Cortisol and subjective stress responses to acute psychosocial stress in fibromyalgia patients and control participants

Eline Coppens (1,2,4), MA; Stefan Kempke (1), MA, PhD; Peter Van Wambeke (3,4), MD; Stephan Claes (2), MD, PhD; Bart Morlion (4,5), MD, PhD; Patrick Luyten (1, 6), MA, PhD; Lukas Van Oudenhove (7,8), MD, PhD

(1) Faculty of Psychology and Educational Sciences, University of Leuven, Belgium
(2) Department of Psychiatry, University Hospitals Leuven, University of Leuven, Belgium
(3) Department of Physical Medicine and Rehabilitation, University Hospitals Leuven, University of Leuven, Belgium
(4) The Leuven Centre for Algology & Pain Management, University Hospitals Leuven, University of Leuven, Belgium
(5) Department of Anesthesiology and Algology, University Hospitals Leuven, University of Leuven, Belgium
(6) Research Department of Clinical, Educational and Health Psychology, University College London, UK
(7) Translational Research Center for Gastrointestinal Disorders (TARGID), Department of Clinical & Experimental Medicine, University of Leuven, Belgium
(8) Consultation-Liaison Psychiatry, University Hospitals Leuven, Belgium

Correspondence to:
Eline Coppens, MA
eline.coppens@ppw.kuleuven.be
University Hospitals Leuven, University of Leuven
Leuven Centre for Algology & Pain Management (LAC)
Weligerveld 1
3212 Pellenberg
Belgium
tel +32 16 33 84 51 - fax +32 16 33 84 53

Funding: Research-Foundation Flanders Bijzondere Onderzoeksbeurs (Eline Coppens); KU Leuven Special Research Fund (Bijzonder Onderzoeksfonds, BOF) (Lukas Van Oudenhove); Stefan Kempke is a Postdoctoral Researcher of the Research Foundation Flanders (FWO); Stephan Claes is a Senior Clinical Investigator of the Research Foundation Flanders (FWO).

In press: Psychosomatic Medicine

We declare no conflict of interest.

Word Count: 6891; Figures: 5; Tables: 1; Supplementary materials: Figure S1, Table S2
Short Title: Stress responsivity in fibromyalgia

Abstract

Objective

Hypothalamic-pituitary-adrenal (HPA) axis dysfunction may play a role in fibromyalgia (FM) pathogenesis, but remains understudied in this disorder. Furthermore, early childhood adversities (ECA) are common in FM, but whether they moderate stress reactivity is unknown. Hence, we investigated cortisol and subjective responses to acute psychosocial stress in FM and controls, while adjusting for ECA.

Methods

Twenty-seven female FM patients and 24 age-matched female controls were recruited in a tertiary care center and through advertisements, respectively. The Childhood Trauma Questionnaire was used to measure ECA history. Salivary cortisol levels and subjective stress ratings were measured at multiple time points before and after the Trier Social Stress Test (TSST) was administered.

Results

Significant main effects of group [$F(1,43)=7.04$, $p=0.011$, lower in FM] and ECA [$F(1,43)=5.18$, $p=0.028$, higher in participants with ECA] were found for *cortisol* responses. When excluding controls with ECA (n=5), a significant group-by-time interaction was found [$F(6,39)=2.60$, $p=0.032$], driven by a blunted response to the stressor in FM compared with controls ($p=0.037$). For *subjective stress* responses, a significant main effect of group [$F(1,45)=10.69$, $p=0.002$, higher in FM] and a trend towards a group-by-time interaction effect [$F(6,45)=2.05$, $p=0.078$, higher in FM 30 minutes before and 30 and 75 minutes after the TSST, and impaired recovery (difference immediately after – 30 minutes after the TSST) in FM] were found.

Conclusions

Blunted cortisol responsivity to the TSST was observed in FM patients compared with controls without ECA. FM patients had higher subjective stress levels compared with controls, particularly at baseline and during recovery from the TSST. In FM patients, ECA history was not associated with cortisol or subjective stress levels, or with responsivity to the TSST. Future research should investigate the mechanisms underlying HPA axis dysregulation in FM.

Keywords: TSST, stress reactivity, cortisol, early childhood adversity, fibromyalgia

Abbreviations

HPA: hypothalamic-pituitary-adrenal

FM: fibromyalgia

ECA: early childhood adversities

TSST: Trier Social Stress Test

ACR: American College of Rheumatology

FSS: functional somatic syndromes

CFS: chronic fatigue syndrome

IBS: irritable bowel syndrome

DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, fourth edition

MINI: Mini-International Neuropsychiatric Interview

PTSD-ZIL: Zelfinventarisatielijst Posttraumatische Stressstoornis

CTQ-SF: Childhood Trauma Questionnaire – short form

T0: baseline measure

T1: pre-TSST measure

T2: immediate after-TSST measure

T3: measure 10 minutes after TSST

T4: measure 20 minutes after TSST

T5: measure 30 minutes after TSST

T6: measure 75 minutes after TSST

SEM: standard error of the mean

PTSD: posttraumatic stress disorder

AUCg: area under the curve with respect to the ground

AUCi: area under the curve with respect to increase

BMI: body mass index

ACTH: adrenocorticotropic hormone

CRH: corticotropin-releasing hormone

CRF: cerebrospinal fluid corticotropin-releasing factor

Introduction

Fibromyalgia (FM) patients suffer from chronic pain, often accompanied by other symptoms such as chronic fatigue, subjective cognitive impairment and sleep disturbances (1, 2). The prevalence of FM is estimated to be around 3% (2, 3), with a female:male ratio of 9:1 (4, 5). Although new conceptualizations and criteria of FM have been developed more recently (6), in this study FM was defined by the 1990 American College of Rheumatology (ACR) criteria, designed for research (1). According to these criteria, chronic, widespread pain (described in detail by Wolfe et al. (1)) must be present for at least three months and patients must report pain in at least 11 of 18 specified tender point sites on digital palpation. Furthermore, FM is often considered part of a spectrum of "functional somatic syndromes" (FSS) (7) or "central sensitivity syndromes" (8) including chronic fatigue syndrome (CFS), irritable bowel syndrome (IBS) and functional dyspepsia, among others. These syndromes show substantial overlap (7). Additionally, the level of emotional distress in these syndromes seems to be higher than in comparable medical conditions and positively related to the number of functional complaints (9-11).

