A Naturalistic Evaluation of Cortisol Secretion in Persons with Fibromyalgia and Rheumatoid Arthritis

Delwyn Catley, Alan T. Kaell, Clemens Kirschbaum, and Arthur A. Stone

Objective. *To compare cortisol levels, diurnal cycles of cortisol, and reactivity of cortisol to psychological stress in fibromyalgia (FM) and rheumatoid arthritis (RA) patients in their natural environment, and to examine the effect on results of accounting for differences among the groups in psychological stress and other lifestyle and psychosocial variables.*

Methods. *Participants were 21 FM patients, 18 RA patients, and 22 healthy controls. Participants engaged in normal daily activities were signaled with a preprogrammed wristwatch alarm to complete a diary (assessing psychosocial- and lifestyle-related variables) or provide a saliva sample (for cortisol assessment). Participants were signaled to provide 6 diary reports and 6 saliva samples on each of two days. Reports of sleep quality and sleep duration were also made upon awakening.*

Results. *FM and RA patients had higher average cortisol levels than controls; however, there were no differences between the groups in diurnal cycles of cortisol or reactivity to psychological stress. While the groups differed on stress measures, surprisingly, the patient groups reported less stress. Furthermore, statistically accounting for psychosocial- and lifestyle-related differences between the groups did not change the cortisol findings.*

Conclusion. *The results provide additional evidence of hypothalamic–pituitary–adrenal axis disturbance in FM and RA patients. While such elevations are consistent with other studies of chronically stressed groups, the elevations in cortisol in this study did not appear to be due to ongoing daily stress, and there was no evidence of disturbed cortisol reactivity to acute stressors.*

Key words. *Rheumatoid arthritis; Fibromyalgia; Cortisol; Stress.*

INTRODUCTION

Fibromyalgia (FM) and rheumatoid arthritis (RA) are both disorders associated with chronic pain. This study attempts to integrate two sets of disparate findings that have emerged about both of these diseases. First, there are findings suggesting psychological stress may be associated with the onset of FM and with disease activity and pain flares in RA (1–5). Specifically, FM patients report high levels of stressful life events (1) and daily hassles (2,3) and frequently attribute the onset of their illness to stress, emotions, or trauma (6). With RA patients, it has been shown that daily event stressors and emotional distress are associated with more intense daily pain (5,7), declines in soluble interleukin-2 receptor levels (8), and higher erythrocyte sedimentation rates (4).

Second, hypothalamic–pituitary–adrenal (HPA) axis disturbances have been observed in both FM

Delwyn Catley, PhD (current address: University of Kansas Medical Center, Kansas City), Department of Psychology, Alan T. Kaell, MD, Clinical Professor, Department of Medicine, and Arthur A. Stone, PhD, Professor, Department of Psychiatry, State University of New York at Stony Brook; and Clemens Kirschbaum, PhD, Assistant Professor, Center for Psychobiological and Psychosomatic Research, University of Trier, Trier, Germany.

Address correspondence to Arthur A. Stone, PhD, Department of Psychiatry, Putnam Hall, State University of New York at Stony Brook, Stony Brook, NY 11794-8790.

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and RA patients. There is evidence that FM patients have normal "peak" and elevated "trough" (evening) plasma cortisol levels suggesting a "flattened" diurnal cycle and higher overall cortisol levels, although 24-hour urinary free cortisol levels have been low (9,10). FM patients also have an inability to suppress plasma cortisol levels in dexamethasone suppression tests (10,11) and adrenal hyporeactivity to corticotropin-releasing hormone stimulation (12). While studies of cortisol levels in RA patients have not always produced consistent results, there is evidence that plasma cortisol levels may be elevated (13), diurnal cycles of cortisol flattened (14), and cortisol reactivity to HPA axis stimulation diminished (15–17).

The rationale for examining psychological stress and HPA function together is based on the strong body of work showing that psychological stress activates the HPA axis and its major secretory product, cortisol (e.g., 18–20; for review, see ref. 21). Acute stressors including public speaking (22), examinations (23), parachute jumps (18), and daily stressors (24) produce acute elevations in cortisol levels. Chronic stressors, such as living near a damaged nuclear power plant (25), being held in captivity (26), or having a chronic illness (27), have also been associated with higher cortisol levels. Furthermore, other chronic stressors such as job stress or being unemployed may lead to alterations in the diurnal cycles of cortisol (28,29). Studies have also found alterations in cortisol reactivity to acute stressors among those who are chronically stressed. For example, parents chronically stressed by their child's fatal illness were found to have reduced corticosteroid reactivity to their child's ongoing acute stressful medical events (30), and community members experiencing chronic stress showed evidence of delayed cortisol recovery following acute stress associated with a mental arithmetic task (31).

