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The associations between basal salivary cortisol and illness symptomatology in chronic fatigue syndrome

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Abstract

Hypocortisolism has been reported in chronic fatigue syndrome (CFS), with the significance of this finding to disease etiology unclear. This study examined cortisol levels and their relationships with symptoms in a group of 108 individuals with CFS. CFS symptoms examined included fatigue, pain, sleep difficulties, neurocognitive functioning, and psychiatric status. Alterations in cortisol levels were examined by calculation of mean daily cortisol, while temporal variation in cortisol function was examined by means of a regression slope. Additionally, deviation from expected cortisol diurnal pattern was determined via clinical judgment. Results indicated that fatigue and pain were associated with salivary cortisol levels. In particular, variance from the expected pattern of cortisol was associated with increased levels of fatigue. The implications of these findings are discussed.

Keywords

salivary cortisol; chronic fatigue syndrome; cognitive functioning; pain; fatigue

Chronic fatigue syndrome (CFS) is an illness characterized by severe, disabling fatigue that is not alleviated with rest, and by flu-type symptoms including sore throats, muscle pain, joint pain, headaches, memory/concentration difficulties, unrefreshing sleep, and post-exertional malaise (Fukuda, et al. 1994). The causes of CFS are currently unknown, and, as of yet, no clear diagnostic marker for this illness has emerged. Physiological studies have suggested the presence of dysfunction in the immune, endocrine, and neurological systems,

and researchers have found numerous abnormalities in these systems. However, many of these physiological abnormalities have not been replicated across studies, and a confusing picture still exists regarding the pathophysiology of this illness. Most recently, it has been suggested that individuals with CFS constitute a heterogeneous population, and little progress in clearly delineating the physiology of this illness may have resulted because of the presence of distinct subgroups within this larger umbrella diagnosis (Jason, Corradi, Torres-Harding, Taylor, & King, 2005).

However, some abnormalities have been more consistently reported in the literature as affecting a subset of individuals with CFS. One finding frequently reported is a dysfunction of the hypothalamic-pituitary-adrenal axis, (HPA) with resulting hypocortisolism (Addington, 2000; Cleare, 2003). Cortisol, a glucocorticoid, is the major end product of the HPA axis and is involved in regulation of several bodily systems. Some researchers have noted that approximately 20–25% of individuals with CFS and related disorders such as fibromyalgia or post-traumatic stress disorder have exhibited hypocortisolism and downregulation of the HPA axis (Fries, Hesse, Hellhammer, & Hellhammer, 2005). Cleare (2003), in a review of the literature examining HPA dysfunction in CFS, found that many, but not all, studies of cortisol in CFS found lower levels of baseline cortisol and changes in sensitivity in the HPA axis in at least some patients with CFS. In addition, Roberts et al. (2004) found lowered cortisol responses on awakening in individuals with CFS.

While there may be a decrease in the overall secretion of cortisol in individuals with CFS, there may also be changes in the sensitivity or responsiveness of the HPA axis in association with fatigue (Cleare, 2003). Changes in the responsiveness of the HPA axis, a major arm of the stress response, could imply a heightened or decreased stress response. This is comparable with literature characterizing CFS as a stress related disorder in that many individuals with CFS reported increased symptomatology and/or illness flare-ups or relapses after periods of severe stress (Lutgendorf et al., 1995), and stress has been proposed by some researchers to cause dysfunction in both the endocrine and immune systems in CFS (Pothelaikoff, 1998). Hypocortisolism has been found to occur in several physiological and psychological disorders, including CFS, fibromyalgia, chronic pelvic pain, irritable bowel syndrome, and post-traumatic stress disorder (Fries, Hesse, Hellhammer, & Hellhammer, 2005). This has led some to propose a common endocrinological pathway that may underlie the development of ‘stress-related’ disorders and which would potentially help explain common symptoms of enhanced stress sensitivity, fatigue, and pain (Fries, Hesse, Hellhammer, & Hellhammer, 2005).

However, the cause of hypocortisolism in some patients with CFS remains unclear. Some evidence suggests that it may be caused by central nervous system signaling of the adrenal glands, such as limited adrenocorticotrophic hormone (ACTH) output; decreased adrenal gland body size (Addington, 2000); compensatory shift towards hypocortisolism after a period of HPA hyperactivity following chronic stress (Fries, Hesse, Hellhammer, & Hellhammer 2005); and enhanced negative feedback of the HPA-axis and reduced response of ACTH accompanied by normal cortisol release following HPA stimulation, suggesting a diminished release of CRH at the central level (Ehlert, Gaab, & Heinrichs, 2001). This dysfunction does not seem to be related to peripheral changes in free cortisol, such as increased synthesis of cortisone from cortisol (Jerjes, Cleare, Wessely, Wood, & Taylor, 2005).

