ARTHRITIS & RHEUMATISM
Vol. 52, No. 11, November 2005, pp 3660–3669
DOI 10.1002/art.21372

Momentary Relationship Between Cortisol Secretion and Symptoms in Patients With Fibromyalgia

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Objective. **To compare the momentary association between salivary cortisol levels and pain, fatigue, and stress symptoms in patients with fibromyalgia (FM), and to compare diurnal cycles of cortisol secretion in patients with FM and healthy control subjects in a naturalistic environment.**

Methods. **Twenty-eight patients with FM and 27 healthy control subjects completed assessments on salivary cortisol levels and pain, fatigue, and stress symptoms, 5 times a day for 2 consecutive days, while engaging in usual daily activities. Only those participants who adhered to the protocol (assessed via activity monitor) were included in the final analyses.**

Results. **Twenty FM patients and 16 healthy control subjects adhered to the protocol. There were no significant differences in cortisol levels or diurnal cortisol variation between FM patients and healthy controls. Among women with FM, a strong relationship between cortisol level and current pain symptoms was observed at the waking time point ($t = 3.35$, $P = 0.008$) and 1 hour after waking ($t = 2.97$, $P = 0.011$), but not at the later 3 time points. This association was not due to differences in age, number of symptoms of depression, or self-reported history of physical or sexual abuse. Cortisol levels alone explained 38% and 14% of the variation in pain at the waking and 1 hour time points, respectively. No relationship was observed between cortisol level and fatigue or stress symptoms at any of the 5 time points.**

Conclusion. **Among women with FM, pain symptoms early in the day are associated with variations in function of the hypothalamic–pituitary–adrenal axis.**

Fibromyalgia (FM) is a common clinical syndrome defined by the presence of chronic widespread pain and tenderness (1). Recent studies have identified altered central nervous system (CNS) pain processing in individuals with FM, suggesting a neurobiologic basis for the disorder (2,3). However, the precise pathophysiologic mechanisms responsible for FM remain poorly understood.

The hypothalamic–pituitary–adrenal (HPA) system is the primary endocrine stress axis in humans and has been implicated in the pathophysiologic development of FM. The function of the HPA axis in patients with FM has been extensively examined, but study findings have been inconsistent. The majority of studies have identified abnormalities consistent with chronic

Presented in part at the 68th Annual Scientific Meeting of the American College of Rheumatology, San Antonio, TX, October 2004.

The opinions and assertions expressed herein are those of the authors and are not to be construed as reflecting the views of the USUHS or the US Department of Defense.

Supported by the Department of Army (grant DAMD-17002-0018) and the NIH (grant M01-RR-13297 from the General Clinical Research Center Program of the National Center for Research Resources). Dr. McLean's work was supported by NIH grant K12-RR-017607-01.

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Dr. Williams has received consulting fees (less than $10,000 per year) from Cypress Biosciences and owns stock in Cypress Biosciences. Dr. Gracely has received consulting fees (less than $10,000 per year) from Cypress Biosciences and Pierre Fabre. Dr. Crofford has received consulting fees (less than $10,000 per year) from Pfizer, Wyeth, and Lilly. Dr. Clauw has received consulting fees (more than $10,000 per year) from Cypress Biosciences and owns stock in Cypress Biosciences.

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Submitted for publication November 30, 2004; accepted in revised form July 21, 2005.

hyperactivity of the HPA axis, including elevated cortisol levels (4–8) and a blunted response to acute stressors (5,8–11).

Most previous studies of the function of the HPA axis in FM have maximized internal validity by assessing patients in highly controlled, inpatient environments and by excluding FM patients with comorbid depression. A limitation of this approach is that the generalizability of the HPA axis findings from these studies to unselected FM populations (30–50% of whom have active depression [12–14]) is unclear. In addition, few studies have examined the relationship of HPA axis function to FM symptoms. Catley et al (6) performed the only previous study that examined cortisol levels and symptoms among FM patients and controls in a naturalistic setting. They found elevated salivary cortisol levels in FM patients that were not accounted for by differences in self-reported psychological stress, sleep quality, or demographic or psychosocial factors (6). No association was observed between cortisol level and patients' stress symptoms (6).

In their study, Catley et al (6) focused on the relationship of cortisol to psychosocial stress and did not examine the relationship between salivary cortisol levels and pain and fatigue symptoms in FM. Such a relationship would be indicative of the important immediate influence of cortisol on other, recently identified aspects of CNS function, such as emotional processing and memory. Among healthy controls, momentary cortisol levels have been associated with negative mood, anxiety, fear, and avoidant responses (15–19).