Prior studies of cortisol secretion in FM and RA patients have usually been conducted in controlled settings where natural variation of environmental factors, such as the level of psychological stress, is restricted. We have advocated the study of psychobiologic associations in participants' typical environments as well as in the laboratory in order to allow naturally occurring events (and other environmental variations) to affect those associations (32). Although this may decrease the homogeneity of the setting, ecological validity and generalizability are increased.

In this study we examine cortisol secretion in patients subject to chronic stress due to FM and RA and investigate the impact of acute stressors associated with usual daily life. In addition, we explore whether statistically accounting for differences between FM and RA patients and healthy control participants on lifestyle and psychosocial variables has any impact on the findings. We advance two hypotheses: that relative to non-ill participants, there will be 1) a difference in cortisol levels and diurnal variation, and 2) differences in the way patients' HPA axes respond to stressful circumstances. Based on prior investigations of plasma cortisol in FM and RA patients, we predict that there will be elevations in overall levels of cortisol in both RA and FM patients, a flattened diurnal cycle, and reduced cortisol responsiveness to acute psychological stressors.

PATIENTS AND METHODS

Participants. Patients were 21 FM and 18 RA volunteers recruited from a community rheumatology practice. Patients were eligible if they met American College of Rheumatology (ACR; formerly the American Rheumatism Association) criteria for FM (33) or ACR criteria for RA (34), had no endocrine disorders, were not pregnant, were not being treated with corticosteroids, and did not work night shifts. Approximately 45% of patients approached about the study completed the protocol. In addition, one RA participant was an outlier on a measure of psychiatric symptoms and was, therefore, excluded from analyses. A control group was formed from data previously collected in a study of stress among community residents that had an identical design (29). The community residents were recruited via newspaper advertisements for a study concerning cortisol and daily activities. The same exclusionary criteria were applied to the healthy volunteers.

Materials. Naturalistic evaluation of cortisol was achieved using ecological momentary assessment (EMA; 32), an intensive monitoring strategy that involves taking multiple assessments throughout the day. This technique minimizes reporting biases because phenomena are assessed as they occur. Assessment of cortisol was made by means of saliva, because salivary cortisol levels are considered valid and reliable correlates of serum/plasma free cortisol concentrations (35,36) and can be collected with minimal disruption of participants' daily activities (35,36). Saliva samples were collected by means of a small cotton roll contained in a special plastic tube (Salivette; Sarstedt, Rommelsdorf, Germany). Cotton rolls were placed in the mouth until saturated with saliva, and then resealed in the salivettes. Salivettes

were prelabeled with the date, and space was provided for participants to indicate the time the saliva sample was taken. A programmable watch (Seiko RC 2000) with an audible signal was used to prompt participants to provide saliva samples.

The watches were also used to prompt completion of short questionnaires that assessed stress, disease-related symptoms, and other psychological and situational variables. The questionnaires were contained in two small booklets (one for each day of the study) that participants kept with them. Each questionnaire asked about the current time and assessed mood using an adjective checklist that yields an index of positive affect, negative affect, and arousal (37). Each mood adjective was rated from 0 ("not at all") to 6 ("extremely"), and the affect index scores were based on means of the composite adjectives. Participants also indicated "at the moment of the beep" what activity they were engaged in, where they were, who they were with, how many people were present, and whether this was pleasant company.

Psychological stress was assessed by asking participants to indicate if anything significant had happened since the last beep (either "yes" or "no"). Significant stressful events were not predefined but were rather based on what events participants subjectively perceived to be significant. Pain flares were therefore not specifically included or excluded as potential stressful events. In the case of a significant event, participants were also asked to provide ratings of their stress level at the moment and at the time of the event; however, participants responded inconsistently to the direction to report their current stress levels only if a significant event had occurred, so these ratings were not included in analyses. The measure of significant stressful events has been successfully used in prior EMA research (29).

The final portion of the questionnaire assessed whether participants had taken any medication or consumed tobacco, caffeinated drinks, or alcohol since the last beep. A separate section of each booklet was used to record the time of awakening, the duration of the night's sleep (in hours and minutes), and sleep quality.