FSS may be associated with dysfunction of the cortisol stress response system, i.e., the hypothalamic-pituitary-adrenal (HPA) axis (12, 13). A meta-analysis of studies measuring basal cortisol levels found that female FM patients showed on average mild hypocortisolism compared with female controls. However, almost a third of the studies in this meta-analysis found hypercortisolism in patients with FM (12). This heterogeneity may be due to possible moderators of HPA axis function, such as early childhood adversities (ECA). Furthermore, most studies on HPA axis functioning in FM have almost exclusively focused on basal cortisol levels. To our knowledge, only one study investigated cortisol reactivity to a (psychosocial) stressor in FM (14). In this study, FM patients and controls showed similar salivary cortisol responses to the Trier Social Stress Test (TSST).

Meta-analyses found elevated levels of ECA in FM, with odds ratios in case–control studies ranging from 1.92 to 3.31 (15-17). However, although ECA has been proposed as a vulnerability factor for FM, a causal role has not been proven (18). Furthermore, most studies tend to focus selectively on sexual and physical ECA without taking emotional types of ECA into account. ECA may play a role in

HPA axis dysregulation found in stress-related disorders in general and in FSS such as FM in particular (19, 20), based on animal models of early life stress (e.g., maternal separation) (21, 22), as well as research in maltreated or traumatized children (23, 24), and adults who experienced ECA (24-26). Several studies in participants with a history of ECA have revealed disrupted HPA axis reactivity (24, 27-29). Studies on the association between ECA and HPA axis reactivity in FM are lacking, but in other FSS, an association was found between ECA and HPA axis responses during the TSST; however, both decreased and increased responses were found in individuals with ECA (30-32), again stressing the need for further research.

The aim of this study was therefore to investigate cortisol and subjective stress responses to the TSST in female FM patients and age-matched controls, while adjusting for history of ECA. We hypothesized that there would be a reduced HPA axis response in the TSST in FM patients compared with controls, based on studies suggesting that FM is associated with HPA axis hyporeactivity (33-35). Given that FM is associated with high levels of subjective stress (36-39), we expected to observe increased levels of subjective stress in FM patients compared with controls in the TSST.

Methods

Participants

From to 2011 to 2014, 27 consecutive female patients were recruited to the study by physicians specializing in FM diagnosis and treatment at the department of Physical Medicine and Rehabilitation and the Centre for Chronic Pain of the University Hospitals Leuven (Belgium). Patients were diagnosed with FM if they met the 1990 ACR criteria for FM (chronic widespread pain with a minimal duration of 3 months and at least 11 of 18 specified tender points present) (1) and if they had no other disorder that would sufficiently explain the widespread pain. Besides a thorough assessment and physical examination, the following investigations were done: chest X-ray and laboratory testing with at least a full blood count and measurement of C-reactive protein, creatine kinase and thyroid-stimulating hormone (40). We chose to use the 1990 ACR criteria because we believe more recent

criteria are inherently more subjective (only anamnestic information is gathered and no clinical examination is required). We are aware that the 1990 ACR criteria were designed for research and not for diagnostic purposes.

When diagnosing patients, physicians screened patients carefully for other medical conditions that could explain symptoms or influence the HPA axis. To avoid gender differences as a potentially confounding factor for the analyses we decided to recruit only female patients. As noted above, the vast majority of FM patients are women (5). Twenty-five age-matched female controls, recruited via advertising on the University Hospital's intranet, were included. Exclusion criteria for both patients and controls were the use of corticosteroids and benzodiazepines. Controls were healthy: they were asked not to apply to take part in the study if they were aware of any illness; in addition, controls could not participate in the study if they reported any illness at the time of the study. Additional exclusion criteria for controls were the use of corticosteroids or psychotropic medication, or diagnosis of a psychiatric disorder according to DSM-IV criteria (41) based on the Mini-International Neuropsychiatric Interview (MINI) (42). FM patients were also screened for psychiatric disorders using the MINI and all patients filled in the well-validated Post-Traumatic Stress Disorder Self-Inventory (PTSD-ZIL) questionnaire. Patients whose pain symptoms were considered secondary to a psychiatric disorder were excluded.

All participants went through an informed consent procedure before participation. The study was approved by the Sociaal-maatschappelijke ethische commissie of the University Hospitals of the University of Leuven and the Medical Ethical Commission of the University Hospitals Leuven.

Questionnaires

Participants completed the Childhood Trauma Questionnaire – short form (CTQ-SF (43)), a well-validated 25-item self-report instrument, to measure exposure to ECA (44). The CTQ has been proven to be a reliable and valid instrument, with good internal consistency (0.63–0.95), criterion-related validity (0.50–0.75) in clinical and community samples, high convergent validity with therapist assessments of ECA, and good specificity and sensitivity of cut-off scores to classify participants with

ECA (43, 44). The CTQ measures five domains of ECA: emotional, physical, and sexual abuse, and emotional and physical neglect. To identify patients with moderate to severe ECA, we used the established cut-off scores for each domain (43, 45). Cut-off scores are 13 or higher for emotional abuse, 10 or higher for physical abuse, 8 or higher for sexual abuse, 15 or higher for emotional neglect, and 10 or higher for physical neglect. Finally, we created a dichotomous variable reflecting the presence of significant early childhood adversity (no ECA in any of the five domains versus at least one ECA in any of the five domains) (43).