In this study we examined cortisol secretion in FM patients and healthy controls in a naturalistic setting, and included FM patients with severe symptoms of depression. In addition, we examined the momentary relationship between salivary cortisol level and concurrent pain, fatigue, and stress symptoms among women with FM. We hypothesized that there would be differences in cortisol secretion between patients and controls, and that salivary cortisol levels would be associated with current FM pain and fatigue symptoms. We also hypothesized that no momentary association would be identified between cortisol level and patients' stress symptoms, given the lack of relationship between cortisol level and stress symptoms in the study by Catley et al (6). In addition, because of increasing recognition of the important influence of self-reported physical or sexual abuse on the function of the HPA axis in adults (20,21), cortisol patterns were compared between women with FM and a history of abuse and those without a history of abuse, and abuse history was included as a covariate in regression model analyses.

PATIENTS AND METHODS

Participants. Participants comprised 2 groups: 28 patients with FM (of whom 22 were women; mean ± SD age 43 ± 9 years, 16 white [57%], 7 African American [25%], and 5 other race [18%]) and 27 healthy control subjects (of whom 12 were women; mean ± SD age 38 ± 9 years, 13 white [48%], 11 African American [41%], and 3 other race [11%]). There were significantly more women in the FM group.

Patients with FM and control participants were recruited via local print advertisements, and patients with FM were also recruited from local clinic samples. All participants received an initial specialized evaluation to determine if they met the American College of Rheumatology (ACR) 1990 criteria for the classification of FM (1). Patients who reported a history of FM but did not meet the ACR criteria at the time of the study were excluded. The presence of psychiatric disorders was assessed using the Composite International Diagnostic Interview (22). In addition, participants were asked if they had ever been the victim of physical or sexual abuse. No specific definition of physical or sexual abuse was provided. If a participant did report abuse, they were asked to report the age at which the physical or sexual abuse occurred or began.

To exclude other medical conditions, all participants underwent a detailed evaluation, comprising a medical history review, physical examination, and laboratory studies, which included determination of the complete blood cell count, levels of serum electrolytes, blood urea nitrogen, creatinine, and thyroid-stimulating hormone, the erythrocyte sedimentation rate, and C-reactive protein concentration. General exclusion criteria were 1) cigarette smoking, 2) substance abuse in the past 2 years, 3) medical conditions known to cause symptoms similar to those of FM, including obesity (body mass index >30 kg/m^2), autoimmune or inflammatory diseases, cardiopulmonary disorders, chronic asthma, uncontrolled endocrine or allergic disorders (e.g., hypothyroidism, diabetes, allergic rhinitis), or malignancy, or 4) schizophrenia or major depression with suicidal ideation. All participants were required to discontinue taking psychoactive medications at least 2 weeks prior to the study (4 weeks for longer-acting compounds such as fluoxetine). Menstruating women were scheduled to undergo study evaluations during days 3–7 of the follicular phase of their menstrual cycle.

Self-report instruments. Participants completed the following self-report instruments, each of which was chosen based on its psychometric properties and applicability to the FM population.

Center for Epidemiologic Studies Depression Scale (CES-D) (23). The CES-D is a 20-item measure that assesses multiple components of depression symptoms: depressed mood, feelings of guilt and worthlessness, feelings of helplessness and hopelessness, loss of appetite, sleep disturbance, and psychomotor retardation. The CES-D has demonstrated strong associations with other measures of depression symptoms (23) and has been validated in pain populations (24,25).

State-Trait Personality Inventory (STPI) (26). The STPI Form Y includes assessment of anger and anxiety symptoms.

This measure possesses strong psychometric properties for the assessment of these mood symptoms. The items have been well validated as parts of larger instruments such as the State-Trait Anxiety Inventory and the State-Trait Anger Inventory (27).

Short Form 36 (SF-36) health survey (28). The SF-36 includes 1 multi-item scale that assesses 8 health concepts: 1) limitations in physical activities because of health problems, 2) limitations in social activities because of physical or emotional problems, 3) limitations in usual role activities because of physical health problems, 4) bodily pain, 5) general mental health (psychological distress and well-being), 6) limitations in usual role activities because of emotional problems, 7) vitality (energy and fatigue), and 8) general health perceptions.

Procedure. Following the initial evaluation, participants completed assessments of ambulatory symptoms and salivary cortisol levels for 2 consecutive days at the following 5 time points: 1) upon awakening and prior to getting out of bed, 2) 1 hour after awakening, 3) 5 hours after awakening, 4) late afternoon between 3:00 and 4:00 PM, and 5) 30 minutes before going to bed. At each time point, participants were instructed to rate their pain, fatigue, and stress symptoms during the previous 30 minutes on a 10-point Likert scale, using an electronic storage device. No specific definitions of pain, fatigue, or stress were provided. Real-time assessments of ambulatory symptoms were performed because such assessments are not influenced by recall biases and are superior to retrospective symptom reports in many settings (29,30).