The clinical significance of hypocortisolism and/or an abnormal flattened diurnal pattern is unclear (Stone, et al. 2001). However, in research conducted with either non-clinical samples or in individuals with other chronic medical or psychiatric conditions, changes in HPA functioning have been demonstrated to be related to other physiological and

psychiatric symptoms, such as sleep patterns, cognitive changes, depression, pain. Hypocortisolism may be associated with cognitive functioning. For example, Sephton et al. (2003) examined the association between neuroendocrine functioning and neurocognitive functioning in a group of 50 women with fibromyalgia (FM), a related disorder. These researchers found that lower cortisol was associated with poorer performance on tests of visual immediate and delayed recall, and delayed verbal recall. They also found that depressive symptoms were associated with memory dysfunction. They concluded that hypocortisolism and depressive symptoms may impact cognitive dysfunction in fibromyalgia (Sephton et al. 2003). In another illness group, HPA functioning, as measured by baseline cortisol, was related to cognitive impairment in a group of 15 patients with multiple sclerosis (Heesen, et al. 2006).

In addition, adrenal corticosteroids are known to be associated with sleep (Buckley & Schatzberg, 2005; Dimitrov, Lange, Fehm, & Born, 2004). For example, some researchers note that acute cortisol administration increases slow-wave sleep and inhibits REM sleep (Steiger, 2002; Breslau, 2006). Cortisol also occurs in a circadian rhythm, with waking levels highest upon first awakening, peaking after awakening, and decreasing throughout the course of the day. Sleep deprivation is associated with HPA axis activation, and sleep fragmentation or nocturnal awakenings have been shown to be associated with pulsatile cortisol release (Buckley & Schatzberg, 2005). Thus, it is possible that the sleep difficulties observed in CFS might be associated with daily variations of baseline cortisol levels.

Hypocortisolism may also be related to pain symptoms. In a study of 131 individuals with fibromyalgia, individuals 'at risk' for developing chronic pain, and healthy controls, cortisol levels were found to be lower in individuals with FM and at risk for developing FM when compared to the control group (McBeth, et al. 2005). However, in this study, salivary cortisol levels were not associated with other psychosocial factors, including self-report sleep difficulties, a measure of general distress, reported somatic symptoms, illness behavior, health anxiety, nor the presence of stressful recent life events. Similarly, McLean et al. (2005) found that pain and salivary cortisol levels were associated in 28 patients with fibromyalgia.

Finally, rates of comorbid depressive symptoms in CFS are 50% higher when compared to other chronic medical conditions (Parker, Wessely, & Cleare, 2001). In CFS, where hypocortisolism is frequently reported, the association between cortisol dysfunction and depression, which is frequently associated with hypercortisolism, is unclear. Indeed, many studies of hypocortisolism and CFS fail to control for depressive symptoms, and this may partially account for the negative findings that have been reported by some researchers when comparing cortisol levels between CFS patients and controls (Parker, Wessely, & Cleare, 2001). One study found that individuals with CFS with or without severe depression, as measured by the BDI, had lower levels of serum cortisol when compared to healthy controls (Gur, Cevik, Nas, Coplan, & Sarac, 2004). However, in that study, the association between depression and cortisol levels themselves was not examined, so it is unknown whether the presence or severity of depression might be associated with cortisol levels. Other variables such as age and gender have been reported to impact cortisol levels (Kirschbaum, et al., 1999) and these may be potential confounding variables.

This investigation explored the associations between salivary cortisol levels, symptoms of CFS, and psychiatric status. It was expected that individuals with CFS who demonstrated evidence of abnormal basal cortisol levels or hypocortisolism would exhibit increased fatigue, increased pain, and sleep difficulties when compared to individuals with CFS with higher cortisol levels. Further it was expected that basal cortisol levels would be associated

with psychiatric status, including having a depression and PTSD diagnosis, and with overall depression and anxiety levels.

Method

All data were collected in the context of a larger study investigating the effectiveness of cognitive behavioral therapies for individuals with CFS (Jason et al., 2007). The study was approved by the DePaul University Institutional Review Board, and carried out in several phases. First, individuals were recruited and screened for eligibility. Participants were recruited from a variety of sources, including physician referrals. Information about the treatment trial study was disseminated to medical colleagues through mailings, phone communication, and invited grand rounds, and through announcements in local media (i.e. newspapers, radio, cable access network). All participants were required to be at least 18 years of age, not pregnant, able to read and speak English, and considered to be physically capable of attending the scheduled sessions. Persons who use wheelchairs and those who were bedridden or housebound were excluded due to the practical difficulties of keeping therapy appointments. Referrals to local physicians who treat CFS and to support groups were offered to these individuals. After a consent form was filled out, prospective participants were initially screened using a structured questionnaire. Because CFS is a diagnosis of exclusion, prospective participants were screened for identifiable psychiatric and medical conditions that may explain CFS-like symptoms. These measures were completed at DePaul University and took approximately two hours. After the initial interview was completed, the patients' information was reviewed to ensure that they met all eligibility requirements.

All eligible participants attended a medical appointment with the study physician in order to confirm the diagnosis of chronic fatigue syndrome. After confirmation that the individual fully met the criteria for CFS according to the Fukuda et al. (1994) case definition, individuals completed a battery of baseline measures (described below). They were also assigned randomly to one of 4 treatment conditions, and completed measures at three follow-up testing periods. However, only the data obtained at baseline will be considered in the current investigation. One hundred fourteen individuals were found to be eligible and recruited into the present study, and completed the initial baseline measures. Of these 114 participants, 108 completed the salivary cortisol sampling protocol, and the current investigation will examine results from these participants. Of these 108 individuals, 18 (16.7%) were male and 90 (83.3%) were female. Regarding ethnicity, 5 of the 108 were African-American (4.6%), 94 were Caucasian (87.0%), 5 were Latino (4.6%), and 4 were Asian-American (3.7%).