At a training session, FM and RA participants completed a battery of questionnaires including demographic information and assessments of stress (Perceived Stress Scale; 38), trait–anxiety (State–Trait Anxiety Inventory; 39), and psychological symptoms (Symptom Checklist-90-Revised [SCL-90-R]; 40). The Perceived Stress Scale is a widely used measure of stress shown to have adequate internal and test–retest reliability as well as significant correlations with other stress measures and health outcomes (38). The Spielberger State–Trait Anxiety Inventory is also a widely used instrument with adequate internal consistency and test–retest reliability. Numerous studies have documented its validity with respect to personality scales, objective stressors, and psychophysiologic indexes (39). The SCL-90-R is a well-established measure of psychiatric symptoms with adequate reliability and validity (40). Demographic information assessed included the number of years since participants were diagnosed, any history of endocrine disorders, any psychiatric and drug/alcohol abuse history, current medications, and health behaviors (i.e., smoking, exercise, dieting). Participants in the control group had previously completed a battery of questionnaires that included the same measures of stress and trait–anxiety (29).

The patients' rheumatologist (ATK) completed rating scales that included a checklist of the diagnostic criteria for FM and RA as well as a Likert scale for the physician's assessment of disease activity (0 = asymptomatic, 4 = very severe). Ratings were based on information from the patient's chart and were generally made on the day the patient was recruited for the study.

Procedure. Participants were recruited during their routine visits to a community rheumatology practice. Volunteers were scheduled as soon as possible (usually within two weeks) for a training session at our laboratory and two days of EMA. At the training session, participants completed the questionnaires and were instructed in the use of the watch, booklets, and salivettes. To encourage adherence to the watch schedule, participants were instructed to make every effort to respond to the watch signals immediately, but if they were unable to respond within 5 minutes, to wait for the next signal. Participants were instructed to complete the sleep information section of the booklets immediately upon awakening, and then to respond to prompts by the watch for the remainder of the day. The alarms were programmed to prompt participants for 6 booklet reports and 6 saliva collections between 8:00 AM and 9:00 PM. Each saliva collection prompt was programmed approximately 25 minutes after each booklet prompt because changes in cortisol in response to stimulation tend to peak after 20–30 minutes (35). Each pair of signals (booklet then saliva) was separated by a 2.5-hour interval, although in order to make it difficult for participants to anticipate the signals, prompts were programmed in a 10-minute window around the 25-minute inter-beep interval

and in a 20-minute window around the 2.5-hour inter-beep interval.

Participants were instructed to store used salivettes in the freezer. Materials were returned as soon as possible after completing the study (typically the next day), and salivettes were kept in a freezer at our laboratory before being analyzed.

Cortisol assay. A time-resolved fluorescence immunoassay with a biotin-cortisol conjugate as a tracer and a streptavidin-europium label was used for salivary cortisol assessment (41). The lower detection limit of this assay is 0.43 nmol for a 50 μl saliva sample. Saliva samples were spun at 3,000 revolutions per minute for 5 minutes, resulting in clear saliva of low viscosity. A 100 μl sample was used for duplicate analysis, and all samples from a participant were analyzed in one assay run. The inter- and intra-assay coefficients of variation were less than 10%. Although the analyses for the FM and RA participants were conducted at a different time than the analyses for the control group, internal control samples were used to ensure the assay performed comparably over time.

Statistical analyses. The repeated daily assessments for each person in this study pose analytic challenges due to the lack of independence among observations and multiple levels of observation (i.e., self-reports and cortisol samples nested within persons) (42). Traditional linear modeling procedures can bias inferential tests and limit the generalizability of results for hierarchical data because they treat persons as fixed factors. We therefore used multilevel random effects models that allow both persons and self-reports/saliva samples to be treated as random effects in the same model. The MIXED procedure in the SAS software package (SAS Institute, Cary, NC) was used to construct linear models that used each self-report/saliva sample as the unit of analysis.

Prior to addressing the hypotheses of this study, we checked for demographic differences between the groups. Demographic variables that were significantly different between the groups were used as covariates in the main analyses. We also identified group differences in lifestyle (i.e., use of alcohol, sleep quality, activities) and psychosocial (i.e., stress and mood) variables that might affect cortisol, so that we could examine the effect of including these variables in the models in the main analyses. Tests for differences in between-subject variables were based on one-way analyses of variance (for continuous variables) and chi-square tests (for categorical variables), while tests for differences between the groups on variables assessed repeatedly during the EMA portion of the study were based on multilevel random effects models. Both overall differences between the groups (e.g., differences in frequency of exercise or use of caffeine) and differences in the daily pattern of activity or consumption (i.e., interactions between group and time) were evaluated. This latter test is important for repeatedly assessed variables, because differences over time could confound the observed diurnal cycle of cortisol secretion.