Symptom ratings at each time point were recorded using an electronic keypad placed on an ambulatory activity monitor (Actiwatch-Score; Mini Mitter, Bend, OR). The activity monitor is a wristwatch-size (37 × 29 × 9 mm), lightweight (17 grams) device with data entry and alarm-prompt capability that has been previously validated (31,32). Symptom ratings were made using the electronic keypad to improve compliance; activity patterns were used for validation of wake-up time assessments (33,34). To optimize participants' compliance with symptom recording and salivary cortisol determinations during daily activities, the monitors provided patients with 3 alerts (1 hour, 5 hours, and 9 hours after waking) that were preset based on each participant's self-reported usual wake-up time. The first and last entries were not accompanied by an alert, in order to minimize interference with participants' usual sleep–wake patterns. Care was taken for proper placement of the actigraph using a standardized mounting and positioning protocol (31).

Participants were also instructed to collect a salivary cortisol sample at each time point, after entering their symptom data. Salivary cortisol levels are reliable correlates of serum/plasma-free cortisol concentrations, and can be obtained with minimal interference with daily activities (35,36). Participants were instructed to refrain from eating and drinking for 30 minutes prior to obtaining a saliva specimen, and to collect their salivary samples before brushing their teeth (e.g., in the morning and evening). Participants chewed on a cotton swab for 45–60 seconds and then placed the swab in a special plastic tube (Salivette; Sarstadt, Newton, NC). Participants were asked to store the tube in their refrigerator prior to returning the samples to the research team on the day after protocol completion.

After the specimens were received from the study participants, the cotton swabs were centrifuged and saliva was divided into aliquots and deposited in a collecting vial, which was then topped, labeled, and frozen at −70°C until shipped for analysis. Salivary cortisol levels were determined by enzyme immunoassay (Salimetrics High Sensitivity Cortisol Kit; Salimetrics, State College, PA). All saliva samples were analyzed at the Michigan Diabetes Research and Training Center at the University of Michigan, and were run in a single batch to maximize reliability.

Quality control procedure for ambulatory data. Given the often-poor adherence of participants to research protocols in naturalistic settings (32), data quality was carefully assessed and data that appeared to be of poor quality were excluded. For each day of data, the time that the participant entered self-reported wake-up values into the electronic keypad was compared with actual wake-up time (estimated via activity monitor [31,32]). If the difference between these 2 times exceeded 30 minutes, data from that day were excluded from the data analyses. The average amount of sleep per night (in minutes) was also calculated using actigraphy data from the 2-day period.

Assessment of ambulatory sleep parameters. Actigraphy data were used to assess the duration of sleep, restlessness during sleep, and sleep efficiency of study participants, as described previously (37). Wake-up time and sleep time were based on patients' self reports and were validated using actigraphy data. Based on prior validation studies, patients were deemed awake when activity exceeded 50% of the average daytime activity level, and asleep when activity levels reached 50% below patients' average nocturnal level. Average nocturnal activity levels were assessed for each of the 2 nights prior to salivary cortisol assessment. The sleep fragmentation index was also used as a second, actigraph software–based indicator of restless sleep, and was calculated as follows: (% 1-minute intervals of movement during sleep + % 1-minute intervals of immobility) divided by total 1-minute immobility intervals (determined by actigraphy). Sleep latency was defined as the time between going to bed and actual sleep start, which was determined as the first 10-minute span of immobility (<40 counts per minute). Sleep efficiency, used as a measure of sleep quality, was defined as follows: (time in bed spent asleep divided by total time in bed) multiplied by 100.

Statistical analysis. The data were evaluated for the presence of outliers via box plots, and salivary cortisol variability at each time point was assessed using coefficients of variation. The Wilcoxon–Mann-Whitney test was used to compare continuous variables, and Fisher's exact test was used to compare categorical variables between the FM and control groups. Differences in cortisol patterns between FM and control participants were evaluated via repeated-measures analysis of variance. In addition, among women with FM, unadjusted morning (defined as AM hours) cortisol values and diurnal variation of cortisol between those reporting and those not reporting a history of physical or sexual abuse were compared using Spearman's rank correlation.

Among women with FM, the momentary association between cortisol level and pain, fatigue, and stress symptoms at each time point was evaluated using a linear regression model with repeated measures, with unstructured variance–covariance within subjects across days. This regression model was also used to assess the association between sleep quality measures and morning cortisol and symptom levels. In addi-

