Psychoneuroendocrinology (2012) 37, 671—684

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Evidence for an association between an enhanced reactivity of interleukin-6 levels and reduced glucocorticoid sensitivity in patients with fibromyalgia

Andrea Geiss [a,b,*], Nicolas Rohleder [c], Fernand Anton [a,d]

[a] *Department of Psychobiology, University of Trier, D-54290 Trier, Germany*
[b] *University of Cologne, D-50931 Köln, Germany*
[c] *Brandeis University, MS 062, PO Box 549110, Waltham, MA 02454, USA*
[d] *Laboratory of Psychobiology and Neurophysiology, FLSHASE/INSIDE, Universite du Luxembourg, L-1511 Luxembourg, Luxembourg*

Received 19 November 2010; received in revised form 27 July 2011; accepted 28 July 2011

KEYWORDS
Fibromyalgia syndrome;
Interleukin 6;
Tumor necrosis factor-α;
Hypothalamus—pituitary—adrenal axis;
Cortisol;
Glucocorticoid sensitivity;
Fatigue;
Pain;
Chronic stress;
Pressure pain thresholds

Summary Pain and fatigue have been identified as core symptoms of fibromyalgia syndrome (FMS). Since both symptoms are also characteristic of hypocortisolemic disorders, reduced cortisol levels have been thought to promote an exacerbation of these FMS core symptoms by an enhanced reactivity of interleukin-6 (IL-6) levels. The aim of the current study was to investigate the pathophysiologic relevance of reduced cortisol levels for manifestation of FMS core symptoms. Twelve female FMS patients with 15 female controls were compared regarding the function of hypothalamus—pituitary—adrenal (HPA) axis and behavioral, endocrine and IL-6 responses after measuring the pressure pain thresholds (PPTs) at tender points. Function of HPA axis was assessed by determining the cortisol awakening response, daytime profile of cortisol secretion, low dose overnight dexamethasone suppression test (DST) and glucocorticoid sensitivity (GC) of inflammatory cytokine production. While endocrine and IL-6 responses were determined by collecting blood and saliva samples behavioral responses were assessed by pain and fatigue recordings of participants before and after PPT measurement using visual analogue scale (VAS). Whereas FMS patients were found not to differ from controls in cortisol awakening response, daytime profile of cortisol secretion and cortisol suppression after overnight DST, they did exhibit a reduced GC sensitivity of inflammatory cytokine production. PPT measurement did induce three times higher cortisol and four times higher IL-6 levels in FMS patients, but no change in their ACTH levels. The enhanced IL-6 reactivity after PPT

* Corresponding author at: University of Cologne, Department of Psychology, Gronewaldstraße 2, D-50931 Köln, Germany. Tel.: +49 0221 470 4723; fax: +49 0221 470 5105.
E-mail address: geissa@uni-koeln.de (A. Geiss).

0306-4530/$ — see front matter
doi:10.1016/j.psyneuen.2011.07.021

measurement was accompanied by an increase in the severity of FMS patients' pain and fatigue ratings. The findings of the present study provide evidence for the pathophysiologic relevance of a disturbed glucocorticoid receptor (GR) function, rather than reduced cortisol levels for the maintenance of FMS core symptoms.

1. Introduction

Fibromyalgia syndrome (FMS) is a chronic disorder characterized by core symptoms widespread musculoskeletal pain, fatigue and sleep disturbances for a duration of at least three months (Wolfe, 1989). FMS is approximately nine times more common among women than men and the prevalence increases with age, affecting nearly 7% of women over 60 years (Wolfe et al., 1995).

Onset of FMS has been found to be preceded by either prior life trauma or high workload and workplace bullying (Imbierowicz and Egle, 2003; Kivimäki et al., 2004). Both types of chronic stressors have been reported to be accompanied by alterations of the hypothalamus–pituitary–adrenal (HPA) axis as this neuroendocrine system is mainly involved in the mediation of the stress response (for review Tanriverdi et al., 2007). Stress-induced glucocorticoid (GC) secretion is currently believed to protect the organism from harmful effects of overshooting defense mechanisms, for example by suppressing the synthesis of pro-inflammatory cytokines, such as interleukin-6 (IL-6) in immune cells (e.g. Sapolsky et al., 2000). Two different patterns of an altered neuroendocrine HPA axis function have been characterized, termed hypocortisolism and hypercortisolemia. Prior life trauma and cumulative adversity have been associated with both patterns of dysfunctional HPA axis function as this chronic stressor was found to increase the risk for developing posttraumatic stress disorder (PTSD) following a later trauma and major depressive disorder (MDD) in later life (Edwards et al., 2003; Keane et al., 2006). A hypocortisolemic pattern is mainly characterized by reduced cortisol levels, enhanced feedback sensitivity and increased sensitivity of peripheral blood mononuclear cells (PBMCs) to glucocorticoids and hence enhanced glucocorticoid (GC) sensitivity (Yehuda et al., 1991, 2004). Main neuroendocrine features of hypercortisolemia are enhanced cortisol levels, a diminished feedback sensitivity and reduced sensitivity of PBMCs to glucocorticoids (Gillespie and Nemeroff, 2005). While pain and fatigue have been identified as core symptoms of hypocortisolemic disorders hypercortisolemia has been reported to be manifested mainly in fatigue and cognitive dysfunction (Geiss et al., 2005; Fries et al., 2005; Miller et al., 2009). Both neuroendocrine patterns have been found to be accompanied by elevated cytokine levels. While a reduced cortisol availability is thought to account for elevated cytokine levels in hypocortisolism an attenuated sensitivity of PBMCs to glucocorticoids has been related to increased cytokine levels in hypercortisolemia (Geiss et al., 2005; Fries et al., 2005; Miller et al., 2009; Rohleder et al., 2010).

There is evidence for both a hypercortisolemic and hypocortisolemic state in FMS. In more detail, FMS patients have been reported to exhibit an elevated cortisol profile (Catley et al., 2000), but also to display an attenuated cortisol response during the first hour after awakening (Weissbecker et al., 2006; Klingmann et al., 2008). Furthermore, FMS patients have been identified to exhibit a lower glucocorticoid receptor (GR) binding of their PBMCs to glucocorticoids and hence reduced GC sensitivity (Lentjes et al., 1997). In contrast, neither Wingenfeld et al. (2008) nor Macedo et al. (2008) did find any difference between FMS patients and controls in their GC sensitivity of circulating monocytes. Inconsistent findings have been reported also for the status of the feedback sensitivity in FMS patients as enhanced feedback sensitivity has been detected by one study (Wingenfeld et al., 2007), but several other studies did show a reduced feedback sensitivity (Ferraccioli et al., 1990; Griep et al., 1998).

Likewise, increased IL-6 levels have been detected previously in supernatants of stimulated FMS patients' PBMCs by Wallace et al. (2001), but recent studies did not find any difference between FMS patients and controls in their IL-6 levels after controlling for depressive symptoms (Gür et al., 2002; Bazzichi et al., 2006; Wang et al., 2008).

Since pain and fatigue have been identified as core symptoms of FMS we assumed that FMS patients' hypocortisolemic pattern might promote an exacerbation of pain and fatigue through increased inflammatory signaling. The aim of the present study was to test whether low cortisol responses to the measurement of pressure pain thresholds (PPTs) at tender points were associated with enhanced reactivity of IL-6 levels in FMS patients as compared with controls.