Trier Social Stress Test

The TSST is the gold standard laboratory procedure (46) to induce psychosocial stress; it consists of a public speech (5 minutes) and an arithmetic task (5 minutes) performed in front of an evaluating audience (two PhDs or MSc students wearing white laboratory coats). Participants have 3 minutes to prepare the public speech. The TSST produces significant increases in cortisol and other neuroendocrine stress markers (47-49). Participants were instructed not to eat, drink (other than water) or smoke for at least 1 hour prior to testing. Salivary cortisol was assessed at baseline (T0), immediately before the TSST (after a relaxation/waiting period of 30 minutes) (T1), immediately after the TSST (T2), and 10 (T3), 20 (T4), 30 (T5), and 75 minutes after the TSST (T6) *(Figure 1)* using Salivette saliva sampling tubes (Sarstedt, Germany). During the 45 minutes prior to the final assessment, participants underwent a semi-structured interview on their experiences during the TSST for qualitative analysis purposes, which will be reported in a separate paper.

Self-reported stress was measured at all time points (T1–T6) using a visual analogue scale, ranging from 0 (not stressed at all) to 100 (as stressed as I could possibly imagine).

Data analysis

Data were analyzed in SAS 9.4 (SAS Institute, Cary, NC, USA) and are shown as mean ± SEM unless otherwise stated. A two-tailed p-value <0.05 was considered significant.

Continuous variables were tested for normality using the Shapiro-Wilk test; logarithmic transformation was used to normalize the distribution where needed in order to fulfill the assumption of normally distributed residuals in general linear model or linear mixed model analyses. Because of a right-tailed skewed distribution of both the cortisol and subjective stress outcome variables, we transformed both logarithmically, which resulted in normalization of the distribution. Where normality could not be achieved after transformation, non-parametric statistics were used.

Demographic characteristics were compared between groups using independent samples Student's t-tests (or Kruskal-Wallis tests when appropriate) for continuous variables and Pearson's χ^2-tests (or Fisher's exact tests when appropriate) for categorical variables. In case of a significant group difference on a given demographic variable, the association between this variable and summary measures for the cortisol and subjective stress response to the TSST was tested using correlation analysis (for continuous variables) or independent samples Student's t-tests (for categorical variables). Three summary measures were calculated for cortisol and subjective stress during the TSST: area under the curve with respect to the ground (AUCg) as an indicator of total output, area under the curve with respect to increase (AUCi) as a measure of response to the stressor (50), and the delta peak value (peak minus baseline) (51). In case of a significant effect of the demographic variable on any of the summary measures, this demographic variable was included in the respective longitudinal analyses described below as an adjustment variable. This approach was chosen over adding all potential confounders as adjustment variables to the models in order to avoid overfitting of the models.

Cortisol and subjective stress levels at each of the 7 time points were log transformed to normalize the distribution.

To analyze the time course of the cortisol and subjective stress response to the TSST, linear mixed models were used. "Time" (7 measurement points, numbered T0–T6, see above and *Figure 1*) was included as a within-subject categorical independent variable; "group" (FM versus controls) was included as a between-subject independent variable. The main effect of group and the group-by-time interaction effect constituted the principal effects of interest. "Presence of ECA" (yes/no) was included

as a between-subject independent variable to adjust for the putative effect of ECA history on the outcomes. Marginal models with an unstructured variance-covariance matrix for the repeated categorical time variable were chosen over linear mixed models with time as a continuous variable (latent growth curve models with random intercept as well as random linear and quadratic effects of time) as the former models fitted the data better (both for cortisol and subjective stress as the dependent variable) based on significantly lower values of Akaike's Information Criterion.

The group-by-time interaction effect in these models was followed up by a priori planned contrasts using independent samples Student's t-tests, with step-down Bonferroni correction for multiple testing. Groups were compared at T0 ("baseline"), T2 or T3 (immediately or 10 minutes after the TSST, the "peak" of the subjective respectively cortisol response after the TSST), T5 (30 minutes after the TSST) and T6 (75 minutes after the TSST). Furthermore, the differences between T0 and T2 or T3 ("response" to the stressor), between T2 or T3 and T5 ("early recovery" from the stressor), and between T2 or T3 and T6 ("late recovery" from the stressor) were compared between groups.

Possible effects of a history of ECA on the dependent variables (cortisol response and subjective distress) was further explored in two ways. First, given the fact that ECA may represent a risk factor for FM, we re-ran the linear mixed model analyses described above excluding the 5 controls with a positive history of ECA. Second, to explore possible effects of ECA on cortisol and subjective stress within the FM group, we ran linear mixed models in the FM group only with "time" as the within-subject independent variable and "presence of ECA" as the between-subject independent variable, including the time-by-ECA interaction effect.

Finally, we report associations between subjective stress levels at T0, T2 and the change between T0 and T2 on the one hand and cortisol levels at T0, T3 and the change between T0 and T3 on the other, for FM patients and controls separately.

Results

Descriptive data

Patients and controls were matched for age; only women were included. One control terminated the experiment prematurely (immediately after stress induction) and her data were removed from all further analyses. Furthermore, the saliva tubes of one control and one patient were empty; these data were removed from the cortisol analyses only. The final study sample therefore included 27 FM patients and 24 controls for subjective stress analysis, and 26 FM patients and 23 controls for cortisol analysis.

Demographic and clinical characteristics are shown in *Table 1*. Twelve FM patients and 5 controls reported at least one ECA (44% versus 22%, p=.014). Groups differed significantly in terms of body mass index (BMI), education level, employment status and smoking status. With regard to psychiatric disorders, 5 patients met the criteria for depression and 2 for posttraumatic stress disorder (PTSD).

The association between education level and cortisol AUCi was marginally significant (higher cortisol AUCi in participants with higher education) (Kruskal-Wallis test $\chi^2(1)=2.83$, p=.092), whereas smoking was associated with a lower cortisol AUCi (Kruskal-Wallis test $\chi^2(1)=4.03$, p=.045). These two variables were not associated with the other two cortisol summary measures, or with any of the subjective stress summary measures. BMI correlated negatively with all three cortisol summary measures (AUCg $\rho=-0.42$, p=.003; AUCi $\rho=-0.27$, p=.064; peak $\rho=-0.30$, p=0.036) but not with any of the subjective stress summary measures. The multivariable models were adjusted for these three variables. Depression and antidepressant use were not associated with any of the summary measures for cortisol or subjective stress.