Measures

The CFS Questionnaire—This screening scale was initially validated by Jason, et al. (1997). This scale is used to collect demographic, health status, medication usage, and symptom data, and it uses the definitional symptoms of CFS (Fukuda et al., 1994). Hawk, Jason, and Torres-Harding (2007) recently revised this CFS Questionnaire, and administered the questionnaire to three groups (those with CFS, Major Depressive Disorder, and healthy controls). The revised instrument, which was used in the present study, evidences good test-retest reliability and has good sensitivity and specificity (Hawk, Jason, & Torres-Harding, 2007). For each Fukuda et al. (1994) case definition symptom, participants rated the intensity of each symptom they endorsed on a scale of 0 to 100, where 0 = no problem and 100 = the worst problem possible.

The Structured Clinical Interview for DSM-IV (SCID) (First, Spitzer, Gibbon, & Williams, 1996) Axis I—This interview was used to establish psychiatric diagnoses. The professionally administered SCID allows for clinical judgment in the assignment of symptoms to psychiatric or medical categories, a crucial distinction in the assessment of symptoms that overlap between CFS and psychiatric disorders, such as fatigue, concentration difficulty, and sleep disturbance (Friedberg & Jason, 1998). A psychodiagnostic study (Taylor & Jason, 1998) validated the use of the SCID in a sample of CFS patients.

Medical Examination—The physician screening evaluation included a general and neurological physical examination. Laboratory tests in the battery were the minimum necessary to rule out other illnesses (Fukuda et al., 1994). Laboratory tests included a chemistry screen (which assesses liver, renal, and thyroid functioning), complete blood count with differential and platelet count, erythrocyte sedimentation rate, arthritic profile (which includes rheumatoid factor and antinuclear antibody), hepatitis B, Lyme Disease screen, HIV screen and urinalysis. A tuberculin skin test was also performed. If the TB skin test was positive, a follow-up chest x-ray was conducted to rule out tuberculosis. The project physician performed a detailed medical examination to detect evidence of diffuse adenopathy, hepatosplenomegaly, synovitis, neuropathy, myopathy, cardiac or pulmonary dysfunction. This medical examination was used to confirm the diagnosis of chronic fatigue syndrome, according to the Fukuda et al. (1994) criteria and to rule out exclusionary medical conditions.

Fatigue Scale (FS)—Krupp et al.'s (1989) Fatigue Severity Scale was used to measure fatigue. This scale includes 9 items rated on 7-point scales and is sensitive to different aspects and gradations of fatigue severity. Previous findings have demonstrated the utility of the Fatigue Severity Scale (Krupp et al., 1989) to discriminate between individuals with CFS, MS, and primary depression (Pepper et al., 1993). In addition, the Fatigue Severity Scale (Krupp et al., 1989) was normed on a sample of individuals with MS, SLE, and healthy controls. A study by Taylor, Jason and Torres (2000) found that, within a CFS-like group, the Fatigue Severity Scale was associated with severity ratings for the eight Fukuda et al. (1994) CFS symptoms.

Beck Depression Inventory (BDI-II)—Because depression is the most commonly diagnosed psychiatric disorder in CFS (Friedberg, 1996), a quantitative measure of depression severity was used. Depressive symptomatology was measured with the BDI-II (Beck, Steer, & Brown, 1996), a 21-item self-report instrument with well-established psychometric. The BDI-II is the only depression rating scale to be empirically tested and interpreted for both depressed and non-depressed patients with CFS (Johnson, DeLuca & Natelson, 1996). Also, the Beck Depression Inventory has shown sensitivity to treatment changes in two cognitive behavioral treatment studies of CFS (Deale et al., 1997).

Brief Pain Inventory—The Brief Pain Inventory (Cleeland & Ryan, 1994) was administered to measure the intensity of pain (pain severity) and the interference of pain in the patient's life (pain interference). Higher scores indicate more severe levels of persistent pain and higher levels of interference with functioning. This measure exhibits adequate levels of reliability to assess pain in non-cancer samples, with coefficient alphas of .70 and above, also evidences good concurrent validity with other generic pain measures, and has been shown to be sensitive to changes in pain status over time (Keller et al., 2004).

Beck Anxiety Inventory (BAI)—Anxiety symptoms were measured with the BAI, a 21-item self-report measure with established and replicated construct validity (Hewitt &

Norton, 1993; Steer, Clark, Beck & Ranieri, 1995). Factor analysis of the BAI and BDI yielded a first-order factor labeled anxiety that had salient loadings for all 21 items on the BAI, but only one item on the BDI. Anxiety symptoms at intake were a predictor of treatment outcome in two cognitive behavioral treatment studies of CFS (Sharpe, 1996).