2. Methods

2.1. Participants

Twelve female patients with FMS were recruited from a local support group in Trier, Germany. All patients were diagnosed with FMS by their rheumatologists according to the 1990 American College of Rheumatology classification criteria (Wolfe et al., 1990). All patients fulfilled the inclusion criteria of (1) being <60 years old; (2) having a body mass index (BMI) <35 kg/m^2; (3) not having a history of major physical (including rheumatic conditions), or psychiatric illness; (4) had not received epidural corticosteroid injections and antidepressant medication at the clinical and low dose during one year preceding the study; and (5) were able to discontinue either anti-inflammatory medication and/or hormone replacement therapy for at least 4 weeks prior to the study. As a control group, fifteen female, pain-free participants matched for age and education were recruited from advertisements placed on bulletin boards and in local newspapers. Donors having elevated Westergen erythrocyte sedimentation rates (WESR) and increased total neutrophil counts, for instance, two weeks preceding the study were excluded from participation. The study was approved by the Ethics Committee of the University of Trier and all participants gave their informed consent to participation.

Table 1 Overview of the experimental examinations the subjects undergoing each day.

	Day 1	Day 2	Day 3	Day 4
Procedure	Collection of saliva samples to determine the cortisol awakening response (CAR) immediately after awakening at 0700 h	Collection of saliva and blood samples before and after measurement of pressure pain thresholds (PPTs) at tender points, starting at 1630 h and ending at 2400 h	Collection of two blood samples to determine the glucocorticoid (GC) sensitivity of inflammatory cytokine production and white blood cell (WBC) count at 11 00 h	Collection of saliva samples to determine cortisol awakening response (CAR) immediately after awakening at 0700 h and daytime profile of cortisol secretion starting at 1000 h and ending at 2200 h
	Collection of saliva samples to determine daytime profile, beginning at 1000 h and ending at 2200 h.		Ingestion of 0.5 mg dexamethasone at 2200 h	

2.2. Experimental protocol

As depicted in Table 1, the experimental examinations were conducted on four consecutive days. On the first day, cortisol awakening response (CAR) and daytime profile of cortisol secretion were assessed by instructing participants to collect one saliva sample immediately after awakening at 0700 h, as well as four saliva samples in intervals of 15 min after awakening. Further seven saliva samples were obtained by the participants at intervals of 2 h, beginning at 1000 h. Participants were asked to refrain from eating for at least 30 min before saliva collection. On the second day, the experimental session was started by inserting a catheter into the antecubital vein (Braun, Melsungen, Germany) within 1 h after arrival of participants in the laboratory of the Center for Psychobiological and Psychosomatic Research, University of Trier (Germany) at 1300 h. In order to avoid a stimulatory effect of physical activity or psychological stress on IL-6 and cortisol levels participants were instructed to take a rest between 1400 h and 1630 h (Ostrowski et al., 1998; Von Känel et al., 2006).

Baseline measurement of differential white blood cell (WBC) count, free cortisol, ACTH, catecholamine and IL-6 levels were performed by taking blood and saliva samples 30, 20, 10 and 1 min before PPT measurement. Participants underwent the algesimetric procedure for a duration of 45 min, starting at 1700 h. Post-algesimetric measurements of free cortisol, ACTH, catecholamines and IL-6 levels were obtained by collecting saliva and blood samples 1, 10, 20, 30, 90, 150, 195, 240, 285, 330 and 375 min after PPT measurement, ending at 2400 h.

Additionally, at all pre- and postalgesimetric time points, participants rated their pain and fatigue intensities by using a 100 mm visual analogue scale (VAS) for pain (VAS-P) and fatigue (VAS-F), respectively. The subjects also measured their core body temperature (CBT) at four time points, beginning at 3 p.m. and thereafter in time intervals of 3 h. Four hours after conclusion of PPT measurement, all participants received a standard meal. On day 3, two blood samples were collected at 1100 h to determine the glucocorticoid (GC) sensitivity of inflammatory cytokine production by circulating monocytes. In the night of day 3 at 2200 h, participants ingested 0.5 mg dexamethasone (DEX; Jenapharm, Jena, Germany) and collected on the day thereafter five saliva samples during the first hour after awakening at 0700 h and additional seven saliva samples in intervals of 2 h (Yehuda et al., 1993).

2.3. Psychological measurements

For assessment of the severity of fatigue symptoms a validated German version of the Multidimensional Fatigue Inventory (MFI) (Gaab et al., 2002a) has been employed. As in the English version, the MFI consists of five scales, each with four items, which address different manifestations of fatigue, including General, Physical and Mental Fatigue. The participants were asked to assess on a seven-point rating scale how frequently they experienced specific fatigue symptoms during previous days.

For assessment of chronic stress levels the validated German version of the Trier Inventory for the Assessment of Chronic Stress (TICS) (Schulz and Schlotz, 1999) has been used. The 39-item TICS consists of six scales which address different origins of subjective stress levels, including work overload, worries, social stress, lack of recognition, work discontent, and intrusive memories. The participants were asked to judge on a five-point rating scale how frequently they experienced specific stress situations during the year preceding the onset of their disease.

Presence of major depressive disorder (MDD) was screened by using the German version of the Center for Epidemiological Studies Depression Scale (CES-D) and the German version of the Patient Health Questionnaire (PHQ-D) in their validated forms (Hautzinger and Bailer, 1993; Löwe et al., 2002). The CES-D assesses the frequency of depressive symptoms during the previous week. Since somatic symptoms comprising depression are thought to be confounded with symptoms of chronic pain patients a cut off score of 19 has been recommended to identify MDD in chronic pain patients (Magni et al., 1994; Turk and Okifuji, 1994). The PHQ-D assesses the frequency of nine depressive symptoms during the previous two weeks and 13 physical symptoms during the previous month according to criteria of Diagnostic and Statistical Manual of Mental Disorders (DSM) (Spitzer et al., 1999).

A sum score greater than 15 provides a threshold diagnosis for MDD and subthreshold diagnosis for somatoform disorder (Kroenke et al., 2001, 2002).

Subjective pain experience was assessed with the validated German version of the McGill Pain Questionnaire (SES) (Geissner, 1995). This questionnaire allows the separate assessment of the sensory (MPQ-S) and affective (MPQ-A) qualities of pain, as well as providing a total pain score. Written instructions were slightly modified to ask patients specifically to describe their musculoskeletal pain.

2.4. Measurement of pressure pain thresholds (PPTs) at tender points

Pressure pain thresholds (PPTs) were determined bilaterally at four different tender points including suboccipital muscle insertions (occiput), the midpoint of the upper border (trapezius) origins, above the scapuala spine near the medial border (supraspinous) and in upper outer quadrants of buttocks in anterior fold of muscle (gluteal) by using a pressure algometer (Somedia Sales AB, Munich, Germany). Ascending series of pressure in a rate of 20 kilopascal (kPa)/s were applied to each muscle until the pressure was first perceived as painful. PPTs were determined twice at each tender point on the right and left side of the body in a randomized order. Application of each pressure series was separated by at least 20 s. The participants underwent the measurement of PPTs in a prone position. For each tender point PPTs were aggregated for the right and left side.

Participants were familiarized with the pressure algometry prior to examination by bilaterally measuring PPTs on the joint capsules of both hands and ankles. This pre-trial assessment was conducted with participants being in a sitting position.

2.5. Biochemical measures

Separate EDTA blood samples were drawn for determination of IL-6, norepinephrine (NE), epinephrine (EPI) and ACTH levels (Sarstedt, Nümbrecht, Germany). In order to prevent IL-6 from being metabolized, blood was drawn into prechilled tubes and centrifuged at $1000 \times g$ for 10 min. Blood samples for determination of catecholamines and ACTH were centrifuged at 3000 rpm for 10 min. Plasma from all samples was aliquoted and stored at $-80\,^{\circ}C$ until later analysis.

Catecholamine levels were determined by high-performance liquid chromatography with electrochemical detection (Chromsystems, Germany; Smedes et al., 1982), and a commercial chemiluminescence assay (Nichols Institute, San Juan Capistrano, USA) was used to detect ACTH levels. Plasma IL-6 levels were determined by a high sensitivity quantitative sandwich enzyme immunoassay (ELISA, R&D Systems, Minneapolis, MN, USA). The lowest limit of detection was 0.156 pg/ml. The intra- and interassay coefficient of variation (CVs) of a concentration at 1.99 pg/ml was 0.15% and 5%, respectively. Saliva samples for cortisol measurement were collected using saliva collection device (Salivette, Sarstedt, Nürnbrecht, Germany) and stored at $-20\,^{\circ}C$ until analysis. Free cortisol in saliva was measured by a time resolved fluorescence immunoassay as described in detail previously (Dressendörfer et al., 1992).