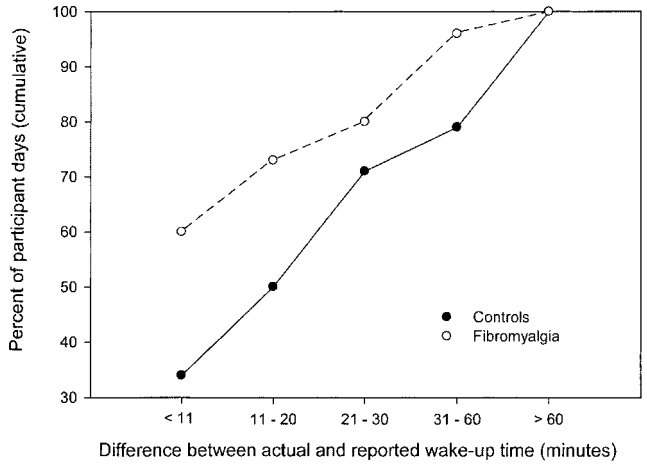

Figure 1. Difference between self-reported wake-up time and actual wake-up time estimated by actigraphy, as a percentage of cumulative participant-days, among patients with fibromyalgia and healthy controls.

tion, the association between wake-up time and cortisol level at wake-up was also evaluated. Age, amount of depression symptoms, and abuse history were included as covariates in each model. All regression models were evaluated for normality, and goodness-of-fit and model aptness were evaluated using residual analysis.

The amount of variance in pain symptoms at each time point accounted for by cortisol level alone was calculated using a coefficient of determination. In addition, for each woman with FM, the association between cortisol level and pain symptoms was calculated by Pearson's correlation coefficient, and a correlation between this value and patients' symptom characteristics was assessed using Spearman's rank correlation. Statistical analyses were performed using SPSS software (SPSS, Chicago, IL).

RESULTS

Data quality assessment. One hundred ten participant-days of data were collected. Ten morning cortisol values were missing, leaving 100 cortisol values at the wake-up time point. Of these 100 values, both self-reported and actigraphy-determined wake-up data were available for 83 participant-days. On 20 (24%) of these days, the difference between the self-reported wake-up time and actual wake-up time was more than 30 minutes. (See Figure 1 for differences between actual and reported wake-up times among FM and control participants.) Due to the questionable nature of this difference, the data from these days were excluded, resulting in the exclusion from the final data set of 8 (29%) of 28 patients with FM and 11 (41%) of 27 control participants. The remaining data, from 20 patients with FM and 16 controls, were used in subsequent analyses.

Of the 360 possible data entry points (36 participants × 2 days × 5 signals per day) for each parameter (cortisol level, pain score, fatigue score, and stress score), participants provided 329 salivary cortisol samples (91.4%), 353 pain scores (98.1%), 344 fatigue scores (95.6%), and 329 stress scores (91.4%). Because differences in the missing cortisol data between the FM patients and control participants could bias the analyses, we examined the overall rate of missing data and the pattern of missing data over the day by group. A generalized linear model analysis indicated that there were no group differences in the rate ($Z = -0.77$, $P = 0.44$) or pattern ($Z = 0.43$, $P = 0.67$) of missing cortisol data, thus allowing the inclusion of random missingness.

Preparation of cortisol data. There were no marked individual outliers, and therefore all values were included in the analyses. The cortisol data exhibited high variability. The coefficients of variation for the 5 salivary cortisol assessment times were 51.53%, 50.24%, 43.72%, 49.74%, and 66.96%.

Participants' symptoms and characteristics. Participants with FM had markedly higher levels of pain, fatigue, stress, and symptoms of depression than did control participants (Table 1). There was a greater

Table 1. Characteristics of the study participants*

Characteristic	Controls (n = 16)	Patients with FM (n = 20)
Age, mean ± SD years	39 ± 9	43 ± 9
BMI, mean ± SD kg/m^2	25 ± 3	26 ± 4
Male, no. (%)	12 (75)	4 (20)†
Race, no. (%)		
White	8 (50)	10 (50)
African American	7 (43)	6 (30)
Hispanic	0	3 (15)
Other	1 (6)	1 (5)
Symptom score, mean ± SD		
Ambulatory pain	1.1 ± 0.2	4.8 ± 1.8‡
Ambulatory fatigue	1.8 ± 0.9	4.7 ± 2.0‡
Ambulatory stress	1.5 ± 0.9	3.2 ± 2.0†
CES-D	4.4 ± 5.8	17.5 ± 8.6‡
Sleep characteristics, mean ± SEM§		
Duration, hours	7.0 ± 0.5	6.8 ± 0.4
Sleep latency, minutes	11.7 ± 6.4	16.0 ± 5.3
Average nocturnal activity level	106.8 ± 18.9	118.8 ± 15.8
Sleep fragmentation index	10.0 ± 3.1	15.5 ± 2.6
Sleep efficiency level	84.9 ± 2.6	85.3 ± 2.2
Actigraphy wake-up time, mean ± SD time, AM§	07:15 ± 1:31	06:17 ± 1:14

* FM = fibromyalgia; BMI = body mass index; CES-D = Center for Epidemiologic Studies Depression Scale.
† $P < 0.01$ versus controls.
‡ $P < 0.0001$ versus controls.
§ Adjusted for age and sex.