California Verbal Learning Test—Second Edition (Delis, Kramer, Kaplan, & Ober, 2000)—This test provides a measure of a participant's ability to learn and remember verbal information. It requires that the participant learn and remember a 16-item word list after repeated presentations, both immediately and after a delay of approximately 20 minutes. This test includes free recall, cued recall, and word recognition of the 16 item list. Split-half reliabilities have been estimated at above .90 with age groups, and $r = .94$ for the standardization sample; and test-retest reliabilities have been reported to be .82 after a mean of 21 days (Delis, et al., 2000).

Digit Span—Digit Span is one of the subtests from the Wechsler Adult Intelligence Scales-Third Edition (WAIS-III). This test measures attention and short-term attention. This subtest includes two parts, digits forward, and digits backward. According to the Technical Manual for the WAIS-III (Wechsler, 1997) test-retest reliability over a 2–12 week interval for digit span for all age groups (16–89), ranged from .83 to .89. Reliability coefficients for the reference group on Digit Span ranged from a low of .84 (85–89 year olds), to a high of .93 (55–69 year olds), with an average of .91.

Rey-Osterreith Figure Drawing—This test involves the reproduction of an abstract visual stimulus. Administration involves copying a complex figure by hand. Next, the participant is asked to reproduce the figure from memory immediately after the first administration, and a third third time at 30 minutes after the first trial. This test measures visuo-spatial, visual organizational and integration abilities, motor abilities, and immediate and delayed memory for complex visual stimuli (Lezak, 1995).

NES-2 Continuous Performance Test—The continuous performance test is a subtest of the Neurobehavioral Evaluation System (Letz & Baker, 1988), a computer-administered test of neurocognitive functioning that has been used extensively in occupational health studies (Arcia & Otto, 1992). The continuous performance test consists of a test where letters flash briefly on the screen (for approximately 50 ms), at the rate of one per second. The participant is instructed to press the response button when the letter 'S' flashes on the screen, but not for any other letter. Cognitive abilities measured on this test include response speed and attention. The mean latency of response for all responses (in milliseconds) was examined. The continuous performance test mean latency response evidenced acceptable test-retest reliability (.66) (Arcia & Otto, 1992).

Trailmaking Test, Trails A and B—This is a brief, easily administered test of attention, sequencing, mental flexibility, visual search, and motor functioning (Spren & Strauss, 1998). This test consists of two parts (A and B). The participant is first instructed to connect consecutively numbered circles on a worksheet (part A), and then is asked to consecutively connect numbered and lettered circles in order, alternating between the numbers and the letters (part B). The participant is encouraged to work as quickly as they can. The scoring used on this measure is the seconds to completion for part A and for part B. Reliability coefficients for this test generally fall between .60 to .90, with most falling into the .80 range (Spren & Strauss, 1998).

Grooved Pegboard—The grooved pegboard is a manipulative dexterity test. It consists of a unit pegboard containing 25 holes, and the individual must place the pegs into randomly

positioned slots on the pegboard. It measures complex visual motor coordination, manual dexterity, and processing speed. The pegboard is completed twice, once with each hand. The score is time to completion in seconds. Good test-retest reliability has been found for this test (Lezak, 1995).

Sleep Difficulties—Sleep disturbances were examined by using the *Pittsburgh Sleep Quality Index*, which was developed to measure sleep quality in psychiatric research (Buysse, et al., 1989). This index measures sleep disruptions and sleep quality. There are nineteen questions (on 0–3 scale) which generate an overall score. Acceptable measures of internal homogeneity, consistency (test-retest reliability), and validity have been reported for this measure. A global PSQI score greater than 5 yielded a diagnostic sensitivity of 89.6% and specificity of 86.5% ($\kappa = 0.75$, $p < 0.001$) in distinguishing good and poor sleepers (Buysse, et al. 1989).

Cortisol variables

Salivary cortisol—Individuals completed 5 samples of salivary cortisol. Saliva was collected using Salivettes® brand collection tubes. Over the course of one day, samples were collected immediately upon first awakening and 45 minutes afterward. Awakening levels of cortisol were measured because it has been shown that they can be assessed in a wide variety of settings, are non-invasive, and are easy to employ (Wust et al., 2000). Furthermore, use of oral contraceptives, smoking, time of awakening, or sleep duration do not have a strong impact on free cortisol levels after awakening, suggesting that this might be a particularly robust measure of free salivary cortisol. In addition, salivary cortisol was assessed at 9 AM, 4 PM, and 9 PM. These fixed time sampling collection were implemented in order to provide opportunity to assess and compare morning, afternoon, and evening salivary cortisol levels. The two awakening times and the three fixed times yielded a total of five samples.

The saliva collection kit consisted of cotton swabs inside small plastic tubes, which are placed into a storage container. Patients were instructed how to properly collect saliva samples. They first were shown how to place the cotton swab in their mouth and gently chew for 30–45 seconds. Participants were then instructed to deposit the moistened swab into its plastic tube and the tube into the container. The container recorded the exact time that they placed the plastic tube into it. These samples were shipped to the E.M. Papper Clinical Immunology Laboratory at the University of Miami for laboratory analysis of salivary cortisol by a high sensitivity enzyme linked immunosorbent assay (Salimetrics, State College, PA). All participants provided 4–5 samples of saliva in order to test for salivary cortisol levels. All saliva samples were collected over the course of one day. Because the goal of the study was to elicit naturally occurring baseline levels of cortisol, individuals were instructed to provide samples on a ‘typical’ day, or on a day where they would not experience any unusual stress or change in their routine. Two participants unexpectedly experienced a significant or unexpected severe stress on the day that they were collecting saliva samples. They then completed their samples on another day, and this second sampling collection was used in the current study. Because salivary cortisol levels change over the course of a day, cortisol levels were operationalized in several ways, yielding three outcome variables.