Glucocorticoid sensitivity of inflammatory cytokine production by circulating monocytes was measured as described earlier (DeRijk et al., 1996). In brief, 5 ml whole blood was diluted 10:1 with 0.9% saline, 400 μl of diluted whole blood was coincubated with 50 μl of lipopolysaccharide (LPS) (*E. coli*, DifcoSigma, Augsburg, Germany) and 50 μl of dexamethasone (DEX) in different concentrations (Sigma, Deisenhofen, Munich, Germany) on a 24-well-plate (Greiner, Nuertingen, Germany) for 6 h at 37 °C in a humidified incubator containing 5% CO_2. The final concentrations were 30 ng/ml LPS, and 0, 10^{-10}, 10^{-9}, 10^{-8}, 5×10^{-8}, 10^{-7} and 10^{-6} M DEX. After centrifugation for 10 min at 4 °C and $2000 \times g$ the supernatants were aliquoted and stored at −80 °C until analysis. Supernatant concentrations of tumor necrosis factor-α (TNF-α) and IL-6 were determined by employing a commercial ELISA (BD Pharmingen, San Diego, CA, USA). The detection limits of the ELISAs were 9.4 and 15.5 pg/ml for IL-6 and TNF-α, respectively. The intra- and interassay CVs were below 10%.

2.6. Statistical analysis

Cortisol awakening responses (CAR) were assessed by computing two indices: The total cortisol secretion within 1 hr postawakening was quantified using the area under the curve (AUC) with respect to ground (AUC_{G1}), and the increase of cortisol after awakening was quantified using the AUC with respect to the baseline (AUC_I) (Pruessner et al., 2003). Daytime cortisol secretion was estimated by computing the AUC with respect to ground for cortisol levels between 1000 h and 2200 h (AUC_{G2}; Wüst et al., 2000). Reactivity of IL-6 levels and changes in pain and fatigue ratings after PPT measurement were quantified by computing the AUC with respect to ground between baseline and 375 min post-testing (Pruessner et al., 2003).

Based on previous findings, feedback sensitivity of the HPA axis was assessed by quantifying the extent and duration of cortisol suppression after overnight dexamethasone suppression test (DST) (Yehuda et al., 1991, 1993).

An enhanced HPA axis feedback sensitivity is characterized by post-dexamethasone (post-DEX) free cortisol levels which are significantly reduced in comparison with controls and below 2 nmol/L during the first hour after awakening and time interval ranging from 1000 h to 2200 h (Gaab et al., 2002b; Geiss et al., 2005). A reduced feedback sensitivity has been characterized by post-DEX levels which are significantly elevated in comparison with controls and above 5 nmol/L during the first hour after awakening and time interval ranging from 1000 h to 2200 h (Lieb et al., 2004). To quantify the extent and duration of cortisol suppression areas under post-DEX cortisol values with respect to ground were calculated for the first hour after awakening (AUC_{D1}), and between 1000 h and 2200 h (AUC_{D2}).

Glucocorticoid sensitivity (GC) of circulating monocytes was assessed by determining the dosage of DEX required to induce a 50% inhibition of LPS-induced TNF-α and IL-6 production (IC_{50}; Geiss et al., 2005).

The time course of free cortisol, ACTH, catecholamines and IL-6 levels was analyzed by performing two-way analyses of variance (ANOVA) for repeated measurements with the between subjects variable "group" and the within-subjects

variable "time". For analyzing DEX inhibition of LPS-induced TNF-α and IL-6 production, two-way analyses of variance (ANOVA) for repeated measurements with the variables "group" and "dose" were computed. Degrees of freedom were adjusted where appropriate employing the Greenhouse-Geisser approach to correct for violation of the sphericity assumption (Hays, 1981). Subsequent univariate analyses (ANOVAs) were employed to ascertain which subgroup, dose or time point accounts for the revealed significant main effect. Comparisons between groups in demographic characteristics and psychometric variables were conducted by performing multiple Student's *t*-tests. A Bonferroni correction of the error probability was performed taking into account the multiple comparisons (Hays, 1981). Since the aim of the present study was to test whether measurement of PPTs at tender points is a suitable method to induce enhanced IL-6 levels in FMS patients and not to analyze the change process of IL-6 levels after PPT measurement we did not take advantage of more potent analytical approaches such as multilevel modeling. Statistical significance was determined at an alpha level of 0.05. For relating psychological parameters to IC_{50} and total-time integrated in vivo IL-6 response to PPT measurement, Pearson correlation coefficients and partial correlation coefficients were computed. Effect sizes (ES) were additionally computed. While product moment (r) was computed for between-subject comparisons partial eta squared (η^2) was calculated for within-subject comparisons (Cohen, 1987).

3. Results

3.1. Demographic and psychometric variables

As reported in Table 2, FMS patients did not differ from controls on most demographic characteristics, with the exception that the majority of FMS patients did take anti-inflammatory medication including Devil's claw extract and nonsteroidal anti-inflammatory drugs such as diclofenac until four weeks before examinations. FMS patients scored higher on the TICS subscales "lack of recognition" [$F(1,24) = 6.512$, $p < 0.018$] and "work discontent" [$F(1,24) = 4.826$, $p < 0.038$]. Furthermore, FMS patients did score significantly higher on all MFI subscales (all Ps < 0.05), and the German version of McGill Pain Questionnaire (SES) indicating that they experienced the sensory [$F(1,23) = 14.125$, $p < 0.001$] and affective [$F(1, 23) = 92.576$, $p < 0.000$] qualities of pain more intensely. FMS patients reported a significantly higher frequency of depressive symptoms than controls [$F(1, 22) = 5.938$,

Table 2 Demographic and psychometric characteristics of patients with fibromyalgia syndrome (FMS) and controls.

	Patients with FMS	Controls
Demographic characteristics		
Age (years)	50 ± 2.07	41 ± 2.98
Time since diagnosis of fibromyalgia (in months)	143.70 ± 33.925	
Body mass index (means ± SE)	26.30 ± 0.363	26.47 ± 1.01
Anti-inflammatory medication and/or hormone replacement therapy (%, *n*)	75 (9)	7 (1)
No medication (%, *n*)	25 (3)	93 (14)
Menopause (%, *n*)	25 (3)	13 (2)
Psychometric characteristics		
Trier Inventory for the Assessment of Chronic Stress (TICS)		
Work overload	24.250 ± 1.919	18.785 ± 2.425
Worries	16.7500 ± 1.349	14.857 ± 0.740
Social stress	15.0000 ± 0.8528	15.500 ± 0.521
Lack of recognition	20.500 ± 1.0624**	17.071 ± 0.848
Work discontent	14.250 ± 0.970*	11.928 ± 0.518
Intrusive memories	16.000 ± 1.193	14.571 ± 0.992
German version of the McGill Pain Questionnaire (SES)		
Sensory qualities of pain	26.90 ± 3.128**	16.60 ± 0.86
Affective qualities of pain	42.20 ± 3.268**	15.47 ± 0.70
German version of Multidimensional Fatigue Inventory (MFI)		
General fatigue	14.800 ± 1.331**	6.500 ± 0.817
Physical fatigue	13.800 ± 1.459**	7.357 ± 0.9978
Mental fatigue	13.600 ± 1.641**	5.714 ± 0.8012
Reduced activation	13.500 ± 1.4624**	5.642 ± 0.874
Reduced motivation	12.100 ± 1.754**	7.142 ± 0.776
German version of Center for Epidemiologic Studies Depression Scale (CES)	24.10 ± 3.99*	14.57 ± 1.695
German version of Patient Health Questionnaire (PHQ)		
Threshold diagnosis of major depressive disorder (%, *n*)	17 (2)	
Subthreshold diagnosis of somatoform disorder (%, *n*)	17 (2)	

Data are represented as mean ± SEM.

