFS in this study was 7.54 yr [95% confidence interval (CI) = 5.27–9.81].

Cortisol results

Salivary cortisol concentrations averaged across all three time points did not differ significantly between the two groups [$F_{(1,177)} = 0.19$; $P = 0.666$]. However, there was a significant interaction effect [$F_{(1.65,292.62)} = 3.46$; $P = 0.041$], indicating different profiles of cortisol concentrations over time between groups, with the CFS group showing an attenuated morning cortisol profile (Fig. 1). Interestingly, we found a significant interaction between sex and cortisol across both groups ($P = 0.009$).

We therefore performed separate analyses for women and men. For all analyses in women, use of hormonal contraceptives or medications for hormone replacement therapy was included as an additional covariate. This covariate did not contribute significantly in any of the following analyses (data not shown). Morning cortisol curves were significantly attenuated among the 57 women with CFS compared with the 82 well female controls [$F_{(1.58,209.04)} = 3.68$; $P = 0.037$; Fig. 2A]. However, there were no significant differences in the profiles of the 17 men with CFS and the 28 well male controls [$F_{(1.74,67.81)} = 2.45$; $P = 0.101$; Fig. 2B]. This finding indicates that the above reported cortisol differences between CFS and well subjects likely reflect differences between female CFS cases and well participants.

The cortisol awakening response was computed from the awakening and the 30-min sample. Using the previously described criterion of an increase of cortisol levels of at least 2.5 nmol/liter (equals 0.09 μg/dl) to qualify for being classified as a responder (36), 72.2% of CFS and 81.9% of well subjects were identified as responders ($P = 0.213$). The mean level of cortisol awakening response was significantly lower in the CFS than in the well group [CFS mean = 0.149 μg/dl (95% CI = 0.088–0.209 μg/dl), *vs.* well mean = 0.231 μg/dl (95% CI = 0.175–0.286 μg/dl); $F_{(1,177)} = 4.55$; $P = 0.034$]. Again, women with CFS showed a significantly lower cortisol awakening response than well women ($P = 0.011$), whereas there was no difference among the men ($P = 0.645$).

Total areas under the curve did not differ between participants with CFS and well controls [CFS mean = 0.564 μg/dl (95% CI = 0.506–0.621 μg/dl) *vs.* well mean = 0.595 μg/dl [95% CI = 0.552–0.638 μg/dl); $F_{(1,177)} = 0.358$; $P = 0.550$]. However, participants with CFS differed from those who were well with regard to the change of cortisol over the course of the first hour after awakening (area under the curve increase) [CFS mean = 0.090 μg/dl (95% CI = 0.047–0.134 μg/ml) *vs.* well mean = 0.153 μg/dl (95% CI = 0.113–0.194 μg/dl); $F_{(1,177)} = 4.91$; $P = 0.028$]. As with overall cortisol profiles and cortisol awakening response, men and women showed different results. Area under the curve increase was significantly lower among women with CFS compared with well women ($P = 0.020$). In contrast, men with CFS and well male controls had similar kinetics as to increase of area under the curve and total output.

The slopes of salivary cortisol response also differed significantly between participants with CFS and well controls [CFS mean $\beta = 0.0440$ (95% CI = −0.0011–0.0891); well mean $\beta = 0.0999$ (95% CI = 0.0640–0.1358); $F_{(1,177)} = 4.13$; $P = 0.044$]. This difference was not affected by sex (*i.e.* differences in change were similar when comparing women with CFS and well women and when comparing men with CFS and well men).

It needs to be noted that, except for sex, none of the covariates included in the analyses (*i.e.* age, race, BMI, and SDS scores) showed a main effect on the variables of interest. Also, an analysis of the FDR on the above results, controlling the FDR to less than 20%, supports the idea that most of the P values < 0.05 above are in fact significant, although one of them is likely a false-positive result.

We computed correlations between cortisol awakening response, areas under the curve, cortisol slope, and CFS-related symptoms (as measured by SF-36, MFI, and CDC SI) for the two groups. None of the SF-36 or CDC SI scales were correlated with the cortisol indices in any of the two groups. However, the Physical Fatigue Score from the MFI was related to several cortisol indices. For the cortisol awakening response, a correlation close to significance was found ($r = -0.220$; $P = 0.058$) for the CFS but not for the well group. There were no significant correlations for the area under the curve total for either group. A negative association was found with the area under the curve in-