Group differences in levels and diurnal cycles of cortisol were examined using multilevel random effects models with cortisol as the dependent variable. Level differences were examined with the group main effect while diurnal cycle differences were examined with the group by time interaction term. To account for the diurnal cycle of cortisol, all models controlled for day and time. The influence of demographic, psychosocial, and lifestyle differences between the groups was examined by repeating the analyses with these variables in the model. Because previous studies have implicated stress and sleep disturbance in the onset or exacerbation of FM and RA, we examined the effect of these psychosocial variables separately.

Cortisol reactivity to acute stress was also examined using multilevel random effects models. A stress variable by group interaction term was used to test for evidence of differences between the groups in cortisol reactivity. To evaluate the impact of lifestyle and psychosocial differences on these results, we repeated the analyses with these variables in the model.

RESULTS

Prior to presenting the main analyses, we describe the treatment of missing data, the preparation of cortisol data, and the preliminary analyses identifying demographic and lifestyle/psychosocial differences between the groups that could affect cortisol secretion. The main analyses are then described with examination of group differences in cortisol levels and diurnal cycles first, and group differences in reactivity to psychological stress presented last.

Missing data. Of the 732 total signals for recording (61 participants $\times$ 2 days $\times$ 6 signals per day), participants provided 594 (81%) cortisol samples and 623 (85%) booklet reports. However, because we were interested in psychosocial- and lifestyle-related

variables that might affect cortisol, we limited analyses to cortisol samples where participants had responded to the preceding booklet signal close to the intended 25-minute time lag between booklet completion and cortisol sampling. Samples were therefore excluded if both samples were not within 18 and 32 minutes of each other (i.e., 25 ± 7 minutes). This reduced the number of samples to 540 (74%).

Because group differences in missing data could bias analyses, we examined both the overall rate of missing data and its pattern over the day. A multilevel random effects model indicated there was no main effect of group, $F(2, 58) = 0.14$, $P > 0.20$, or time of day, $F(5, 665) = 1.44$, $P > 0.20$, on missing data; however, there was a significant interaction between group and time of day, $F(10, 655) = 1.96$, $P < 0.04$. The missing data of the control group had a U-shaped pattern, with more missing data than the other groups at the first and last time points. The RA group had the least missing data at the first two time points, but increased in missing data in the middle of the afternoon with a slight decline over the evening. The FM group had relatively high levels of missing data at the first 3 time points and less missing data at the last 3 time points.

Preparation of cortisol data. Cortisol data were examined for outliers by standardizing and eliminating values more than 4 standard deviations from the mean. Eight observations (1.5%) were eliminated. Raw cortisol values were also negatively skewed and were therefore logarithmically transformed prior to analysis.

Group differences on demographic, lifestyle, and psychosocial variables. To ensure our groups were comparable with respect to demographics and to identify group differences in lifestyle and psychosocial variables that could potentially affect cortisol secretion, preliminary analyses of group differences were conducted. Group differences in between-subject variables (i.e., demographic and other background variables assessed prior to the EMA portion of the study) are shown in Table 1. Because significant variables were to be used as covariates in the primary analyses, we adopted a liberal alpha level (0.10). Differences between the 3 groups were observed only for age, $F(2, 58) = 3.85$, $P < 0.03$, and employment status, χ^2 (8) = 27.16, $P < 0.001$. The RA group was older and had more retirees than the other two groups. The FM group had more part-time workers and homemakers, while the control group had more full-time workers, compared with the other groups.

Random effects models used to evaluate group differences on lifestyle and psychosocial variables that were repeatedly assessed during the EMA portion of the study (i.e., positive affect, negative affect, arousal, current stress, daily event, eating/drinking, exercise, tobacco, caffeine, alcohol, sleep quality, sleep duration) revealed a number of group differences (Table 2). With 0.10 alpha level, significant main effects of group emerged for arousal, $F(2, 58) = 7.71$, $P < 0.002$, occurrence of a daily event, $F(2, 58) = 9.37$, $P < 0.001$, and quality of sleep, $F(2, 57) = 13.31$, $P < 0.001$. A significant interaction between group and time emerged for eating/drinking, $F(10, 449) = 1.65$, $P < 0.090$. Arousal and sleep quality were lower in the FM group compared with the other two groups. Surprisingly, both the FM and RA groups had *fewer* daily events. These differences were greatest at the second, third, and fourth time points (i.e., late morning and afternoon).