* Indicates significant differences between FMS patients and controls ($p < 0.05$).

** Indicates significant differences between FMS patients and controls ($p < 0.01$).

$p < 0.023$] and two FMS patients did conform with criteria for a threshold diagnosis of a moderately severe MDD.

3.2. Baseline measurements

3.2.1. Pressure pain thresholds (PPTs) at tender points

FMS patients had significantly lower PPTs at all examined tender points, including suboccipital muscle insertions (occiput) [FMS patients: 219.56 kPa ± 47.08; Controls: 433.19 kPa ± 15.63], the midpoint of the upper border (trapezius) origins [FMS patients: 249.25 kPa ± 55.26; Controls: 431.72 kPa ± 7.11], above the scapuala spine near the medial border (supraspinous) [FMS patients: 255.75 kPa ± 49.38; Controls: 446.28 kPa ± 10.32] and in upper outer quadrants of buttocks in anterior fold of muscle (gluteal) [FMS patients: 258.87 kPa ± 46.96; Controls: 437.35 kPa ± 11.33] (all *P*s < 0.001).

3.2.2. Differential white blood cell (WBC) count

FMS patients did differ from controls on subsets of white blood cells, showing significantly higher leukocyte [FMS patients: 7.18×10^6 cells/ml ± 0.75; Controls: 5.54×10^6 cells/ml ± 0.37; $F(1, 21) = 4.79$, $p < 0.04$], neutrophil [FMS patients: 4.29×10^6 cells/ml ± 0.65; Controls: 2.81×10^6 cells/ml ± 0.36; $F(1, 21) = 4.67$, $p < 0.04$] and monocyte counts [FMS patients: 0.55×10^6 cells/ml ± 0.07; Controls: 0.37×10^6 cells/ml ± 0.01; $F(1, 21) = 10.47$, $p < 0.004$].

3.3. Assessment of HPA axis function

3.3.1. Cortisol levels in response to awakening and during the day

Fig. 1 displays free cortisol levels during the first hour after awakening and the interval ranging from 1000 h to 2200 h. ANOVA for repeated measures revealed a significant main effect of time [$F(2.04, 51.13) = 9.66$, $p < 0.001$, $\eta^2 = 0.28$], but no significant group by time interaction [$F(2.05, 51.14) = 0.85$, $p < 0.44$, $\eta^2 = 0.03$], thereby indicating that FMS patients and controls did exhibit a similar cortisol response during the first hour after awakening.

In further support of this notion FMS patients did not differ significantly from controls in their cortisol secretion during the first hour after awakening (AUC_{G1}), and adrenocortical response to awakening (AUC_I) [$F(1, 25) = 2.72$, $p < 0.11$, $r = 0.07$; $F(1, 25) = 1.331$, $p < 0.26$, $r = 0.04$, respectively].

Comparison between FMS patients and controls regarding their cortisol levels during the day revealed neither a significant group by time interaction [$F(4.14, 91.05) = 1.53$, $p < 0.20$, $\eta^2 = 0.07$] nor main effect of group [$F(1, 22) = 0.06$, $p < 0.81$, $\eta^2 = 0.003$] indicating similarity of FMS patients and controls in their cortisol levels throughout the day. Furthermore, there was no significant difference between FMS patients and controls in their AUC of cortisol levels between 1000 h and 2200 h (AUC_{G2}) [$F(1, 22) = 0.12$, $p < 0.72$].

3.3.2. Feedback sensitivity of the pituitary—adrenal axis

Fig. 2 displays the post-DEX cortisol values during the first hour after awakening and the interval ranging from 1000 h to 2200 h. Statistical analysis of the post-DEX cortisol values during the first hour after awakening revealed a significant

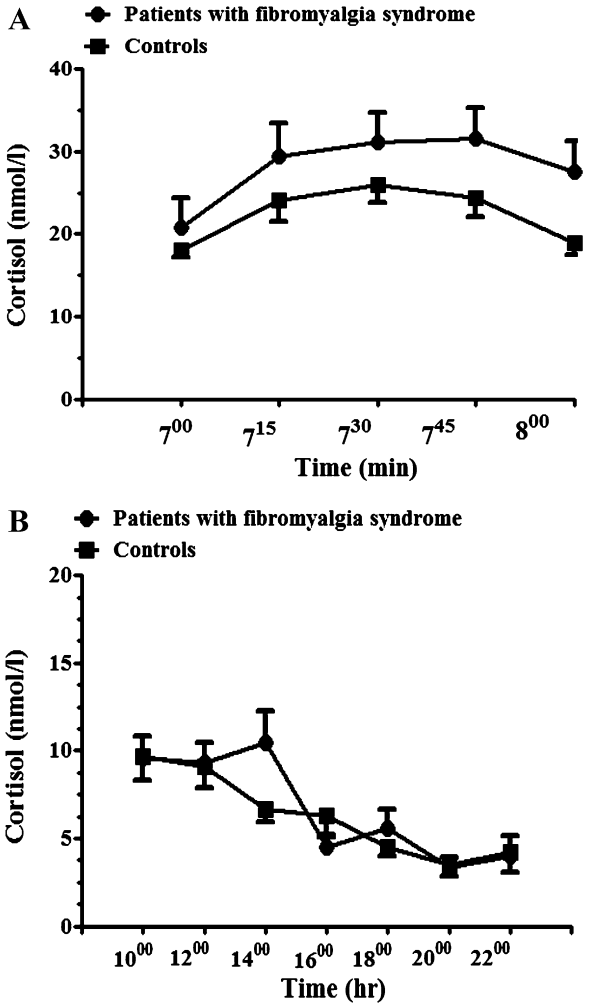

Figure 1 Comparison of the groups regarding free cortisol levels (A) during the first hour after awakening and (B) interval ranging from 1000 h to 2200 h. Data are expressed as means ± S.E.M.

group by time interaction [$F(1.76, 44.00) = 3.60$, $p < .04$, $\eta^2 = 0.13$]. *Post hoc* analysis ascertained FMS patients as displaying significantly higher post DEX cortisol values 45 min [$F(1,25) = 6.59$, $p < 0.01$] and 60 min [$F(1, 25) = 3.94$, $p < 0.05$] postawakening. There was a statistical trend for a higher post-DEX cortisol secretion within 1 hr postawakening (AUC_{D1}) in FMS patients as compared with controls [$F(1, 25) = 3.21$, $p < 0.09$, $r = 0.10$].

Again, comparing post-DEX cortisol values between 1000 h and 2200 h revealed a significant group by time interaction [$F(1.45, 33.42) = 4.63$, $p < 0.03$, $\eta^2 = 0.17$]. *Post hoc* univariate ANOVAs confirmed that FMS patients had similar post-DEX values as controls between 1000 h and 2000 h, but significantly higher post-DEX values at 2200 h [$F(1,24) = 7.41$, $p < 0.01$]. FMS patients did display similar AUC_{D2} [$F(1, 23) = 0.001$, $p < 0.98$, $r = 0.00$] as controls.