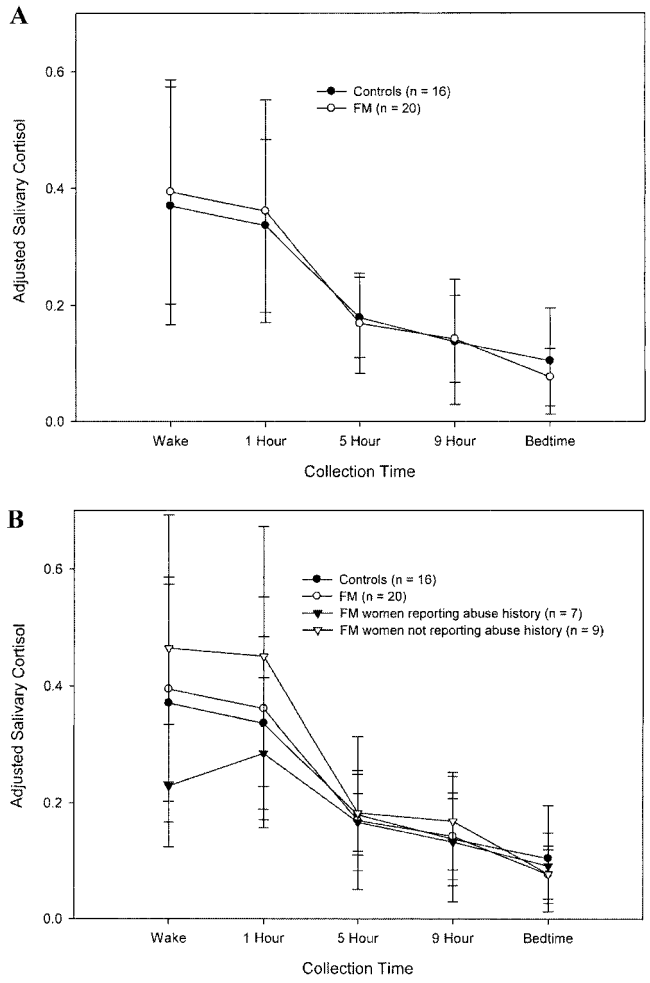

Figure 2. Salivary cortisol values (in μg/dl), adjusted for age and sex, across the 4 time points among patients with fibromyalgia (FM) and healthy controls **(A)**, and among women with FM reporting and those not reporting a history of abuse **(B)**. Bars show the mean ± SD.

number of women in the FM group. Consistent with prior observations (38), chronic fatigue syndrome, as defined by the Centers for Disease Control and Prevention criteria (39), often coincided with FM in the patients (16 of 20 patients, or 80%). Ten of the 20 participants with FM had significantly increased ratings of depression symptoms (CES-D score ≥16), compared with only 1 of the 16 control participants. Participants with FM had a longer sleep latency, more restless sleep (higher average nocturnal activity level and sleep fragmentation index), and an earlier wake-up time than did controls.

Adjusted group differences in salivary cortisol levels. Plots of adjusted salivary cortisol values by group at each time point, adjusted for age and sex, are shown in Figure 2A. In repeated-measures analysis, time-of-day and group effects were not significantly different between patients with FM and control participants.

Association between self-reported history of physical or sexual abuse and salivary cortisol secretion among women with FM. Of the 16 women in the FM group, 7 (44%) reported a history of physical or sexual abuse. Four reported a history of abuse during the preteen years (ages 6–13 years), 2 reported a history of abuse as young adults, and 1 participant did not provide information regarding the time period of abuse. One of the women with a history of abuse met the diagnostic criteria for posttraumatic stress disorder. None of the men with FM and none of the control participants reported a history of abuse. There was no significant difference in the mean number of depression symptoms among the women with FM when comparing those with and those without a self-reported history of abuse (mean ± SD 20.5 ± 7.7 versus 17.3 ± 10.2; $P = 0.33$).

Among the women with FM, those reporting a history of physical or sexual abuse had a lower unadjusted morning cortisol level (mean ± SD 0.23 ± 0.11 μg/dl versus 0.46 ± 0.23 μg/dl; $P = 0.028$) and decreased diurnal cortisol variation (0.13 ± 0.12 μg/dl versus 0.39 ± 0.21 μg/dl; $P = 0.011$) than those without a history of abuse. There was no difference in mean wake-up time between those reporting a history of abuse (mean ± SD 6:07 ± 1:23 AM) and those not reporting a history of abuse (6:41 ± 1:29 AM) ($t = 1.32$, $P = 0.20$). Although the group numbers were too small to allow meaningful repeated-measures analysis, mean cortisol values at individual time points suggest that diurnal