Group differences in cortisol level and diurnal cycle. The raw cortisol means for each group at each time of day are displayed in Figure 1. A multilevel random effects model that tested level differences between the groups revealed significant main effects for time of day, $F(5, 465) = 65.11$, $P < 0.001$, and group, $F(2, 58) = 7.63$, $P < 0.002$. The results from a separate model testing diurnal cycle differences indicated that there was no significant group by time of day interaction, $F(10, 445) = 1.54$, P not significant. These results indicated that there were significant overall level differences between the groups but no significant difference in the diurnal cycles. Post hoc *t*-tests indicated the control group had significantly lower levels of cortisol than both the RA, $t(58) = 2.81$, $P < 0.007$, and the FM groups, $t(58) = 3.72$, $P < 0.001$; no difference between the RA and FM groups, $t(58) = 0.77$, P not significant, was observed. Mean levels of cortisol (based on the anti-logs of the least-squared means) were 7.10, 6.50, and 4.72 nmol/l for the FM, RA, and control groups, respectively.

When the analyses were repeated with the different sets of demographic and psychosocial variables (those on which the groups differed) included in the models, the pattern of results remained the same. With the addition of age, employment status, and eating/drinking, the significant main effect for group was only slightly reduced, $F(2, 53) = 5.88$, $P < 0.005$, and the nonsignificant interaction between time and group remained, $F(10, 448) = 1.48$, P not significant. As before, the post hoc tests revealed that the control group had lower levels of cortisol than both the FM and RA groups, $t(53) = 3.34$, $P < 0.002$, and $t(53) = 2.08$, $P < 0.042$, respectively. The mean adjusted

Table 1. Descriptive statistics for demographic and other background variables of rheumatoid arthritis (RA), fibromyalgia (FM), and control groups

	Control (n = 22)		RA (n = 18)		FM (n = 21)		Test of difference between groups	
	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)	χ^2	F
Age	–	46.64 (6.25)	–	52.83 (8.35)	–	47.86 (7.47)	–	3.85*
Number of children	–	2.05 (1.46)	–	2.35 (1.22)	–	2.24 (1.39)	–	0.25
Years of education	–	15.25 (2.51)	–	14.19 (2.04)	–	14.76 (2.64)	–	0.84
Perceived stress	–	1.72 (0.52)	–	1.55 (0.49)	–	1.87 (0.54)	–	1.89
Trait anxiety	–	1.89 (0.49)	–	1.52 (0.46)	–	1.82 (0.69)	–	2.32
Years since diagnosis	–	–	–	11.50 (8.26)	–	3.89 (3.03)	–	12.00†
Physician rating of disease activity	–	–	–	2.50 (0.82)	–	1.89 (0.99)	–	3.78
Symptom checklist	–	–	–	0.45 (0.38)	–	0.69 (0.35)	–	4.46*
Sex (female)	73	–	67	–	86	–	2.03	–
Ethnicity	–	–	–	–		–	6.30	–
White	100	–	94	–	90	–	–	–
Latino	0	–	0	–	10	–	–	–
American Indian	0	–	6	–	0	–	–	–
Employment	–	–		–	–	–	27.16‡	–
Working full-time	86	–	50	–	52	–	–	–
Working part-time	13	–	0	–	29	–	–	–
Homemaker	0	–	0	–	9	–	–	–
Retired	0	–	44	–	10	–	–	–
Disabled	0	–	6	–	0	–	–	–
Endocrine disorder history (thyroid)	7	–	2	–	10	–	0.64	–
Eating disorder history	0	–	0	–	0	–	–	–
Drug/alcohol abuse history	3	–	6	–	10	–	2.22	–
Psychiatric history	9	–	0	–	5	–	1.98	–
Currently dieting	12	–	22	–	25	–	2.03	–
Exercise regularly	60	–	40	–	42	–	1.13	–
Smokers	34	–	6	–	15	–	0.85	–
Oral contraceptives	3	–	9	–	5	–	0.92	–
Medications§	–	–		–	–	–	–	–
Antidepressants	–	–	17	–	43	–	3.12–	–
NSAIDs	–	–	78	–	38	–	6.21*	–
Analgesics	–	–	11	–	19	–	0.47	–
Anxiolytics	–	–	0	–	10	–	1.81	–
Muscle relaxants	–	–	6	–	19	–	1.58	–
SAARDs	–	–	83	–	5	–	0.44	–
Other	–	–	63	–	52	–	0.20	–