3.3.3. Glucocorticoid (GC) sensitivity of inflammatory cytokine production by circulating monocytes

As depicted in Fig. 3A, significantly higher concentration of DEX was necessary to induce a 50% inhibition of FMS patients' LPS-induced IL-6 production in comparison with controls

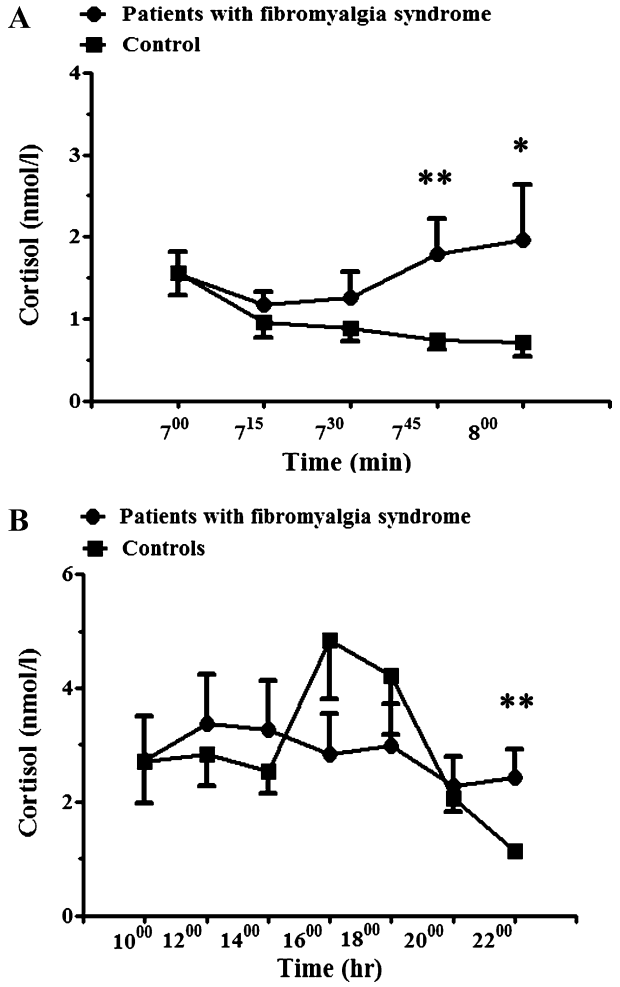

Figure 2 Cortisol suppression after ingestion of 0.5 mg dexamethasone (A) during the first hour after awakening and (B) interval ranging from 1000 h to 2200 h. * Indicates significant differences between FMS patients and healthy controls ($p < 0.05$). ** Indicates significant differences between FMS patients and healthy controls ($p < 0.01$). Data are expressed as means ± S.E.M.

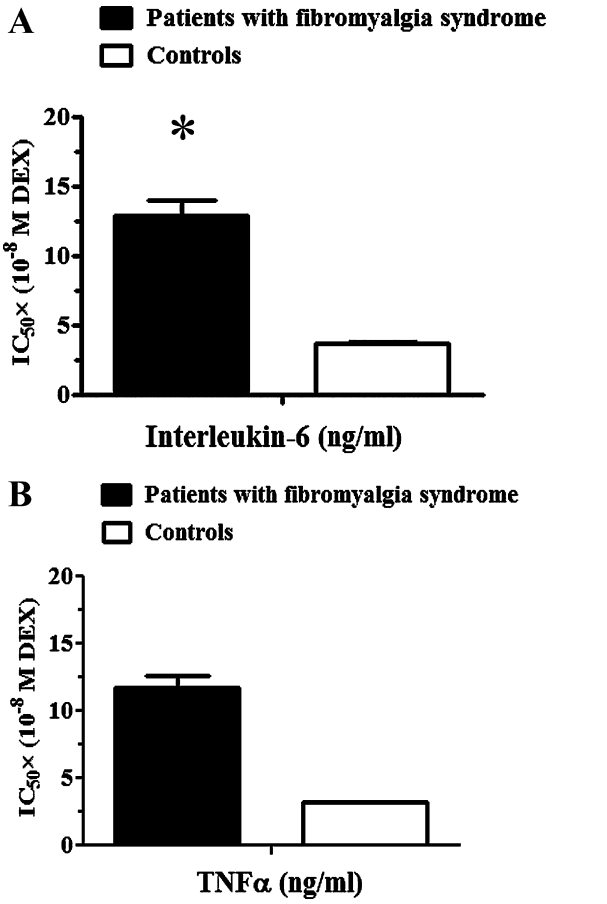

Figure 3 Differences between FMS patients and controls in dosages of dexamethasone being required to induce a 50% inhibition of the LPS-induced (A) IL-6 production and (B) TNFα-production in peripheral blood monocytes (IC_{50}). The IC_{50} is inversely related to the sensitivity of monocytes to the immunosuppressive effects of cortisol. A higher IC_{50} indicates a lower sensitivity of monocytes to the immunosuppressive effects of cortisol. * Indicates significant difference between FMS patients and controls ($p < 0.05$). Data are expressed as means ± SEM.

[$F(1, 20) = 4.38$, $p < 0.05$, $r = 0.18$]. For the DEX dose being required for a 50% inhibition of the LPS-induced TNFα production the difference between FMS patients and controls in their IC_{50} failed to reach significance [$F(1,20) = 2.13$, $p < 0.16$, $r = 0.09$].

3.3.4. Bivariate associations of psychological variables with glucocorticoid sensitivity

In all participants, dosage of DEX which is required to induce a 50% inhibition of LPS-induced TNF-α and IL-6 production was significantly correlated with subscales of the MFI, including "General Fatigue [$r = 0.475$, $p < 0.01$ and $r = 0.533$, $p < 0.008$, respectively], "Mental Fatigue" [$r = 0.375$, $p < 0.05$ and $r = 0.531$, $p < 0.008$, respectively], "Reduced motivation" [$r = 0.407$, $p < 0.03$ and $r = 0.57$, $p < 0.004$, respectively] and "Reduced Activation" [$r = 0.405$, $p < 0.03$ and $r = 0.375$, $p < 0.05$, respectively]. Whereas MFI subscale "Physical Fatigue" was significantly correlated with IC_{50} for LPS-induced TNFα production [$r = 0.361$, $p < 0.05$] the correlation with the DEX dose being required to induce a 50% inhibition of the LPS-induced IL-6 production failed to reach significance [$r = 0.283$, $p < 0.11$]. With the exception of the correlation between MFI subscale "General Fatigue" and required DEX dose to induce a 50% inhibition of LPS-induced IL-6 production the correlations between MFI subscales and required DEX dose to induce a 50% inhibition of LPS-induced TNFα and IL-6 production did disappear after controlling for the effect of total-time integrated in vivo IL-6 response to PPT measurement [all Ps > 0.07].

3.4. Behavioral, endocrine and interleukin-6 responses to pressure pain threshold (PPT) measurement

3.4.1. Fatigue and pain response to PPT measurement

The time courses of fatigue and pain ratings before and after PPT measurement are depicted in Fig. 4. Since FMS patients