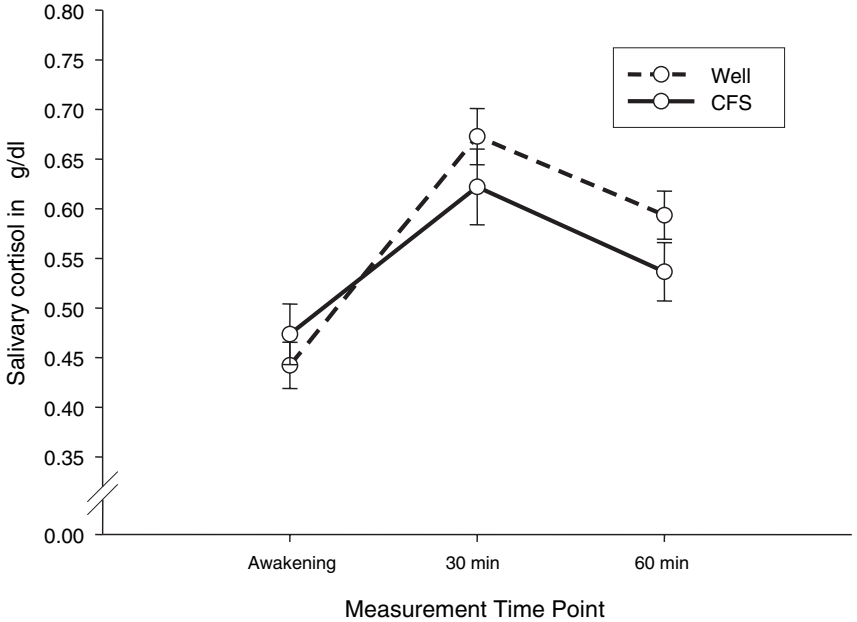

FIG. 1. Morning profile of salivary cortisol (awakening and 30 and 60 min) in cases with CFS (n = 75) and well controls (n = 110). There was a significant interaction effect ($P = 0.041$), indicating different profiles of cortisol concentrations over time between groups.

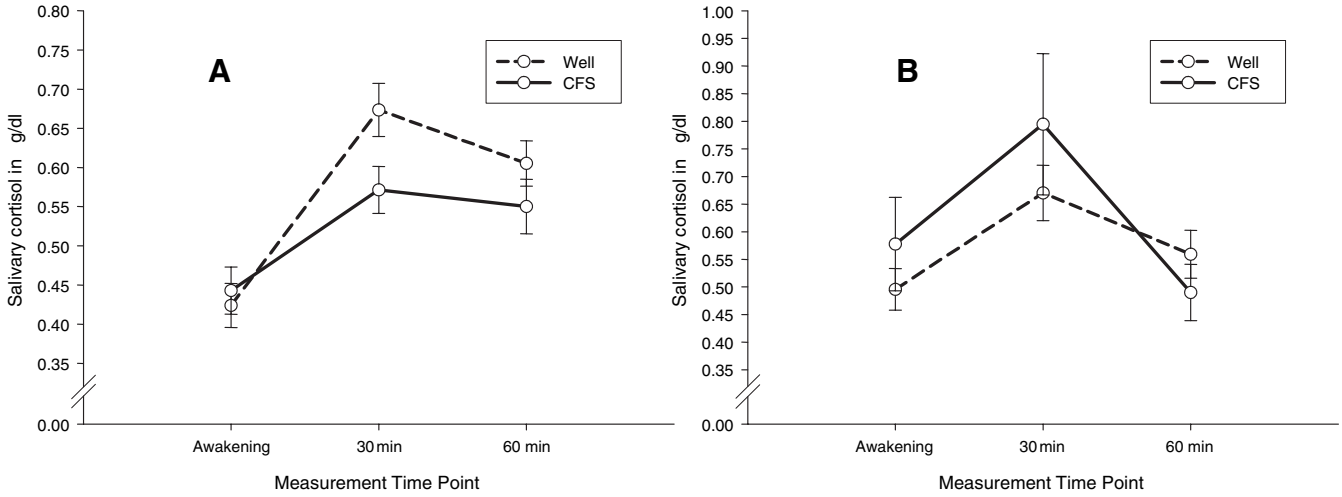

FIG. 2. Morning profile of salivary cortisol (awakening and 30 and 60 min) in female cases with CFS (n = 57) and well controls (n = 82) (A) and male cases with CFS (n = 17) and well controls (n = 28) (B). Female cases with CFS differed significantly from well controls ($P = 0.037$), whereas male cases with CFS did not differ from well controls ($P = 0.101$).

crease ($r = -0.255$; $P = 0.028$) in the CFS group but not in the well group, and a positive correlation was shown with the cortisol slope ($r = -0.259$; $P = 0.025$) in the CFS group but not in the well group. These results suggest that among the CFS group, lower cortisol awakening response was associated with worse physical fatigue.

Discussion

Our main finding of attenuated morning cortisol profiles in CFS corroborates a previous study reporting lower salivary cortisol in the morning in a sample of CFS (21). However, although these authors found a reduction of overall cortisol output within the first hour after awakening, we found differing dynamics of specific morning cortisol indices but not regarding total cortisol output. In contrast, the only other study measuring morning salivary cortisol profile in CFS we are aware of did not find any differences compared with a healthy control group (20). A recent study in severely fatigued girls found no differences in morning salivary cortisol (37). Studies of 24-h sampling of serum cortisol again report no significant differences between CFS and healthy (23) and fibromyalgia (22) subjects, although the latter study found numerically lower cortisol levels in the morning hours in the CFS group. The reasons for these discrepant findings might be multifaceted. All previous studies were limited by relatively small sample sizes and did not enroll patients from the general population and thus suffer from recruitment bias. In addition, many do not adequately describe diagnostic (or exclusionary) criteria for CFS.