* $P < 0.05$.
† $P < 0.01$.
‡ $P < 0.001$.
§ NSAIDs = nonsteroidal anti-inflammatory drugs; SAARDs = slow-acting antirheumatic drugs.

cortisol levels were 7.35, 6.56, and 4.88 nmol/l, for the FM, RA, and control groups, respectively.

Models that included the psychological stress variables (i.e., daily event and arousal) also revealed a significant main effect for group, F(2, 58) = 8.05, $P < 0.001$, and no significant interaction, F(10, 427) = 1.48, P not significant. Post hoc t-tests revealed the same pattern as before, with the control group having significantly lower levels of cortisol than both the FM and RA groups, t(58) = 3.78, $P < 0.001$, and t(58) = 2.97, $P < 0.005$, respectively. The mean adjusted cortisol level for the FM, RA, and control groups was 7.43, 6.84, and 4.84 nmol/l, respectively.

With sleep quality included in the model, there was again a significant main effect for group, F(2, 57) = 4.76, $P < 0.013$, and no significant interaction, F(10, 447) = 1.55, P not significant. As before, post

Table 2. Group differences on psychosocial- and lifestyle-related confounding variables*

	Control		Rheumatoid arthritis		Fibromyalgia		Main effect of group		Group × time of day interaction	
	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)	df	F	df	F
Positive affect	–	2.23 (1.35)	–	2.68 (1.37)	–	2.22 (1.28)	2, 58	0.18	10, 520	0.32
Negative affect	–	0.86 (1.20)	–	0.51 (0.86)	–	0.85 (1.09)	2, 58	0.31	10, 519	0.52
Arousal	–	3.60 (1.27)	–	3.85 (1.03)	–	2.91 (1.20)	2, 58	7.96†	10, 516	0.85
Sleep duration in minutes	–	405.19 (63.67)	–	419.42 (72.18)	–	430.49 (75.75)	2, 56	1.38	–	–
Sleep quality	–	5.32 (1.21)	–	5.09 (1.79)	–	3.42 (1.60)	2, 57	11.28†	–	–
Event	37	–	11	–	15	–	2, 58	11.33†	10, 509	1.67
Eating/drinking	10	–	12	–	11	–	2, 58	0.31	10, 521	1.97‡
Exercise	2	–	1	–	2	–	2, 58	0.47	10, 521	0.91
Tobacco use	6	–	5	–	12	–	2, 58	0.64	10, 507	0.98
Caffeine use	25	–	35	–	19	–	2, 58	2.67‡	10, 522	0.60
Alcohol use	2	–	1	–	2	–	2, 58	0.36	10, 507	0.72

* % = percentage of self-reports with positive responses. df = degrees of freedom.
† $P < 0.001$.
‡ $P < 0.10$.

hoc *t*-tests indicated that the control group had significantly lower cortisol levels than the FM and RA groups, $t(57) = 2.63$, $P < 0.011$, and $t(57) = 2.66$, $P < 0.011$, and there was no difference between the RA and FM groups, $t(57) = 0.06$, P not significant. The adjusted mean cortisol level was 6.71, 6.65, and 4.90 nmol/l for the FM, RA, and control groups, respectively.

Reactivity to stress. Multilevel random effects models that included each stress variable (daily event, positive affect, negative affect, and arousal) by group interaction term revealed no significant interaction effects for any of the stress variables (Table 3). There was, however, a marginally significant main effect for daily event, $F(1, 447) = 2.75$, $P < 0.098$. Thus, cortisol levels rose following significant daily