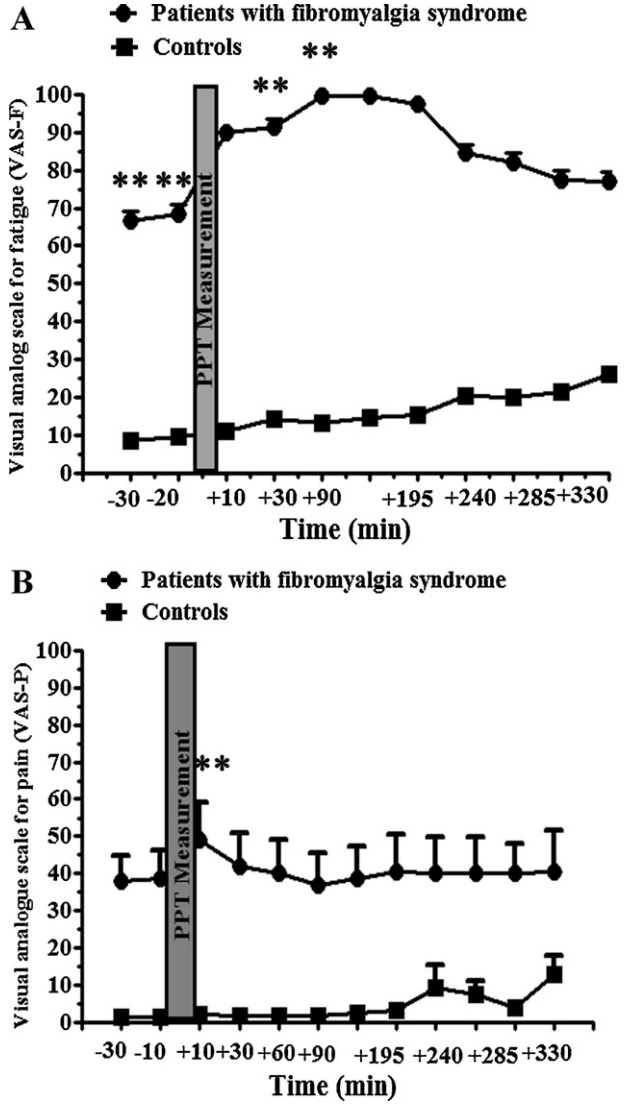

Figure 4 Changes in the severity of (A) fatigue and (B) pain ratings after conclusion of pressure pain threshold (PPT) measurement. Subjects scored their fatigue and pain intensity on a visual analogue scale for fatigue (VAS-F) and visual analogue scale for pain (VAS-P), respectively. ** Indicates significant differences between FMS patients and healthy controls ($p < 0.01$). Data are expressed as means ± S.E.M.

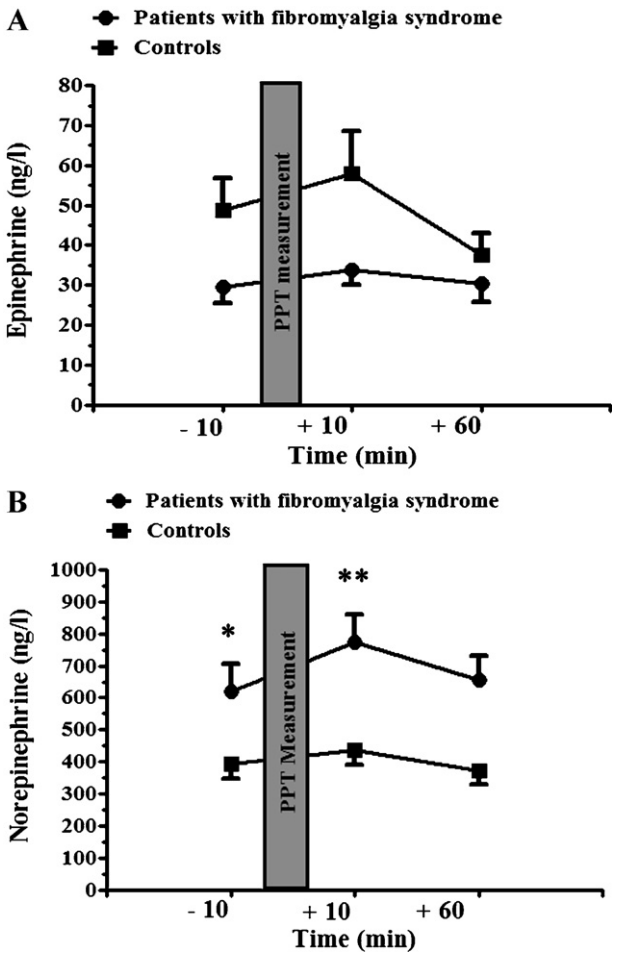

Figure 5 Changes in plasma (A) epinephrine (EPI) and (B) norepinephrine (NE) levels after conclusion of pressure pain threshold (PPT) measurement. * Indicates significant differences between FMS patients and healthy controls ($p < 0.05$). ** Indicates significant differences between FMS patients and healthy controls ($p < 0.01$). Data are expressed as means ± S.E.M.

reported significantly higher fatigue scores 30 min [$F(1, 23) = 779.64$, $p < 0.0001$] and 20 min [$F(1, 23) = 781.23$, $p < 0.0001$] before PPT measurement the averaged fatigue scores were considered as a covariate in the subsequent analysis. ANOVA for repeated measures revealed a significant interaction between group and time [$F(3, 69.07) = 89.96$, $p < 0.0001$, $\eta^2 = 0.80$] (Fig. 4A). *Post hoc* analysis showed that pressure application on tender points did aggravate sleepiness in FMS patients as their VAS-F scores increased significantly 60 min [$F(1,23) = 1726,74$, $p < 0.0001$] and 90 min post-testing [$F(1,23) = 22417.72$, $p < 0.001$].

For changes in pain ratings after PPT measurement, there *was a statistical trend for group by time interaction* [$F(2.19, 43.8) = 2.7$, $p < 0.08$, $\eta^2 = 0.12$] after controlling for FMS patients' significantly higher pain scores under pre-algesimetric conditions [$F(1, 23) = 39.01$, $p < 0.001$]. Univariate analyses revealed that VAS-P scores of FMS patients did increase by 10 units on average within 10 min after pain measurement [$F(1, 20) = 43.05$, $p < 0.0001$], thereby revealing that PPT measurement exacerbated sleepiness and pain intensity in FMS patients.

3.4.2. Catecholamine response to PPT measurement

Fig. 5 shows epinephrine (EPI) and norepinephrine (NE) levels before and after PPT measurement. For epinephrine levels, ANOVA for repeated measures indicated a statistical trend for group by time interaction [$F(2,44) = 2.89$, $p < 0.07$, $\eta^2 = 0.12$]. Univariate analyses confirmed a statistical trend for higher epinephrine levels 30 min pre-testing [$F(1, 22) = 3.69$, $p < 0.07$] in controls and identified significant lower epinephrine levels 60 min post-testing in this subgroup [$F(2,44) = 5.55$, $p < 0.007$] (Fig. 5A). For norepinephrine levels, ANOVA for repeated measures showed a significant main effect of group [$F(1,22) = 11.3$, $p < 0.003$, $\eta^2 = 0.34$], but group by time interaction failed to reach significance

[$F(2,44) = 2.22$, $p < 0.12$, $\eta^2 = 0.09$] (Fig. 5B). *Post hoc* analyses revealed that the significant main effect is due to FMS patients' significantly elevated norepinephrine levels 10 min before [$F(1,22) = 6.04$, $p < 0.02$] and 10 min after PPT measurement [$F(1,22) = 13.08$, $p < 0.002$], thereby pointing to their higher sympathoadrenal activity.

3.4.3. ACTH response to PPT measurement

Compared with controls, FMS patients exhibited significantly lower ACTH levels 30 and 10 min before PPT measurement ($F(1,21) = 7.24$, $p < 0.02$; $F(1, 21) = 5.69$, $p < 0.03$, respectively) (Fig. 6A). In order to control for this group difference, the average of the baseline ACTH levels was added to the repeated measures ANOVA as a covariate. A significant interaction between group and time emerged for ACTH levels ($F(1.78, 37.37) = 7.4$, $p < 0.003$, $\eta^2 = 0.26$) after controlling for baseline ACTH levels. While controls' ACTH levels declined from pre- to 10 min post-testing [$F(1,21) = 3.71$, $p < 0.07$], FMS patients' ACTH levels did not change during the observation period.