Analysis of cortisol and subjective stress responses to the TSST

Cortisol

Comparison of fibromyalgia and controls, adjusted for ECA (Figure 2A)

The main effects of group [F(1,43)=7.04, p=.011, lower average over all time points in FM (1.25±0.08) versus controls (1.60±0.10)], and time [F(6,43)=12.0, p<.0001] were significant. The group-by-time interaction effect was not significant [F(6,43)=1.85, p=.11]. However, planned contrast analyses showed that FM patients had lower cortisol levels at 10 minutes (T3, "peak"), 30 minutes (T5), and 75 minutes (T6) after the TSST (all p-values <.03), but not at 30 minutes before (T0, "baseline") the TSST (p=.37). Furthermore, a trend towards a stronger response to the TSST (increase from T0 to T3) in controls compared with FM was found (p=.076). The effect of smoking was not significant [F(1,42)=0.08, p=.78], whereas the effects of education [F(1,42)=5.88, p=.020], log BMI [F(1,42)=6.75, p=.013] and, interestingly, ECA [F(1,43)=5.18, p=.028, higher average over all time points in participants with ECA (1.52±0.09) versus participants without ECA (1.32±0.07)], were.

In sum, FM patients had lower cortisol levels compared with controls after, but not before, the stressor, and showed a trend towards a blunted response to the stressor. Participants (both FM and controls) with an ECA history had higher cortisol levels compared with participants without ECA.

Comparison of fibromyalgia and controls without ECA (Figure 2B)

The main effect of group [F(1,39)=1.04, p=0.31] was not significant, contrary to the main effect of time [F(6,39)=9.29, p<.0001] and the group-by-time interaction effect [F(6,39)=2.60, p=.034]. Planned contrast analyses showed that cortisol levels in FM patients did not differ significantly from those in controls at any of the pre-specified time points (all p-values >.11). However, the response to the TSST (increase from T0 to T3) was significantly stronger in controls than in FM patients (p=.037); in contrast, in the "early recovery" (decrease from T3 to T5) (p=.36) and the "late recovery" (decrease from T3 to T6) (p=.74) there was no difference between controls and FM patients. The effect of smoking was not significant [F(1,39)=0.30, p=.59], whereas the effects of education [F(1,39)=8.21,

p=.007] and log BMI [F(1,39)=7.14, p=0.011] were, with higher cortisol levels being recorded in participants with lower education, and lower levels in participants with higher BMI.

In sum, these results indicate that FM patients showed a blunted cortisol response to the stressor compared with controls without ECA. The fact that the main effect of group found in these analyses, which included controls with a history of ECA (n=5), was no longer significant when these controls were excluded, indicates that the higher cortisol levels in controls found in the above analysis are driven by this small subgroup of controls with ECA.

Effect of ECA within fibromyalgia (Figure 3)

The main effect of ECA was non-significant [F(1,24)=0.41, p=.53], the main effect of time was significant [F(6,24)=13.5, p<.0001], and the ECA-by-time interaction effect was non-significant [F(6,24)=0.74, p=.62]. In sum, FM patients with and without ECA did not differ in terms of cortisol response to the stressor.

Subjective stress ratings

Comparison of fibromyalgia and controls, adjusted for ECA (Figure 4)

The main effects of group [F(1,45)=10.69, p=.002, higher average over all time points in FM patients (3.06±0.21) versus controls (1.86±0.30)] and time [F(6,45)=13.70, p<.0001] were significant. The group-by-time interaction effect showed a trend [F(6,45)=2.05, p=.078]. Planned contrast analyses showed that FM patients had higher subjective stress levels at 30 minutes before (T0, "baseline"), and at 30 and 75 minutes after the TSST (all p-values <.02), but not immediately after the TSST (T2, "peak") (p=.12). Furthermore, a trend towards a stronger response to the TSST (increase from T0 to T2) in controls compared with FM patients was found (p=.099), as well as a significantly stronger early recovery (decrease from T2 to T5) (p=.01) and a trend towards a stronger late recovery (decrease from T2 to T6) (p=.099). The effects of smoking, education, and log BMI were not significant (all p-values >.29), and nor was the effect of ECA [F(1,45)=0.28, p=.60].

In sum, FM patients had higher subjective stress levels compared with controls at baseline and at 30 and 75 minutes after the stressor, but not immediately after the stressor. This resulted in a trend

towards a blunted subjective stress response (which may be due to a ceiling effect given the significantly higher baseline) and a significantly impaired recovery among FM patients. ECA history was not associated with the subjective stress response to the TSST.

Comparison of fibromyalgia patients and controls without ECA (see Supplementary materials Figure S1)

Except for some minimal numerical differences (slightly higher p-value for the omnibus test of the interaction effect, slightly lower p-values for planned contrast analyses), the results of the analysis comparing FM patients with controls without ECA were virtually identical to those of the comparison with all controls reported in the previous section (data not shown).

Effect of ECA within fibromyalgia (Figure 5)

The main effect of ECA was non-significant [$F(1,25)=0.24$, $p=.63$], the main effect of time was significant [$F(6,25)=10.30$, $p<.0001$], and the ECA-by-time interaction effect was non-significant [$F(6,25)=0.41$, $p=.87$]. In sum, FM patients with and without ECA did not differ in terms of subjective stress response to the TSST.

Correlations between cortisol and subjective stress responses (see Supplementary materials Table S2)

We investigated the correlations between subjective stress levels at T0, T2 and the change between T0 and T2 on the one hand and cortisol levels at T0, T3 and the change between T0 and T3 on the other, for FM patients and controls separately. None of these correlations were significant, with the exception of the correlation between subjective stress on T0 with cortisol levels on T0 ($r=.42$, $p=.045$) and T3 ($r=.42$, $p=.044$) in controls.