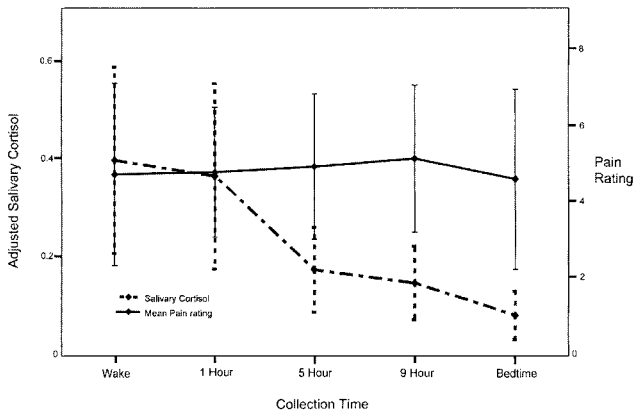

Figure 3. Ambulatory salivary cortisol values (in μg/dl) versus ambulatory pain ratings (on a 10-point Likert scale), adjusted for age, sex, and abuse history, among patients with FM. Bars show the mean ± SD.

Table 2. Linear regression models examining the momentary relationship of cortisol level to pain symptoms among women with fibromyalgia (n = 16)

Dependent measure, independent variable*	Beta	*t*	*P*
Waking cortisol			
Waking pain	0.0629	3.347	0.008
Abuse history	0.3364	3.464	0.005
CES-D score	0.0048	1.196	0.274
Age	0.0052	0.964	0.356
Day	−0.0317	−0.341	0.737
1 hour cortisol			
1 hour pain	0.0624	2.974	0.011
Abuse history	0.3362	3.154	0.011
CES-D score	0.0020	0.392	0.704
Age	0.0075	1.318	0.219
Day	−0.0047	−0.049	0.962
5 hour cortisol			
5 hour pain	0.0247	1.889	0.078
Abuse history	0.0370	0.546	0.597
CES-D score	−0.0014	−0.420	0.684
Age	0.0000	0.009	0.993
Day	−0.0214	−0.583	0.577
9 hour cortisol			
9 hour pain	0.0069	0.668	0.517
Abuse history	0.0715	1.294	0.226
CES-D score	0.0041	1.391	0.194
Age	0.0017	0.552	0.593
Day	−0.0228	−0.796	0.465
Bedtime cortisol			
Bedtime pain	−0.0022	−0.327	0.749
Abuse history	−0.0275	−0.764	0.465
CES-D score	−0.0023	−1.298	0.231
Age	0.0018	0.928	0.382
Day	−0.0060	−0.206	0.839

* CES-D = Center for Epidemiologic Studies Depression Scale.

cortisol pattern may differ in women with FM according to whether there is a history of abuse (Figure 2B).

Momentary salivary cortisol level and patients' symptoms among women with FM. Mean unadjusted cortisol levels and ratings of pain symptoms at the 5 time points are shown in Figure 3. Among women with FM, ratings of momentary pain symptoms were strongly associated with cortisol levels at the waking and 1 hour time points, but not at the 5 hour time point, 9 hour time point, or bedtime (Table 2). These results did not change in models that included all data on all women with FM in the original patient study group. Cortisol level alone explained 38% and 14% of the pain variance at the waking and 1 hour time points, respectively.

To determine if the daily HPA axis "starting point" value might influence pain symptoms later in the day, regression models were performed to examine the relationship between salivary cortisol levels at the waking time point and pain symptom ratings at each of the later 3 time points; none of the associations were found to be significant. Moreover, there was no association between cortisol level and ratings of stress or fatigue symptoms at any of the 5 time points (for example, at the waking time point, $t = 1.21$, $P = 0.27$ for the relationship between cortisol and stress; $t = 1.27$, $P = 0.23$ for the relationship between cortisol and fatigue). There was no association between wake-up time and wake-up cortisol level ($t = 0.64$, $P = 0.56$).

Association between sleep quality measures and waking cortisol level and waking symptoms among women with FM. The association between sleep quality (average nocturnal activity level, sleep fragmentation index, and sleep efficiency level) and waking cortisol level and waking symptoms was also assessed by linear regression analysis. There was no association between the 3 sleep-related measures and waking cortisol level (for example, $t = 0.26$, $P = 0.63$ for the relationship between sleep efficiency and waking cortisol levels; $t = 0.01$, $P = 0.92$ for the relationship between sleep fragmentation index and waking cortisol levels). Furthermore, there was no association between these 3 measures of sleep quality and waking pain or stress levels (for example, $t = 0.02$, $P = 0.99$ for the relationship between the sleep fragmentation index and pain levels). Ratings of fatigue at the waking time point were associated with the sleep fragmentation index, showing a trend toward significance ($t = 1.84$, $P = 0.09$); there was no association between waking fatigue level and the other 2 sleep quality measures.