3.4.4. Cortisol response to PPT measurement

Similar to ACTH, FMS patients exhibited significantly lower cortisol levels 30 min ($F(1,23) = 4.50$, $p < 0.04$) before PPT measurement (Fig. 6B). ANOVA for repeated measures revealed a significant group by time interaction after

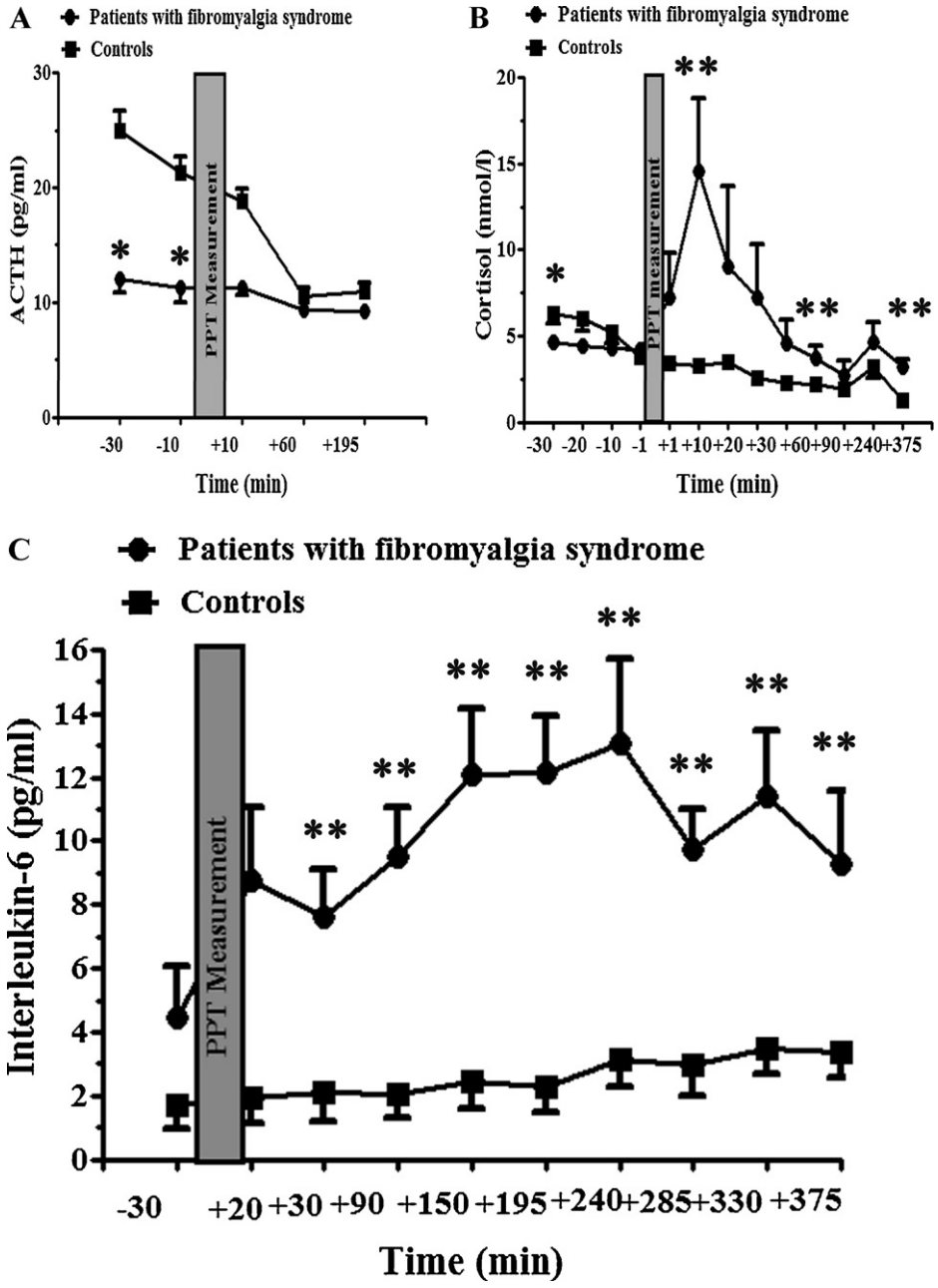

Figure 6 Changes in plasma (A) ACTH levels, (B) free cortisol levels and (C) IL-6 levels after conclusion of pressure pain threshold (PPT) measurement. * Indicates significant differences between FMS patients and healthy controls ($p < 0.05$). ** Indicates significant differences between FMS patients and healthy controls ($p < 0.01$). Data are expressed as means ± S.E.M.

controlling for differences in pre-algesimetric cortisol values [$F(2.09, 46.02) = 4.9$, $p < 0.011$, $\eta^2 = 0.18$]. *Post hoc* analyses revealed that FMS patients had significantly higher cortisol levels after PPT measurement for 10 min [$F(1,23) = 11.04$, $p < 0.003$], 90 min [$F(1,23) = 7.01$, $p < 0.01$] and 375 min [$F(1,23) = 27.22$, $p < 0.0001$]. From baseline levels, cortisol increased approximately 300% at 10-min post-testing, then returned below baseline levels and increased again at 240-min post-testing, thereby indicating that PPT measurement induced considerable adrenocortical activation in FMS patients.

3.4.5. Interleukin-6 response to PPT measurement

Statistical comparison of pre-algesimetric with post-algesimetric IL-6 levels revealed a significant group by time interaction [$F(3.23, 61.35) = 4.77$, $p < 0.004$, $\eta^2 = 0.20$] (Fig. 6C). While FMS patients did not differ from controls in IL-6 levels before PPT measurement [$F(1, 21) = 2.71$, $p < 0.11$] they exhibited significantly enhanced IL-6 levels during the whole post-testing session including 30 min [$F(1,21) = 10.5$, $p < 0.004$], 90 min [$F(1,20) = 22.22$, $p < 0.0001$], 150 min [$F(1, 21) = 22.53$, $p < 0.0001$], 195 min [$F(1,21) = 28.57$, $p < 0.0001$], 240 min [$F(1,21) = 15.62$, $p < 0.001$], 285 min [$F(1,21) = 18.51$, $p < 0.001$], 330 min [$F(1, 21) = 15.03$, $p < 0.001$] and 375 min [$F(1,20) = 7.81$, $p < 0.011$] post-testing. In terms of FMS patients' IL-6 increase, their IL-6 levels peaked at approximately 300% above baseline at 240-min post-testing and did not return to baseline levels at the end of post-testing session, thereby revealing their enhanced IL-6 reactivity in response to PPT measurement. In further support of FMS patients' enhanced IL-6 reactivity after PPT measurement they exhibited a significantly higher total-time integrated IL-6 response as compared with controls [$F(1, 19) = 16.26$, $p < 0.001$, $r = 0.42$]. IL-6 AUC was significantly associated with fatigue ($r = 0.77$, $p < 0.0001$) and pain ratings ($r = 0.60$, $p < 0.001$) AUC. There was a statistical trend for a positive correlation between *in vitro* GC sensitivity of LPS-induced IL-6, but not TNFα production with total-time integrated in vivo IL-6 response to PPT measurement [($r = 0.34$, $p < 0.09$) and ($r = 0.21$, $p < 0.21$), respectively].

4. Discussion

This study was conducted to examine whether FMS patients' reduced cortisol levels are associated with an exacerbation of pain and fatigue ratings, which may be mediated by an increased IL-6 reactivity after PPT measurement. This is to the best of our knowledge the first study to test for endocrine and immune characteristics in FMS patients that are in agreement with the hypothesized pathophysiological relevance of a hypocortisolemic pattern for manifestation of FMS core symptoms. In summary, FMS patients' awakening response, daytime profile of cortisol secretion and cortisol suppression after ingestion of a low dose DEX were found to be normal. In contrast, IL-6 production in circulating monocytes of FMS patients was found to be less sensitive to the immunosuppressive actions of glucocorticoids. While PPT measurement did not change ACTH levels in FMS patients, we found elevated norepinephrine levels, together with three times higher cortisol levels and four times higher IL-6 levels in FMS patients compared with controls. The enhanced IL-6 reactivity was accompanied by a reduced sensitivity of circulating monocytes to glucocorticoid inhibition of IL-6 production and by an aggravation of pain and fatigue ratings after PPT measurement.

Overall, the findings of the present study are in agreement with the hypothesis of a pathophysiological relevance of a disturbed GR signaling pathway, rather than reduced cortisol levels for the maintenance of FMS core symptoms fatigue and pain. The reduced sensitivity of circulating monocytes to the immunosuppressive effects of glucocorticoids may permit the maintenance of FMS core symptoms fatigue and pain by an insufficient inhibition of cytokine production.