Discussion

We investigated cortisol and subjective stress responses to the TSST in female FM patients and age-matched controls. As expected, we found a blunted cortisol response to an experimental stressor in FM patients compared with controls, particularly when controls with as history of ECA were excluded

from the analyses. These findings contrast with those of the only previous study on salivary cortisol reactivity in FM patients versus controls in response to the TSST (14). In that study, a dissociation was found between total plasma cortisol and salivary cortisol responsivity in FM patients. FM patients in the previous study showed reduced cortisol reactivity in plasma total cortisol release, but not in salivary free cortisol, compared with controls. There were no differences in terms of cortisol baseline levels between FM and controls. FM patients in the previous study were, as in our study, diagnosed based on the 1990 ACR criteria but, contrary to our study, they were recruited from local support groups, general practitioners' offices and the gynecological department of a general hospital. The FM patients in our study were recruited from a tertiary care center and may therefore represent more disabled patients, possibly explaining the discrepancy in the results between the two studies. Future research is needed to examine this assumption.

Different explanations have been formulated for the cortisol hyporeactivity observed in FM. For example, a reduced adrenal cortisol response to adrenocorticotropic hormone (ACTH) has been proposed (52). Decreased adrenocortical sensitivity to ACTH may be attributed to downregulation of adrenal receptors, but genetic variation and morphological changes of the adrenal gland, such as atrophy or decreased volume, may also contribute (14). In contrast, undersecretion of corticotropin-releasing hormone (CRH) by the hypothalamus and secondary atrophy of the adrenals due to chronic understimulation by reduced ACTH levels was proposed as a possible explanation for hypofunction of the HPA axis in FM in a review on neuroendocrine function in FM (53). Several studies using dynamic testing (such as the dexamethasone suppression test) have shown an exaggerated ACTH response but a blunted cortisol response to CRH. Another recent review on the neuroendocrinology of FM (54) has argued that while ECA may alter HPA axis function, resulting in excessive pain, HPA axis dysfunction in FM may also be secondary to chronic painful symptoms. Animal studies suggest in this context that stress can produce local but also widespread hyperalgesia, or can enhance or prolong the hyperalgesic response to mild noxious stimuli (54). In FM patients, there might be a vicious circle of pain, disability and stress. ECA may have initially caused pain symptoms in FM, whereas current disability and pain levels may maintain stress, HPA dysfunction and chronic pain itself.

A history of ECA was not associated with cortisol responsivity in the TSST in FM patients. This observation is in contrast with findings from a study reporting that a subgroup of FM patients were characterized by both a history of ECA and basal hypocortisolism (55). In contrast with that study, we did not find an association of ECA with baseline cortisol in FM. In a study of patients with CFS, another FSS (56), only patients with ECA exhibited decreased concentrations of baseline cortisol compared with controls. Although we did not find an association of ECA with baseline cortisol in FM patients, it is possible that ECA has an impact on awakening cortisol levels. Furthermore, although we did not find an association between ECA and saliva cortisol, another study in FM patients (20) found an association between increased Cerebrospinal fluid Corticotropin-Releasing Factor (CRF) and pain and between decreased CRF and ECA. Furthermore, we investigated the relationship only with ECA in general, and not with particular subtypes of ECA. It is possible that, as has been shown in CFS, emotional types of ECA are associated with the blunted cortisol response (30). Because of the limited sample size in the present study, we could not investigate this.

We also found higher subjective stress levels in FM patients compared with controls at baseline and during recovery, but not immediately after the TSST. It is possible that FM patients show a ceiling effect for subjective stress, i.e., their subjective stress level is already increased at baseline (which may reflect anticipatory stress), preventing a further increase in response to the TSST to a level that is significantly different from that of controls. High subjective stress levels in FM patients might be a consequence of a constant "fight or flight" response mode related to hypervigilance to threat (57). However, FM patients may be particularly vulnerable to social stress, as it has been shown that FM patients use fewer positive affective resources and less effective pain coping strategies during social stress compared with other chronic pain patients (39). In the absence of other studies assessing subjective distress in the TSST in FM patients, further research is needed in this area. However, even if replicated, high subjective distress in response to stress might be associated with high pain or disability levels. Finally, we found no association of a history of ECA with differences in subjective stress levels or responses. As noted above, however, it is possible that ECA might have been responsible for increased vulnerability for pain, in part through its associations with maladaptive pain

coping strategies and high stress levels in FM (58), but is not associated with subjective distress once FM has fully developed.

Taken together, these results reflect a dissociation between subjective and cortisol responses to stress in FM. This dissociation has been described in several FSS (58, 59) and might be crucial in understanding these syndromes (60, 61). More specifically, this finding may suggest a "crash" of the stress system in FM and is in line with a recent study (62) in which no association was found between basal cortisol levels and daily life stress in FM.

Furthermore, PTSD (which may represent the impact of ECA) has been shown to be highly prevalent in FM patients (17) and has been associated with hypocortisolemia (63-65). However, because only two patients in our FM sample met criteria for PTSD, we could not investigate a possible effect of PTSD on our outcomes. We consider it highly unlikely that PTSD plays a role in the blunted HPA axis response in FM in the present study, as our analyses showed very similar results after removing the two FM participants who met criteria for PTSD (data not shown).