Characteristics associated with a stronger correlation between pain symptoms and cortisol level. For each woman with FM, the correlation between pain symptoms and cortisol level was calculated across days and time points, and the association between this value and patients' symptom characteristics was assessed (Table 3). A stronger correlation between pain symptoms and cortisol level was associated with a perception of worsening health on the SF-36, and with increased anger symptoms on the STPI. In addition, a stronger correlation between pain symptoms and cortisol level showed an association with increased STPI anxiety symptoms, with a trend toward significance. The strength of the association between pain symptoms and cortisol level was not due to the degree of elevation in cortisol ($t = -0.97$, $P = 0.34$ for the association between mean morning cortisol level and strength of association between pain symptoms and cortisol level, adjusted for age, depression symptoms, and abuse history).

Table 3. Partial correlation between selected symptom characteristics and amount of correlation between salivary cortisol levels and pain symptoms among women with fibromyalgia*

Characteristic	r	*P*
Symptom score		
Mean ambulatory pain	−0.082	0.762
Mean ambulatory fatigue	−0.197	0.464
Mean ambulatory stress	−0.397	0.128
STPI anger	−0.539	0.038
STPI anxiety	−0.508	0.064
CES-D, SD	0.004	0.990
SF-36 subscale		
Change in health (worsening health)	−0.604	0.017
Bodily pain	−0.293	0.289
General health	0.171	0.541
Physical functioning	−0.104	0.713
Social functioning	−0.157	0.576
Role—emotional	−0.140	0.620
Role—physical	−0.229	0.411
Vitality		
Sleep duration by actigraphy	−0.186	0.508

* STPI = State-Trait Personality Inventory; CES-D = Center for Epidemiologic Studies Depression Scale; SF-36 = Short Form 36.

DISCUSSION

Among participants in our sample, there was no difference in baseline cortisol secretion between FM patients and healthy control subjects. The similar pattern of age- and sex-adjusted cortisol secretion among FM patients and controls is consistent with the heterogeneous results of previous studies of cortisol secretion in FM, which have often failed to find group differences in various HPA axis measures between FM patients and healthy subjects (40). Among the women with FM in our sample, those who reported a history of physical or sexual abuse had a lower waking cortisol level and decreased diurnal cortisol variation compared with those not reporting a history of abuse. Participants of studies of HPA axis function in FM are often recruited from tertiary care clinic populations in which the prevalence of self-reported abuse among FM patients can be 50% or higher (20,41,42). Because of this high prevalence of abuse, assessing participants for a history of abuse and for other forms of early life stress may be important when examining HPA axis function in FM.

Among women with FM, the cortisol level was associated with current pain symptoms upon waking and at 1 hour after waking, but not at the later time points. At these 2 early time points, the cortisol level explained 38% and 14% of the variance in pain symptoms. Patients' pain symptoms tended to generally increase or remain stable during the later portion of the day, whereas cortisol values declined. The factors that limit the strong association between cortisol level and pain symptoms to the earlier part of the day are unknown, but may be related to relatively low levels of cortisol later in the day and/or a greater influence of other factors, such as daily activity level as the day progresses.

The association between cortisol levels and pain symptoms in FM does not imply causality. Cortisol level may influence pain symptoms, pain symptoms may influence cortisol level, and/or cortisol and pain symptoms may be associated via a third variable. The mechanisms by which cortisol might influence pain in FM remain speculative. As noted above, in studies examining the immediate effects of cortisol levels in healthy controls, elevated cortisol levels have been associated with increased negative mood, anxiety, and fear (15–19). Such psychological factors have been found to influence brain activity in pain-sensitive brain regions (43), and thus cortisol may influence pain via short-term mood-induced alterations in CNS pain processing. Individuals with increased anger and anxiety symptoms may be more vulnerable to this influence, which would account for this finding in our sample.

Candidate neurotransmitters through which cortisol might influence pain symptoms include serotonin (5-HT) and norepinephrine (NE). The HPA axis and central 5-HT appear to be closely interrelated; glucocorticoids influence 5-HT_{1A} receptor density and 5-HT synthesis (44,45), and in patients with depression who are treated with serotonergic antidepressants, corticotrophin-releasing factor concentrations are reduced (46). The HPA axis and central NE sympathetic systems also have interconnected function (47,48), and central NE systems appear to be involved in descending inhibitory pain control (49–52). Whether cortisol is capable of influencing pain via any of these mechanisms on a momentary basis is unknown.

In addition to its effects on the brain, cortisol may also influence pain processing via its actions at the spinal cord level or in the periphery. The important nociceptive functions of glucocorticoid receptors in the spinal cord dorsal horn have been increasingly recognized. Dorsal horn glucocorticoid receptors respond to peripheral nociceptive stimulation (53,54), modulate morphine-induced antinociception (55–57), and contribute to neuronal plastic changes resulting from neuronal injury (58). In the periphery, cortisol variation may influence the balance of peripheral proinflammatory cytokines, which might contribute to pain symptoms via peripheral or central mechanisms (59).