Our current and previous (19) findings in population-based samples are similar to studies of convenience samples of patients with CFS suggesting a HPA axis hypoactivity in CFS compared with healthy control groups (reviewed in Ref. 38).

The morning cortisol surge is well studied, and the underlying processes are becoming more and more evident. Central nervous system oscillators, such as the suprachiasmatic nucleus, are involved in the morning cortisol surge (39). Furthermore, cognitive anticipatory factors related to the process of awakening itself seem to be relevant (40). However, it does not appear to be stimulated by physiological changes in blood glucose levels (41).

The functional consequence of an attenuated morning cortisol response is highly relevant to the study of CFS. A previous study suggested that the cortisol awakening response might reflect to some extent the adrenal cortex capacity (42). Reduced adrenal capacity and subsequent reduced availability of cortisol might be a permissive factor for increased activation of the immune system, which is known to be constrained by cortisol. Indeed, Addison's disease, a condition characterized by glucocorticoid insufficiency and increased immune activity, shares many symptoms with CFS (43). Furthermore, it has previously been shown that administration of the cytokine IL-6 during a hypocortisolemic state causes symptoms of CFS (44). IL-6 participates in the genesis of fatigue, and recent studies have reported elevated IL-6 levels in CFS patients (45, 46). Taken together, increased immune activation, in part explained by reduced availability of cortisol, might lead to fatigue and fatigue-related symptoms.

Thus, attenuated morning cortisol might be a potential pathophysiological mechanism in the development of CFS symptoms. Indeed, we found correlations between morning cortisol indices and fatigue in CFS. However, at this point, no statements can be made about the causal relationship of attenuated morning cortisol and symptoms. A recent study has found that lower wake-up cortisol levels predict fatigue later the same day in healthy people (47). Future studies should therefore incorporate prospective designs to reveal temporal relationships between morning cortisol and functional status in CFS.

It might be argued that comorbid depression might influence our cortisol findings. However, the literature suggests the opposite of our findings, *i.e.* higher cortisol levels in depression (48). In our study, only 21.3% of the CFS cases and none of the well cases fulfilled diagnosis for major depressive disorder. Nonetheless, we accounted for current depressivity in our analyses by including SDS scores as covariates. Our results indicate

that current depressivity did not influence cortisol levels in the current sample.

We observed the attenuation of morning cortisol in women with CFS only, but not in men. This stands in stark contrast to previous studies in normal volunteers reporting higher awakening responses in women than in men, with women showing larger responses and a delayed decrease (36, 49). However, given the higher prevalence of CFS in women, sex differences in HPA axis function might in part mediate CFS outcome, thus translating potential risk (sex) into illness (CFS).

Several limitations of our study have to be noted. First, our analyses did not account for some factors potentially having an impact on HPA axis regulation, such as menstrual cycle phase, postmenopausal status, and smoking. Although a recent study has shown that the cortisol awakening response is not influenced by menstrual cycle phase (15), no study has so far been conducted on the impact of postmenopausal status on morning cortisol profiles. We therefore cannot rule out that part of our findings might be explained by postmenopausal status of some of the participants. Regarding smoking behavior, the findings have been mixed. Some studies did not find that smoking habits influenced morning cortisol concentrations (15, 36, 49), whereas one study has shown the cortisol awakening response was greater in smokers than in nonsmokers (50). In our study, smoking behavior was not assessed. We therefore cannot rule out that smoking might have influenced morning cortisol levels in our participants. Second, recent studies have revealed that saliva sampling conducted by the subjects at their home is prone to measurement error due to a lack of compliance to collect the sample at the specified time (27). Specimen collection compliance can be improved by using electronic monitoring devices, but we did not use these or similar devices in our study. Our approach to exclude noncompliant participants from the individual self-reports of collection times may serve only as a proxy for compliance, and future studies should certainly include more elaborate measures of compliance control. Third, results of biological parameters might be influenced by a variety of factors and activities when measured at home and not in the laboratory. Although a recent study showed that home assessment of salivary morning cortisol provides the same results as in the laboratory (40), it might be useful to conduct additional studies in a controlled laboratory environment to control for a number of real-world activities that might influence cortisol concentrations. Fourth, we used unequal sample sizes for men and women, which might have underestimated nondifferences in men due to limited sample size. However, our sample is drawn from a population-based study on the epidemiology of CFS, and the higher number of female participants reflects the five-times higher prevalence of CFS in women (1).

Conclusions

We were able to show subtle changes in normal HPA axis activity in people suffering from CFS. These changes could be related to CFS-related symptoms, as some of our findings indicate, but no statement on a causal relationship can be made at this point. Importantly, the observed changes were present only in women but not in men. These findings might in part explain the higher prevalence of CFS in women.

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