Total mean cortisol—First, the average of cortisol levels throughout the day (mean) was constructed. This variable was used to indicate average cortisol production throughout the day.

Cortisol slope—Next, a raw slope variable was constructed (slope) fitting a regression line to the data points for each individual. The raw-slope of cortisol gave an indication of how cortisol levels changes over the course of a day. A higher value indicated a positive slope, with increasing levels of cortisol throughout the day (lowest in morning, increasing throughout the day). A negative value indicated a negative slope, which means that cortisol generally decreased throughout the day. Ideally, cortisol levels in healthy populations would be expected to show a negative slope, as cortisol levels are highest in the morning and lowest in the evening. A flattened slope suggests abnormal output, in that cortisol levels with a flattened slope (i.e. close to zero) would indicate relatively little change throughout the day.

Clinical classification—Finally, each individual's cortisol levels were examined by a physician who examined the pattern of cortisol and determined whether the 'pattern' was normal or abnormal (clinical). This physician had a large private practice of individuals with CFS, and was blinded to participant identity. Cortisol patterns were examined for variation from expected peak pattern, along with the general slope, in comparison with expected patterns of diurnal variation. Daily cortisol patterns or results exhibiting divergent peak times, decreases in cortisol level followed by sudden increases, or general attenuation of pattern were classified as 'abnormal'. Patterns which matched the expected diurnal pattern were classified as 'normal'. This means of classifying diurnal cortisol pattern has been employed in other research studies as well as in the clinical setting. This classification was done to determine whether clinical judgments of normality or abnormality for a set of daily cortisol results could meaningfully differentiate patients.

Results

Preliminary Analyses

First, the clinical classification method described above found that 51 participants (47.2%) were classified as having an abnormal daily baseline cortisol pattern using clinical judgment. Fifty-seven participants were classified as normal (52.8%), using clinical judgment.

Next, two individuals' results were excluded from the analyses because of elevated cortisol levels (i.e. higher than 5 u/mL), such that all of their daily cortisol levels were extreme outliers. Third, potentially confounding variables were examined to determine whether they might predict results on the cortisol outcome measures (total mean cortisol, cortisol slope, and clinical group membership). Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer (1999) noted that salivary cortisol results may be affected by use of oral contraceptives or hormone replacement therapy, use of antidepressant medication, smoking, and the demographic variables of age and gender. A series of separate t-tests was conducted to examine the association between the continuous salivary cortisol variables (slope, mean) and the independent variables of gender, use of any medication (yes/no), use of antidepressant medication (yes/no), use of hormonal therapies or thyroid medication (yes/no), and current smoking status. Pearson correlations were run using the continuous salivary cortisol variables (slope, mean) and age. A chi-square analyses was run using the clinical cortisol variable (clinical group) and the independent variables of gender, use of any medication (yes/no), use of antidepressant medication (yes/no), use of hormonal therapies or thyroid medication (yes/no), or current smoking status. Finally, a t-test was used to examine the association between age and the clinical cortisol variable (clinical group).

In these analyses, gender significantly predicted mean cortisol levels ($t=2.43$, $df=94$, $p=.01$). A summary of gender, demographic characteristics, and the outcome variables is provided in table 1. In addition, use of antidepressant medication was significantly related to the cortisol slope variable ($t=-3.21$, $df=94$, $p<.01$) and the clinical variable ($\chi^2=6.83$, $df=1$, $p<.01$).

Therefore, these two variables were included as covariates in all of the analyses listed below in order to control for these potentially confounding effects. Age, hormonal treatment/medication, and smoking were not associated with any of the cortisol outcome measures.

Main Analyses

Because of the number of analyses performed below, an *a priori* significance level was set at $p < .01$. The results of the following analyses are summarized in table 2.

Fatigue—First, the association between each of the cortisol measures and the Krupp Fatigue Scale and self-reported fatigue severity, as measured on a 0–100 scale, were examined. Separate linear regression analyses were conducted, with the Krupp Fatigue Scale score or self-reported fatigue as the dependent variable, and either cortisol mean or cortisol slope as the independent variables in separate linear regression analyses, while controlling for gender and antidepressant use. In addition, an ANOVA was conducted, with the clinical groups (abnormal/normal) as the independent variable, gender and antidepressant medication as covariates, and either the Krupp fatigue scale score or self-reported fatigue severity as the dependent variable. In these analyses, the clinical groups variable was significantly related to self-reported fatigue severity ($F=9.27$, $df = 1$, $p < .01$). Those participants rated to be ‘abnormal’ in their daily cortisol pattern self-reported higher mean levels of fatigue ($m=80.51$) when compared to individuals with normal cortisol levels ($m=70.46$). The $\eta^2 = .08$, indicating that group membership accounted for 8% of the variance in the fatigue severity measure.