This study has a number of limitations that should be considered when interpreting the results. First, 24% of participant-days of data were eliminated due to nonadherence to protocol. This rate of nonadherence is similar to that among participants who were unaware of compliance assessment in a recent study by Broderick et al (60). As in that study, our findings suggest that patients with FM are more compliant with research protocols than are healthy controls. The exclusion of nonadherent participants is likely to increase internal validity; however, these exclusions resulted in a relatively small control group and a markedly different sex distribution between groups. This limits the generalizability of our comparisons between FM patients and control participants. It is somewhat reassuring that the adjusted mean morning cortisol level of control participants in our sample (0.37 μg/dl, or 10.2 nmoles/liter) is very close to the mean morning cortisol level of the 22 control participants (73% women) in another naturalistic salivary cortisol study (slightly higher than 10 nmoles/liter, as shown in Figure 1 of Catley et al [6]).

A related issue is that the relatively small sample size, after exclusions, limited our statistical power to detect group differences in diurnal cortisol secretion. This limited statistical power increases the risk that meaningful differences in salivary cortisol levels between groups may have been missed. Of note, power estimates based on *t*-tests using observed mean cortisol values and standard deviations at each time point indicate that to identify even the largest effect size observed between FM patients and control participants with a power of 0.8 (at the fifth time point) would have required 89 participants per group. At other time points, several hundred participants per group would have been required to identify group differences. In contrast, because of the much larger effect sizes observed between women with FM reporting a history of abuse and those not reporting a history of abuse, 30 women with FM in each group would be sufficient to identify between-group differences at the first, second, and fifth time point. Future studies should consider these issues and plan for comparable rates of nonadherence when estimating sample size requirements.

Similarly, the small sample size could also have prevented us from identifying significant associations between fatigue and stress symptoms and momentary cortisol levels. Such associations, if present, are likely to be far weaker than the association between pain symptoms and momentary cortisol level, given that a robust association between pain symptoms and cortisol level was present at some time points, and that tests of association between fatigue and stress symptoms and the momentary cortisol measurement were not significant, even if the alpha level was increased from 0.05 to 0.20.

The wake-up time of FM patients was earlier than that of the controls, which would tend to increase the morning cortisol level (61) in the patients with FM compared with the controls. However, although this influence would have increased the difference in waking cortisol levels between the groups, no significant difference in waking cortisol levels between groups was found. Also, fixed-time measurements were chosen for the study, in order to provide a better ability to compare diurnal cortisol levels between groups. This design increases the possible influence of participant expectation; future studies should consider using a random sampling strategy to minimize this possible influence. Furthermore, because of its naturalistic design, this study did not control for the breadth of daily experiences that might influence baseline cortisol levels.

Another study limitation relates to the manner in which abuse history was assessed. Study participants were asked if they had ever been physically or sexually abused, but were not asked behaviorally specific questions (e.g., "Did anyone ever touch your genitals when you didn't want them to?"). Abuse in this study was thus not specifically defined, and the definition relied solely on participants' own label of their past experience. This kind of label-only abuse assessment is a relatively insensitive method of identifying abuse victims compared with asking behaviorally specific questions, because a significant number of victims do not label their experiences as abuse (62,63). Thus, our method of identifying abuse history may not have identified all participants with an abuse history in the study. Error in classification would tend to lead to an underestimation of the true differences between those with and those without self-reported abuse, and could increase the inaccuracy of regression model estimates. However, the prevalence of self-reported abuse among FM patients in our sample is similar to that reported in other FM tertiary care populations (20,41,42), and the validity of the brief self-report measure used in the study is supported by the nature of the HPA axis changes found among those reporting abuse, which are consistent with the known biologic affects of early life stress (20,21).

Simple 10-point Likert scales were used to assess patients' pain, fatigue, and stress symptoms. It is possible that more sophisticated measures of stress or fatigue would have yielded different findings from these simple assessment measures. Simple, brief symptom measures were used in an attempt to maintain the naturalistic

quality of the study by minimizing disruption from participants' daily activities at each assessment. Similarly, sleep quality was assessed via relatively simple actigraphy-based measures. Such measures may be an optimal method of assessing sleep quality in naturalistic studies, but they have not been validated and their relationship to gold standard sleep laboratory assessments is not known. Finally, because no general self-report measure of recent perceived stress was used, the influence of recent perceived stress on cortisol levels, pain, and the correlation between cortisol and pain could not be assessed.

The results of this study indicate that pain symptoms in women with FM are associated with cortisol concentrations during the early part of the day, but not at later time points. These data support the hypothesis that HPA axis function is associated with symptoms in FM and accounts for the substantial percentage of pain symptom variance during the early part of the day. However, this study does not determine if these changes in cortisol level play any role in causing pain or are, instead, caused by the pain. Further studies examining the relationship between cortisol level and pain symptoms in FM and in other chronic pain disorders are needed to confirm this finding and to determine the mechanisms involved in the association between momentary cortisol level and pain.

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