The findings of this study must be considered in the light of its limitations. First, because of the limited sample size we could not investigate the putative effect of different types of ECA on the stress response. Second, between T6 and T7 we performed a semi-structured interview, which may have affected the results at the last time point. Nevertheless, cortisol levels decreased further between these two time points, demonstrating normal recovery over all participants and rendering the possibility that the recovery of the stress response was affected by the interview unlikely. Furthermore, in another TSST study in FM patients, the cortisol peak appeared later (25 minutes after the end of the TSST) (14) compared with our results (10 minutes after the end of the TSST). Our results are, however, comparable with results found in abused women in a study by Heim et al. (27), whose protocol we followed. In that study, the peak appeared 15 minutes after the end of the TSST. Our results are also comparable with those of a TSST study in IBS patients (66) where the peak was found 7 minutes after the end of the TSST. Third, results may be influenced by the higher prevalence of depression in FM. Depression was more or less equally divided over ECA categories (of the 5 patients with depression,

there were 3 with ECA and 2 without ECA) and not associated with any of the summary measures of the cortisol and subjective stress response. Nonetheless, we re-ran the analysis without depressed patients and obtained similar results. Furthermore, adding depression as a covariate to the models (a) did not affect the effects of the other variables, (b) did not have a significant effect itself on cortisol and subjective outcome measures, and (c) did not significantly improve model fit. A fourth limitation concerns the recruitment procedure of controls (i.e., advertisement on the university hospital's intranet). It is therefore possible that the control group was not very representative of the general population. Even though all controls were carefully screened, we investigated the possible influence of certain factors (e.g. education level) and we did not include participants with any prior contact with the investigators, our results may require replication with a control group that is more representative of the general population.

Despite these limitations, this is the first study that investigated subjective and HPA axis responsivity to a well-validated stressor in FM patients compared with controls while at the same time adjusting for a history of ECA. The findings of this study provide support for a blunted cortisol response and increased subjective stress levels in FM patients, independent of a history of ECA. Future research should investigate the mechanisms underlying HPA axis dysregulation in FM, and the putative influence of different types of ECA on HPA axis function in FM.

References

1. Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DL, Tugwell P, Campbell SM, Abeles M, Clark P. The american college of rheumatology 1990 criteria for the classification of fibromyalgia. Arthritis and Rheumatism. 1990;33:160-72.

2. Branco JC, Bannwarth B, Failde I, Abello Carbonell J, Blotman F, Spaeth M, Saraiva F, Nacci F, Thomas E, Caubère J-P. Prevalence of fibromyalgia: A survey in five european countries. Seminars in Arthritis and Rheumatism. 2010;39:448-53.

3. Spaeth M. Epidemiology, costs, and the economic burden of fibromyalgia. Arthritis Research and Therapy. 2009;11:117.

4. Hauser W, Kuhn-Becker H, von Wilmoswky H, Settan M, Brahler E, Petzke F. Demographic and clinical features of patients with fibromyalgia syndrome of different settings: A gender comparison. Gender Medicine. 2011;8:116-25.

5. Yunus M. The role of gender in fibromyalgia syndrome. Current Rheumatology Reports. 2001;3:128-34.

6. Wolfe F, Clauw DJ, Fitzcharles MA, Goldenberg DL, Katz RS, Mease P, Russell AS, Russell IJ, Winfield JB, Yunus MB. The american college of rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. Arthritis Care and Research. 2010;62:600-10.

7. Wessely S, Nimnuan C, Sharpe M. Functional somatic syndromes: One or many? The Lancet. 1999;354:936-9.

8. Yunus MB, editor. Fibromyalgia and overlapping disorders: The unifying concept of central sensitivity syndromes. Seminars in Arthritis and Rheumatism; 2007: Elsevier.

9. Simon GE, VonKorff M. Somatization and psychiatric disorder. American Journal of Psychiatry. 1991;148:1494-500.

10. Kroenke K, Spitzer RL, Williams JB, Linzer M, Hahn SR, deGruy III FV, Brody D. Physical symptoms in primary care: Predictors of psychiatric disorders and functional impairment. Archives of Family Medicine. 1994;3:774.

11. Wessely S, Chalder T, Hirsch S. Psychological symptoms, somatic symptoms, and psychiatric disorder in chronic fatigue and chronic fatigue syndrome: A prospective study in the primary care setting. Year Book of Psychiatry and Applied Mental Health. 1998;1998:333.

12. Tak LM, Cleare AJ, Ormel J, Manoharan A, Kok IC, Wessely S, Rosmalen JG. Meta-analysis and meta-regression of hypothalamic-pituitary-adrenal axis activity in functional somatic disorders. Biological Psychology. 2011;87:183-94.

13. Harbeck B, Süfke S, Harten P, Haas C, Lehnert H, Mönig H. High prevalence of fibromyalgia-associated symptoms in patients with hypothalamic-pituitary disorders. Clinical and Experimental Rheumatology. 2012;31:S16-21.

14. Wingenfeld K, Heim C, Schmidt I, Wagner D, Meinlschmidt G, Hellhammer DH. Hpa axis reactivity and lymphocyte glucocorticoid sensitivity in fibromyalgia syndrome and chronic pelvic pain. Psychosomatic Medicine. 2008;70:65-72.

15. Davis DA, Luecken LJ, Zautra AJ. Are reports of childhood abuse related to the experience of chronic pain in adulthood?: A meta-analytic review of the literature. The Clinical journal of pain. 2005;21:398-405.

16. Häuser W, Kosseva M, Üceyler N, Klose P, Sommer C. Emotional, physical, and sexual abuse in fibromyalgia syndrome: A systematic review with meta-analysis. Arthritis Care and Research. 2011;63:808-20.

17. Afari N, Ahumada SM, Wright LJ, Mostoufi S, Golnari G, Reis V, Cuneo JG. Psychological trauma and functional somatic syndromes: A systematic review and meta-analysis. Psychosomatic Medicine. 2014;76:2-11.

18. Sommer C, Häuser W, Burgmer M, Engelhardt R, Gerhold K, Petzke F, Schmidt-Wilcke T, Späth M, Tölle T, Üçeyler N. Ätiologie und pathophysiologie des fibromyalgiesyndroms. Der Schmerz. 2012;26:259-67.

19. Van Houdenhove B, Egle UT. Fibromyalgia: A stress disorder? Psychotherapy and Psychosomatics. 2004;73:267-75.

20. McLean SA, Williams DA, Stein PK, Harris RE, Lyden AK, Whalen G, Park KM, Liberzon I, Sen A, Gracely RH. Cerebrospinal fluid corticotropin-releasing factor concentration is associated with pain but not fatigue symptoms in patients with fibromyalgia. Neuropsychopharmacology. 2006;31:2776-82.