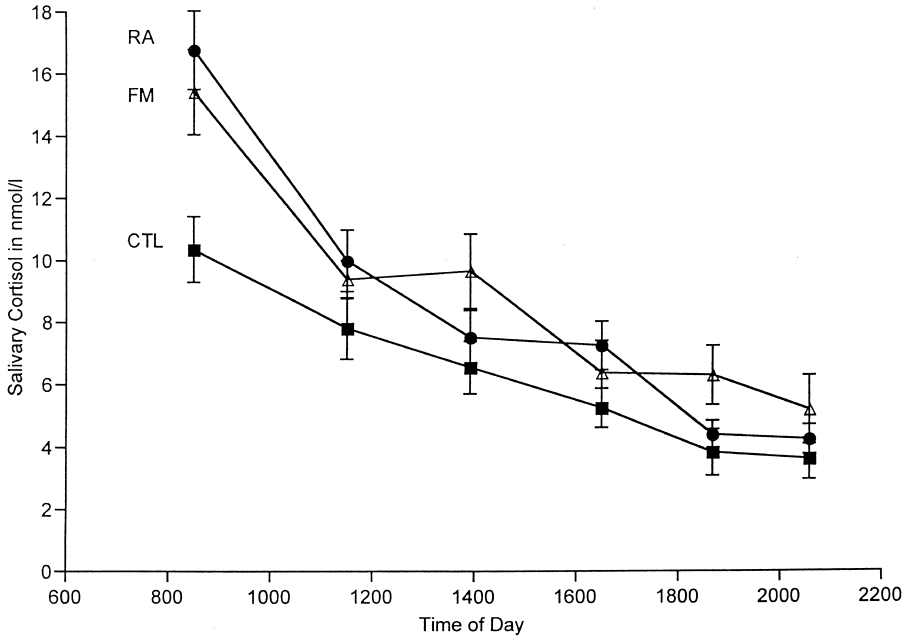

Figure 1. Means of raw cortisol values at each time of day. RA = rheumatoid arthritis; FM = fibromyalgia; CTL = control.

Table 3. Reactivity of cortisol to stress

	Main effect		Interaction with group	
	df*	F	df	F
Event	1, 447	2.75†	2, 445	0.00
Positive affect	1, 498	0.10	2, 496	1.88
Negative affect	1, 497	0.43	2, 495	1.81
Arousal	1, 494	0.49	2, 492	0.31

* df = degrees of freedom.
† $P < 0.10$.

events, but the reactivity of cortisol did not differ between the groups. The magnitude of the cortisol response to a daily event in unlogged terms was 1.12 nmol/l.

Evaluation of group differences in cortisol reactivity in response to stress while including the demographic and psychosocial variables in the models produced very similar results. There remained no indication of group differences in reactivity for any of the stress variables; however, the main effect of daily event was weakened, $F(1, 431) = 2.23$, $P < 0.137$.

DISCUSSION

This study examined 3 aspects of cortisol secretion (level, diurnal cycle, and reactivity to stress) in FM patients, RA patients, and healthy control participants engaged in usual daily activities. We hypothesized that there would be elevations in overall levels of cortisol, flattened diurnal cycles of cortisol, and reduced cortisol reactivity to acute stress in both RA and FM patients. Results indicated that overall cortisol levels were higher in the FM and RA groups than in the control group, but that there were no significant differences between the groups in the pattern of cortisol secretion over the day. There was also no evidence of group differences in cortisol reactivity to stress.

Previous laboratory studies of HPA axis functioning of FM and RA patients have not produced consistent results. In FM patients, Crofford et al (9) found *lowered* 24-hour urinary free cortisol levels; however, morning and evening free plasma cortisol assessments suggested *higher* overall levels and a flattened diurnal cycle. Griep et al (43) also found lower 24-hour urinary free cortisol and total plasma cortisol, but no differences between FM patients and controls in plasma free cortisol. Differences between these results and our findings may be because we conducted a greater number of cortisol assessments and used a statistical model to compare the diurnal cycles of each group. In addition, we measured cortisol only through the waking hours. It is possible that reduced secretion during the late phase of sleep (when cortisol secretion is usually highest) could lead to reduced 24-hour urinary free cortisol levels.

Previous studies of cortisol in RA patients have also been inconsistent. Cash et al (13) reported higher evening basal cortisol levels (prior to a corticotropin-releasing hormone test), while other similar studies found slightly higher basal levels of cortisol that did not reach statistical significance (16,44,45), and Hedman et al (46) reported lower levels. A limitation of these RA studies is that cortisol levels were assessed at a single point in time; however, Crofford et al (17) conducted half-hourly plasma assessments over 24 hours and also found slightly higher levels of cortisol in RA patients that did not reach statistical significance. In light of these results, it is possible that the larger sample size we used (n = 19 compared with 10 or less in these previous studies) provided greater statistical power that allowed us to detect a small but reliable elevation in cortisol among RA patients. In addition, the present study differed from these studies in that we measured the biologically active cortisol fraction (i.e., the steroid levels available at the target tissue level) rather than total cortisol. Salivary cortisol is generally more weakly correlated with total plasma cortisol than with plasma free cortisol (35).