Pain—Next, a series of analyses examined the associations between pain severity and pain interference scores, as measured by the Brief Pain Inventory, and the four cortisol variables. A set of linear regression analyses were conducted with either the BPI pain interference score or BPI pain severity score as the dependent variable, and cortisol slope or cortisol mean as the independent variable, while controlling for gender and antidepressant medication. ANOVA’s were conducted with BPI pain interference and BPI pain severity scale scores as the dependent variables, and clinical group as the independent variable, while controlling for gender and antidepressant medication. Statistically significant results were found for the association between pain severity scores and the slope variable ($\beta=-2.53$, $df = 3$, $p < .01$), and the r^2 was .12, indicating that the slope of the daily cortisol production accounted for 12% of the variance in the pain severity measure. In addition, statistically significant results were found for the clinical group membership ($F= 6.18$, $df = 1$, $p < .01$); and the $\eta^2 = .06$, indicating that group membership accounted for 6% of the variance in the pain severity measure. Individuals who had more positive scores, suggesting an atypical pattern of cortisol change throughout the day, were more likely to report severe pain. In addition, individuals who were classified as abnormal on clinical judgment reported significantly more severe overall pain. In addition, the association between pain interference and the slope variable approached but did not meet statistical significance ($p = .04$).

Next, self-reported sore throat severity, muscle pain, joint pain, headaches, and lymph node pain/tenderness were also examined to assess whether association existed between baseline salivary cortisol and self-reported variables of pain severity on a 0–100 scale. A series of linear regression analyses were conducted with either sore throat severity, joint pain severity, muscle severity, headache severity, or lymph node severity as the dependent variable, and cortisol slope or cortisol mean as the independent variable, while controlling for gender and antidepressant medication. ANOVA’s were conducted with the pain symptoms as the dependent variables, and clinical group as the independent variable, while controlling for gender and antidepressant medication. Results indicated that lymph node pain severity and cortisol slope score approached but did not meet significant ($p=.047$).

None of these self-reported pain symptoms were significantly associated with the cortisol measures.

Sleep—Next, the relationships between sleep difficulties and the cortisol measures were explored. The Pittsburgh Sleep Quality Inventory total score and self-reported unrefreshing sleep, rated on a scale from 0–100, were entered as dependent measures in separate linear regression analyses, with either the cortisol slope or cortisol mean levels as the independent variable, while controlling for gender and antidepressant use. ANOVA's were conducted with the sleep total score and self-reported unrefreshing sleep as the dependent variables, and clinical group as the independent variable, while controlling for gender and antidepressant medication. None of these analyses was statistically significant.

Neurocognitive functioning—Next, the relationship between the cortisol outcome variables and the neurocognitive functioning were explored. Total t-score on the California Verbal Learning Test, mean response time of the NES-2 continuous performance test; copy, trial 1, and trial 2 of the Rey Complex Figure drawing test; scaled score of the WAIS digit span; or Trails A and Trails B time to completion were entered as the dependent variable in a series of linear regression analyses; and the cortisol slope or cortisol mean variables were entered as the independent variable, while controlling for gender and antidepressant use. On these analyses, none were statistically significant, but the association between cortisol slope and the copy score on the Rey complex figure drawing approached significance ($p=.018$), as did the association between cortisol mean scores and delayed memory trail of the Rey complex figure drawing ($p = .04$).

Psychiatric status—Finally, the association between salivary cortisol levels and psychiatric status was explored. Variables examined included the presence of a current or past diagnosis of a depressive disorder and presence of a current or past diagnosis of post-traumatic stress disorder. In addition, depression and anxiety levels, as measured by the Beck Depression Inventory and by the Beck Anxiety Inventory were examined. Total depressive level score and total anxiety level score were entered as dependent variables in separate linear regression analyses, with either the cortisol mean or cortisol slope as the independent variable, while controlling for gender and antidepressant use. The presence of a depressive disorder and of PTSD were entered as independent variable in univariate ANOVA's, with cortisol mean or cortisol slope as the dependent variable, and gender and antidepressant use entered as covariates. Next, the associations between the clinical group status and psychiatric status were tested using either separate univariate ANOVA's, with BDI total or BAI total score as the dependent variable and gender and antidepressant use entered as covariates. In addition, chi-square analyses were conducted examining the associations between the clinical group status and the presence or absence of lifetime depressive disorder or lifetime PTSD. In all of these analyses, there were no statistically significant associations found between the psychiatric status variables and the salivary cortisol variables.

Discussion

This study compared the associations between a range of CFS symptoms and psychiatric functioning, and daily salivary cortisol levels, as measured by daily mean levels, cortisol slope, or change over the course of a day in cortisol levels, and a classification of the abnormality or normality of an individual's overall daily pattern of cortisol levels. Results of this study suggest that there were several potential associations between daily cortisol production, fatigue, and pain.