21. Dettling AC, Feldon J, Pryce CR. Repeated parental deprivation in the infant common marmoset (callithrix jacchus,primates) and analysis of its effects on early development. Biological Psychiatry. 2002;52:1037-46.

22. Sanchez M, Ladd CO, Plotsky PM. Early adverse experience as a developmental risk factor for later psychopathology: Evidence from rodent and primate models. Development and Psychopathology. 2001;13:419-49.

23. Gunnar MR, Vazquez DM. Low cortisol and a flattening of expected daytime rhythm: Potential indices of risk in human development. Development and Psychopathology. 2001;13:515-38.

24. Heim C, Nemeroff C, editors. Neurobiology of early life stress: Clinical studies. Seminars in Clinical Neuropsychiatry; 2002.

25. Carpenter LL, Carvalho JP, Tyrka AR, Wier LM, Mello AF, Mello MF, Anderson GM, Wilkinson CW, Price LH. Decreased adrenocorticotropic hormone and cortisol responses to stress in healthy adults reporting significant childhood maltreatment. Biological Psychiatry. 2007;62:1080-7.

26. Carpenter LL, Tyrka AR, Ross NS, Khoury L, Anderson GM, Price LH. Effect of childhood emotional abuse and age on cortisol responsivity in adulthood. Biological Psychiatry. 2009;66:69-75.

27. Heim C, Newport DJ, Heit S, Graham YP, Wilcox M, Bonsall R, Miller AH, Nemeroff CB. Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. JAMA. 2000;284:592-7.

28. Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nature Reviews Neuroscience. 2009;10:434-45.

29. Loman MM, Gunnar MR. Early experience and the development of stress reactivity and regulation in children. Neuroscience and Biobehavioral Reviews. 2010;34:867-76.

30. Kempke S, Luyten P, De Coninck S, Van Houdenhove B, Mayes LC, Claes S. Effects of early childhood trauma on hypothalamic-pituitary-adrenal (hpa) axis function in patients with chronic fatigue syndrome. Psychoneuroendocrinology. 2014.

31. Van Den Eede F, Moorkens G, Van Houdenhove B, Cosyns P, Claes SJ. Hypothalamic-pituitary-adrenal axis function in chronic fatigue syndrome. Neuropsychobiology. 2007;55:112-20.

32. Videlock EJ, Adeyemo M, Licudine A, Hirano M, Ohning G, Mayer M, Mayer EA, Chang L. Childhood trauma is associated with hypothalamic-pituitary-adrenal axis responsiveness in irritable bowel syndrome. Gastroenterology. 2009;137:1954-62.

33. Giske L, Vøllestad NK, Mengshoel AM, Jensen J, Knardahl S, Røe C. Attenuated adrenergic responses to exercise in women with fibromyalgia–a controlled study. European Journal of Pain. 2008;12:351-60.

34. Griep EN, Boersma J, Lentjes E, Prins A, Van der Korst J, De Kloet E. Function of the hypothalamic-pituitary-adrenal axis in patients with fibromyalgia and low back pain. The Journal of rheumatology. 1998;25:1374-81.

35. Gur A, Cevik R, Nas K, Colpan L, Sarac S. Cortisol and hypothalamic–pituitary–gonadal axis hormones in follicular-phase women with fibromyalgia and chronic fatigue syndrome and effect of depressive symptoms on these hormones. Arthritis Research & Therapy. 2004;6:R232.

36. Sallinen M, Kukkurainen ML, Peltokallio L, Mikkelsson M, Anderberg UM. Fatigue, worry, and fear—life events in the narratives of women with fibromyalgia. Health Care for Women International. 2012;33:473-94.

37. Van Houdenhove B, Egle U, Luyten P. The role of life stress in fibromyalgia. Current rheumatology reports. 2005;7:365-70.

38. Wentz K, Lindberg C, Hallberg L. Psychological functioning in women with fibromyalgia: A grounded theory study. Health Care for Women International. 2004;25:702-29.

39. Davis MC, Zautra AJ, Reich JW. Vulnerability to stress among women in chronic pain from fibromyalgia and osteoarthritis Annals of Behavioral Medicine. 2001;23:215-26.

40. Fitzcharles M-A, Ste-Marie PA, Goldenberg DL, Pereira JX, Abbey S, Choinière M, Ko G, Moulin DE, Panopalis P, Proulx J. 2012 canadian guidelines for the diagnosis and management of fibromyalgia syndrome: Executive summary. Pain Research and Management. 2013;18:119-26.

41. Association AP. Diagnostic and statistical manual of mental disorders. 2000;: Text revision. Washington, DC: American Psychiatric Association.

42. Lecrubier Y, Sheehan D, Weiller E, Amorim P, Bonora I, Sheehan KH, Janavs J, Dunbar G. The mini international neuropsychiatric interview (mini). A short diagnostic structured interview: Reliability and validity according to the cidi. European Psychiatry. 1997;12:224-31.

43. Bernstein D FL. Childhood trauma questionnaire: A retrospective self- report questionnaire and manual. San Antonio: TX: Psychological Corp; 1998.

44. Bernstein DP, Stein JA, Newcomb MD, Walker E, Pogge D, Ahluvalia T, Stokes J, Handelsman L, Medrano M, Desmond D, Zule W. Development and validation of a brief screening version of the childhood trauma questionnaire. Child Abuse and Neglect. 2003;27:169-90.

45. Heim C. Early adverse experience and risk for chronic fatigue syndrome: Results from a population-based study. Archives of General Psychiatry. 2006;63:1258.

46. Kirschbaum C, Pirke KM, Hellhammer DH. The trier social stress test - a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology. 1993;28:76-81.