The most surprising aspect of our findings was that although overall cortisol levels in the FM and RA patients were elevated consistent with studies of individuals subject to chronic stress (25–27), psychological stress variables did not account for the elevation. In fact, the FM and RA groups in this study reported fewer significant events and similar scores on the perceived stress and trait anxiety scales. While this suggests that chronic psychological stress should be ruled out as an explanation for elevated cortisol levels in FM and RA patients, some research indicates that psychological stress factors are relevant only for a subset of patients. For example, Stewart et al (47) found evidence that post-onset life event stress and disease activity were significantly related only among those RA patients without autoantibody rheumatoid factor (i.e., seronegative RA). Alternatively, only particular types of stress may be relevant in RA and FM patients. For example, Zautra et al found evidence suggesting that RA patients were particularly physiologically and psychologically reactive to the stress of interpersonal

conflict (48). Further research examining subtypes of patients and alternative stress indicators is necessary to evaluate these possibilities.

Two additional points are worth noting in regard to the proposed link between stress and cortisol. First, although these results indicate that the overall elevated cortisol levels are not due to chronic psychological stress, this does not exclude the possibility that prior stressful experiences are involved in the onset of illness (7,49,50). Second, even though we found no evidence of disturbed cortisol reactivity to daily stress, stressful events were marginally associated with acute increases in cortisol, indicating that daily stress could influence disease processes via the HPA axis. This is consistent with other research that has demonstrated a link between daily stressful events and immune processes in RA patients (51). The association between stress and cortisol may have been stronger had we limited the types of stressful events participants could report to those situations that are thought to be most strongly associated with cortisol reactivity. Studies suggest that cortisol increases are especially associated with stressful situations involving high ego involvement, low predictability, low controllability, and novelty (36).

Because we assessed cortisol naturalistically, we were also able to examine whether accounting for differences in other psychosocial and lifestyle factors affected the cortisol findings. Although the groups differed on eating/drinking habits and quality of sleep, these differences failed to account for the elevated cortisol levels and had no effect on the findings regarding diurnal cycles or reactivity of cortisol. This result is particularly relevant to FM research because it challenges prior speculation that sleep disturbance in FM patients leads to HPA axis disturbance (52). The fact that there were no group differences on measures of positive and negative affect is also noteworthy, as previous research has linked depression and elevated levels of cortisol (53,54). While formal diagnosis of depression was not conducted in this study, more recent research suggests that overall elevations in cortisol levels are characteristic only of psychotic depression (55,56).

There are a number of limitations of this study that should be considered when interpreting the results. Although patients who were taking corticosteroid medication were excluded, patients in the study were taking other medications. It would have been preferable to evaluate cortisol secretion in unmedicated patients; however, this is very difficult in practice. In addition, although no significant differences between the groups emerged on tobacco use, the use of nicotine by participants in all groups may have affected the cortisol findings. Another limitation of the present study is that the control group data were collected in a previous study. We cannot completely rule out the possibility that unidentified differences between the studies are responsible for the observed difference in levels of cortisol, although the identical design and cortisol analysis make this unlikely. A unique feature of the present study was its naturalistic evaluation of cortisol and the specific assessment of the role of psychosocial- and lifestyle-related factors; however, this approach prevented control over the magnitude and exact timing of stressors and assessments and required reliance on the self-report of patients. In addition, group differences in patterns of missing data that appeared to be related to living with FM and RA could not be completely controlled. Finally, in generalizing these results it should be appreciated that the patients in this study were recruited from a community rheumatology practice and may not be comparable to those seen in hospital settings.

In summary, we found further evidence of HPA axis disturbance among FM and RA patients. While elevations of cortisol have often been seen among chronically stressed individuals, we found no evidence that ongoing psychological stress accounted for the between-group differences in cortisol levels. Furthermore, no other differences in psychosocial and lifestyle variables accounted for the elevation, including the relatively poor sleep quality of FM patients. This suggests that HPA axis disturbances observed in FM and RA patients are more likely to be due to factors not examined in this study, such as chronic pain or disease-related physiologic factors.

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