First, an association was found between self-reported fatigue severity scores and whether an individual had an abnormal or normal pattern of cortisol. HPA axis dysfunction has been suggested by some to underlie the symptoms of chronic fatigue syndrome, including fatigue (Addington, 2000), but to date, the CFS literature has been mixed regarding whether fatigue severity is associated with alterations in cortisol levels, with some researchers reporting negative findings (Gaab, et al., 2004; Rubin, Hotopf, Papadopoulos, & Cleare, 2005; ter Wolbeek, et al, 2007). However, differences in results may have been due to variability in ways that cortisol was measured. For instance, in the ter Wolbeek et al. (2007) study, no association was found between cortisol levels and fatigue severity in adolescent females with chronic fatigue. In the Gaab et al. (2004) study, fatigue severity was correlated with ACTH, and the association was not found to be statistically significant. In the Rubin et al. (2005) study, cortisol was not found to be a predictor of post-operative fatigue. In contrast, in this study, baseline cortisol was measured in three different ways with adults, and only the clinical classification group membership was associated with fatigue severity. Results from the current investigation suggests that looking at the overall daily patterns and whether cortisol levels fall within or outside of expected ranges might be most important, with only the individuals displaying the most atypical patterns experiencing more fatigue. Low cortisol levels or atypical or 'flattened' slopes have been found to be associated with fatigue in other illness groups, such as in vital exhaustion (Nicholson & van Diest, 2000) and in breast cancer survivors (Bower, et al. 2005), but not in fibromyalgia (McLean et al., 2005). More research should focus on the association between fatigue severity and the daily cortisol patterns in CFS to further explore this potential link.

Second, an association was found between pain severity, as measured by the Brief Pain Inventory, and both cortisol group membership (normal versus abnormal) and cortisol slope. The slope variable measured the change in cortisol over the course of a day; and individuals who experienced either positive (an increase in cortisol production over the course of the day) or more flattened patterns (i.e. little change in cortisol throughout the day) reported more severe pain than individuals who had negative slope of daily cortisol (highest levels in morning, lowest levels in evening). Both of these patterns (positive slope and flattened slopes) are atypical and may indicate of HPA axis dysfunction. These results are consistent with results that have been found in people with fibromyalgia (McLean et al. 2001, McBeth et al. 2005). Given the overlap of symptoms and the high comorbidity of symptoms between CFS and fibromyalgia, it is possible that a similar association between altered cortisol functioning and pain severity may exist in these overlapping, yet distinct, syndromes.

Contrary to what was hypothesized, cortisol mean, cortisol slope, or abnormal cortisol levels were not related to neurocognitive symptoms, psychiatric status, or sleep difficulties. Regarding neurocognitive levels, although some analyses approached significance, there were no statistically significant associations between cognitive functioning, as measured by a brief cognitive testing battery, and baseline salivary cortisol levels. These non-significant findings contrast with Sephton and colleagues (2003), who found associations between cortisol and memory functioning in a group of fibromyalgia patients. However, differences between the two studies may be due to the fact that the current investigation and the Sephton et al. (2003) study sampled from CFS and from fibromyalgia, respectively, two overlapping but distinct patient groups. Second, the current investigation did not measure salivary cortisol levels on the same day that the neurocognitive measures were administered, and it is possible that examining the concordance of cortisol levels and neurocognitive functioning at the same time might yield associations that occur on a daily or even hourly basis. However, it is also possible that salivary cortisol levels and general neurocognitive functioning are not directly associated in CFS.

In addition, cortisol levels were not associated with psychiatric status, as measured by either a history of or current diagnosis of depressive disorder or PTSD; or with current depression and anxiety. CFS frequently co-occurs with depression (Parker, et al. 2001). However, past or current depressive disorder was not associated with changes in baseline cortisol. One reason for a lack of association might be that the type of depression experienced in CFS may be different than individuals with a primary depressive disorder, such as melancholic depression. For instance, CFS patients have been found to be less likely to endorse the self-reproach items of the Beck Depression Inventory, and were more likely to endorse items indicating somatic complaints of depression (i.e. sleeplessness, appetite/weight change) that are confounded with the symptoms of CFS (Hawk, Jason, & Torres-Harding, 2006). Similarly, there was no difference in cortisol levels with individuals with a history of current or past post-traumatic stress disorder. However, given that both CFS and PTSD are associated with low levels of cortisol and hypofunctioning of the HPA axis, it is possible that further exacerbations or alterations in hypercortisolism could not be obtained if one had both CFS and history of PTSD when compared to having CFS only.

Finally, there were no statistically significant associations between sleep difficulties and cortisol levels in CFS. This finding was unexpected, given the severe disruptions in sleep patterns that some with CFS report. These individuals frequently report a great deal of variability in their wake-up times, and it might be expected that this could alter the diurnal pattern of cortisol secretion. However, when looking at all overall production (mean), change over the course of the day (slope), and the overall normality of the pattern of salivary cortisol, as judged by a study physician, no association was found. Buckley & Schatzberg (2005) suggest that nighttime awakenings may be associated with increased pulsative cortisol levels, but that night time awakenings might not have any impact on awakening cortisol levels. In addition, it is possible that measuring alterations in cortisol levels during the daytime may not capture nighttime levels, which may be more affected by sleep disruptions. In addition, this study only measured overall sleep quality, and cortisol production may be associated with specific aspects of sleep dysfunction, such as the duration of slow-wave sleep.