47. Allen AP, Kennedy PJ, Cryan JF, Dinan TG, Clarke G. Biological and psychological markers of stress in humans: Focus on the trier social stress test. Neuroscience and Biobehavioral Reviews. 2014;38:94-124.

48. Kudielka B, Wüst S, Kirschbaum C, Hellhammer D. Trier social stress test. Encyclopedia of stress. 2007;3:776-81.

49. Frisch JU, Häusser JA, Mojzisch A. The trier social stress test as a paradigm to study how people respond to threat in social interactions. Frontiers in Psychology. 2015;6.

50. Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology. 2003;28:916-31.

51. Khoury JE, Gonzalez A, Levitan RD, Pruessner JC, Chopra K, Santo Basile V, Masellis M, Goodwill A, Atkinson L. Summary cortisol reactivity indicators: Interrelations and meaning. Neurobiology of Stress. 2015;2:34-43.

52. Wingenfeld K, Wagner D, Schmidt I, Meinlschmidt G, Hellhammer DH, Heim C. The low-dose dexamethasone suppression test in fibromyalgia. Journal of Psychosomatic Research. 2007;62:85-91.

53. Gupta A, Silman AJ. Psychological stress and fibromyalgia: A review of the evidence suggesting a neuroendocrine link. Arthritis research and therapy. 2004;6:98-106.

54. Sluka KA, Clauw DJ. Neurobiology of fibromyalgia and chronic widespread pain. Neuroscience. 2016;338:114-29.

55. Loevinger BL. Delineating psychological and biomedical profiles in a heterogeneous fibromyalgia population using cluster analysis. Clinical Rheumatology. 2012;31:677.

56. Heim C, Nater UM, Maloney E, Boneva R, Jones JF, Reeves WC. Childhood trauma and risk for chronic fatigue syndrome association with neuroendocrine dysfunction. Archives of General Psychiatry. 2009;66:72-80.

57. Crombez G, Eccleston C, Van den Broeck A, Goubert L, Van Houdenhove B. Hypervigilance to pain in fibromyalgia: The mediating role of pain intensity and catastrophic thinking about pain. The Clinical journal of pain. 2004;20:98-102.

58. Luyten P, Van Houdenhove B, Lemma A, Target M, Fonagy P. Vulnerability for functional somatic disorders: A contemporary psychodynamic approach. 2013.

59. Kempke S, Luyten, P., Mayes, L., Van Houdenhove, B., & Claes, S. (in press). Self-critical perfectionism predicts lower cortisol response to experimental stress in patients with chronic fatigue syndrome. Health Psychology Review.

60. Abbass A. Raised consciousness about emotions and health. Dalhousie Medical Journal. 2005;33:23-5.

61. Lumley MA. Beyond cognitive-behavioral therapy for fibromyalgia: Addressing stress by emotional exposure, processing, and resolution. Arthritis Research & Therapy. 2011;13.

62. Catley D, Kaell AT, Kirschbaum C, Stone AA. A naturalistic evaluation of cortisol secretion in persons with fibromyalgia and rheumatoid arthritis. Arthritis Care and Research. 2000;13:51-61.

63. Yehuda R, Bierer LM, Schmeidler J, Aferiat DH, Breslau I, Dolan S. Low cortisol and risk for ptsd in adult offspring of holocaust survivors. American Journal of Psychiatry. 2014.

64. Meewisse M-L, Reitsma JB, De Vries G-J, Gersons BP, Olff M. Cortisol and post-traumatic stress disorder in adults. The British Journal of Psychiatry. 2007;191:387-92.

65. Newport DJ, Heim C, Bonsall R, Miller AH, Nemeroff CB. Pituitary-adrenal responses to standard and low-dose dexamethasone suppression tests in adult survivors of child abuse. Biological Psychiatry. 2004;55:10-20.

66. Kennedy P, Cryan J, Quigley E, Dinan T, Clarke G. A sustained hypothalamic–pituitary–adrenal axis response to acute psychosocial stress in irritable bowel syndrome. Psychological Medicine. 2014;44:3123-34.

Figure legends

Figure 1. Protocol of the Trier Social Stress Test (TSST).

Figure 2. Cortisol stress responses of fibromyalgia patients (FM) and control subjects (CS) on the Trier Social Stress Test (TSST). Figure A. Fibromyalgia patients (FM) and control subjects (CS) both controlled for early childhood adversity (ECA). Figure B. Fibromyalgia patients (FM) and control subjects (CS) without early childhood adversity (ECA). Time points: (0) baseline, (1) pre-TSST, (2) after-TSST, (3) 10' after-TSST, (4) 20' after-TSST, (5) 30' after-TSST and (6) 75' after-TSST.

Error bars show standard errors of the mean.

Figure 3. Cortisol stress responses of fibromyalgia patients (FM) with and without early childhood adversity (ECA) on the Trier Social Stress Test (TSST). Time points: (0) baseline, (1) pre-TSST, (2) after-TSST, (3) 10' after-TSST, (4) 20' after-TSST, (5) 30' after-TSST and (6) 75' after-TSST.

Error bars show standard errors of the mean.

Figure 4. Subjective stress responses of fibromyalgia patients (FM) and control subjects (CS) on the Trier Social Stress Test (TSST). Figure A. Fibromyalgia patients (FM) and control subjects (CS) both adjusted for childhood early adversity (ECA). Figure B. Fibromyalgia patients (FM) and control subjects (CS) without childhood early adversity (ECA). Time points: (0) baseline, (1) pre-TSST, (2) after-TSST, (3) 10' after-TSST, (4) 20' after-TSST, (5) 30' after-TSST and (6) 75' after-TSST.

Error bars show standard errors of the mean.

Figure 5. Subjective stress responses of fibromyalgia patients (FM) with and without early childhood adversity (ECA) on the Trier Social Stress Test (TSST). Time points: (0) baseline, (1) pre-TSST, (2) after-TSST, (3) 10' after-TSST, (4) 20' after-TSST, (5) 30' after-TSST and (6) 75' after-TSST.

Error bars show standard errors of the mean.