Potential limitations of this study included the fact that this study measured only baseline cortisol over the course of one day, and there may have been inter-individual variability that was not measured due to this sampling methodology. However, when measuring between-subject variability, Stone et al. (2001) found consistency of results among different sampling protocols of salivary cortisol measurement, and the sampling protocols in their set of studies varied from 1 day to 24 days. Thus, it is expected that the use of between-subject comparisons in the current investigations over the course of one day is appropriate. Second, Cleare (2003) noted that examining activation of the HPA axis after exposure to stress may yield important information about HPA functioning in CFS; however, HPA axis reactivity after stress could not be inferred from the current study, as only baseline cortisol measurement was employed. Another limitation was that the salivary cortisol measurements and the other measures were not sampled on the same day, and sometimes there was a span of days or weeks between cortisol sampling and completion of the other measures. Thus, direct concordance in time between salivary cortisol and other physiological variables, such as neurocognitive functioning, could not be measured, and no direction of causality could be established. Finally, due to the number of analyses conducted, the likelihood of obtaining a Type 1 error might have increased. In order to minimize the likelihood of Type 1 error, significance level was set at the $p=.01$ level. However, the analyses presented in this investigation should be considered exploratory, and replication of these results in other samples of people with CFS would lend support to the validity of these findings.

In summary, baseline levels of salivary cortisol appeared to be associated with illness parameters in CFS, including fatigue severity and pain. These findings are particularly important, given that fatigue and pain are two of the hallmark symptoms of CFS. These findings are consistent with the hypothesis that hypocortisolism may underlie some of the frequently reported aspects of CFS. Future research should focus on delineating the connections between available cortisol levels and potential effects on neurological and immunological systems. In addition, attention needs to be paid to those individuals who exhibit diurnal dysregulation of cortisol as these individuals may be the ones most likely to experience significant CFS symptoms of fatigue and pain.

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Table 1

Patient Characteristics and Outcome Variable Results by Gender: Means (Standard Deviation) and Percentages

	Males	Females
	n=18	n=90
Age	39.56 (11.54)	43.91 (11.41)
Ethnicity		
White	75.0%	87.5%
African-American	6.3%	5.0%
Latino	6.3%	5.0%
Asian-American	12.5%	2.5%
Mean cortisol results	.33 (.18)	.29 (.16)
Mean cortisol slope	-.00042 (.00049)	-.00044 (.00041)
Clinical classification variable		
Abnormal	62.5%	40.0%
Normal	37.5%	60.0%

Table 2

Association between cortisol measures and symptom variables, controlling for gender and antidepressant use

	<u>Mean cortisol</u>	<u>Raw slope of cortisol change</u>	<u>Clinical rating groups</u>	
			<u>Normal n=54</u>	<u>Abnormal n=47</u>
<u>Fatigue</u>				
Krupp Fatigue severity scale score	.04	.17	5.99	6.18
Fatigue symptom severity self-rating	−.14	−.06	70.46 <i>a *</i>	80.76 [*]
<u>Pain</u>				
Brief Pain Inventory Severity	.00	.23 ^{b*}	3.51 <i>a *</i>	4.50 [*]
Brief Pain Inventory Pain Interference	.00	−.16	3.89	4.59
<u>CFS definitional symptoms</u>				
Sore throat symptom severity	−.03	−.16	23.21	25.67
Tender/sore lymph severity	.01	−.21	27.19	26.18
Muscle pain severity	.07	−.13	60.64	57.81
Pain in multiple joints severity	−.08	−.06	45.23	49.22
Impaired memory & concentration severity	.04	−.18	62.70	65.16
Headache severity	.09	.03	49.88	59.10
Post-exertional malaise severity	−.10	−.02	72.90	79.10
<u>Sleep</u>				
Pittsburgh Sleep Quality Inventory global score	.10	−.08	8.52	7.98
Unrefreshing sleep severity	−.08	−.17	77.41	84.83
<u>Psychiatric status</u>				
Beck Depression Inventory Total	.00	−.07	19.56	18.73
Beck Anxiety Inventory Total	.12	−.01	13.30	12.10
Lifetime depressive disorder diagnosis present				
Lifetime PTSD diagnosis present				
<u>Cognitive functioning</u>				
CVLT total score	.05	−.14	56.46	55.47
CVLT short delay free recall score	−.03	−.07	.26	.27
CVLT long delay free recall score	.01	.01	.32	.21
NES score Mean RT	.08	−.02	459.48	450.15
ROCF (trial 1) copy scaled score	.01	.24	10.32	10.65
ROCF (trial 2) immed. recall scaled score	.22	−.09	10.16	10.77
ROCF (trial 3) delayed recall scaled score	.22	−.08	10.09	10.67
Grooved Pegboard dominant hand	−.05	.01	99.86	94.92
Grooved Pegboard non-dominant hand	−.04	−.08	111.21	105.04
WAIS digit span- scaled score	−.01	−.21	11.51	11.57
Trailmaking A- time (in seconds)	.02	−.13	30.99	28.69
Trailmaking B- time (in seconds)	.02	−.13	53.21	49.88

* significant at the $p < .01$ level

Italicized numerals indicate results approached but did not meet the a priori statistical significance level of $p < .01$

^a results were found to be statistically significant using ANOVA controlling for gender and antidepressant use

^b results were found to be statistically significant using a linear regression analysis, controlling for gender and antidepressant use

^c results were found to be statistically significant using an ANOVA analysis, while controlling for gender and antidepressant use