The revealed similarity between FMS patients and controls in their cortisol awakening response does conflict with previous findings as FMS patients were found to exhibit an attenuated cortisol awakening response (Weissbecker et al., 2006; Klingmann et al., 2008). Lack of control for confounding variables of cortisol awakening response such as trauma history or gestational length may account for the inconsistency.

During the first hour after awakening FMS patients' post-DEX cortisol values were found to be below the cut-off value of 2 nmol/L, but significantly elevated in comparison with controls. During the remainder of the post-DEX study day, FMS patients did exhibit similar post-DEX cortisol values as controls. On average, FMS patients' post-DEX cortisol values were below 5 nmol/L during the time interval ranging from 1000 h to 2200 h. Since FMS patients' post-DEX cortisol values did not fulfill all criteria of either an enhanced or a reduced HPA axis feedback sensitivity the present findings do point to a normal HPA axis feedback sensitivity in this population. Differences between current and previous studies in DEX dosage, amount of post-DEX cortisol measurements and measured cortisol fraction may account for the inconsistent findings. While Ferraccioli et al. (1990) did use 1 mg DEX for assessing feedback sensitivity subjects in the present study did ingest 0.5 mg DEX. Both dosages have been reported to differ in their sensitivity to discriminate normal from altered feedback sensitivity as a dosage of 1 mg DEX causes a total and a dosage of 0.5 mg DEX induces a partial suppression of the adrenal cortisol secretion (Yehuda, 1997). Limitation to one post-DEX total cortisol measurement at 0800 h in Wingenfeld's study may influence DST results as post-DEX cortisol values have been shown to vary markedly 9 and 16 h following DEX ingestion (Lowy and Meltzer, 1987). DST results may be also influenced by limitation of the measured cortisol fraction to total cortisol levels because total cortisol levels are modulated by corticosteroid-binding globulin (CBG) levels (Rosner, 1990). Since FMS patients have been shown to exhibit reduced CBG levels, exaggerated cortisol suppression in Wingenfeld's study may reflect, in part, FMS patient's lower CBG levels (Lentjes et al., 1997).

In the current study a significantly higher DEX dose was found to be required to induce a 50% inhibition of the LPS-induced IL-6 production in FMS patients. This finding is indicative of a reduced GC sensitivity of circulating monocytes in these patients. The reduced sensitivity of circulating monocytes to the immunosuppressive effects of glucocorticoids is accompanied by a higher frequency of experienced fatigue symptoms, thereby suggesting the pathophysiologic relevance of reduced GC sensitivity of circulating monocytes for FMS core symptoms.

A reduced GC sensitivity of circulating monocytes has been related to a disturbance within the transduction process of the cortisol signal into cytokine inhibition. Interference of the hormone-activated GR with nuclear factor (NF)-κB

signaling pathway by means of an increased production of glucocorticoid inducible leucine zipper (GILZ) in monocytes has been identified as an important step within this transduction process (Clark, 2007; Eddleston et al., 2007; De Bosscher and Haegeman, 2009). PBMCs of FMS patients have recently been found to contain significantly lower levels of GILZ in comparison with controls (Macedo et al., 2008). This raises the possibility that FMS patients' higher IC_{50} for LPS-induced IL-6 production does partly reflect a reduced expression of GILZ and hence abnormal NF-κB activation in their circulating monocytes (Eddleston et al., 2007).

Exclusion of subjects having taken long-term antidepressant medication from participation and examination of mainly premenopausal women in the current study may provide an explanation for the inconsistency between present and findings by Wingenfeld et al. (2008) and Macedo et al. (2008) as participants in Macedo's study were allowed to participate after discontinuing medication for two weeks, and Wingenfeld et al. did examine mainly postmenopausal women. There is *in vitro* evidence suggesting that transduction of cortisol signal into cytokine inhibition may be decelerated by reduced estradiol availability during menopause as fewer estrogen receptors (ER) are occupied by this hormone (Liu et al., 2005). Long-term antidepressant medication may accelerate the transduction of cortisol signal into cytokine inhibition because incubation of whole blood samples for 24 h with amitriptyline has been demonstrated to induce an upregulation of GR expression (Vedder et al., 1999).

The revealed reduced GC sensitivity of circulating monocytes in FMS patients is indicative of a hypercortisolemic pattern in this population. In conflicting with a hypercortisolemic pattern FMS patients did exhibit a normal daytime cortisol profile and HPA axis feedback sensitivity. FMS patients were, on average, above the threshold for a diagnosis of MDD on the CES-D. The PHQ-D identified two FMS patients as conforming with criteria for a threshold diagnosis of MDD indicative of MDD presence in a subgroup of FMS patients. In line with this finding, subgroups and not the general population of FMS patients have been reported previously to experience significant depressive symptomatology (Ahles et al., 1987; Ferraccioli et al., 1990). Although threshold diagnosis of MDD in FMS patients needs to be confirmed by structured interviews it seems unlikely that a depressive-like hypercortisolemia may account for altered HPA axis function in FMS patients.

It might be argued that exclusion of FMS patients having taken antidepressant medication for a long time increases the risk of having a sub-group of patients which are not representative of FMS patients in general. Exclusion of FMS patients having taken antidepressant medication for a long time was necessary as long-term amitriptyline medication has been identified as a confounder of GC sensitivity of circulating monocytes in *in vitro* and our pilot studies (Okugawa et al., 1999; Vedder et al., 1999). The currently examined FMS patients are representative of FMS patients in general as they are comparable to previous populations on psychosocial variables. For instance, the mean age of the currently studied FMS patients is representative of the age group having a high prevalence for this disorder (Wolfe et al., 1995). Consistent with previous findings a minority of the currently examined FMS patients did experience significant depressive symptomatology (Ahles et al., 1987; Ferraccioli et al., 1990).

Whereas FMS patients did not differ from controls in their IL-6 levels before PPT measurement, their IL-6 levels increased approximately 300% above baseline after PPT measurement. The enhanced IL-6 reactivity in FMS patients after pressure application on their tender points provides the first line of evidence for elevated IL-6 levels after painful stimulation in this population.

Muscle cells may represent a cellular origin of enhanced IL-6 reactivity after pressure application on tender points in FMS patients as painful stimulation of muscles has been demonstrated to increase circulating IL-6 levels in humans and animals (Loram et al., 2007; Edwards et al., 2008). In more detail, muscle-derived IL-6 may elevate circulating IL-6 levels by the demonstrated potency of this cytokine to sensitize muscle nociceptors through induction of prostaglandin $(PG)E_2$ synthesis (Lahiri et al., 2001). Sensitized muscle nociceptors release the monocyte attractant neuropeptide substance P (SP) which has been shown *in vitro* to be able to increase circulating IL-6 levels by binding to a non-neurokinin SP receptor on monocytes (Kavelaars et al., 1994; Schratzberger et al., 1997; Mense, 2003).

Although FMS patient's epinephrine concentrations remained at low levels at all time points before and after PPT measurement their norepinephrine concentrations did increase significantly from 10 min pre-testing to 10 min post-testing. In line with present findings low epinephrine levels have been detected consistently in FMS patients after exposure to different types of stressors (Giske et al., 2008; Kadetoff and Kosek, 2010).

High norepinephrine along with low epinephrine levels are indicative of a reduced cortisol production in the adrenal cortex as decreased cortisol production gives rise to a lower norepinephrine to epinephrine conversion in the adrenal medulla (Wurtman and Axelrod, 1966). A stress or inflammation-related dissociation between the hypothalamic—pituitary-system and the adrenal cortex has been related to an adrenal insufficiency (Nussdorfer, 1996; Ehrhardt-Bornstein et al., 1998).

Elevated cortisol levels after PPT measurement which were not preceded by an ACTH increase in FMS patients are indicative of a dissociation between the hypothalamic—pituitary-system and adrenal cortex. Norepinephrine and IL-6 have been identified as ACTH-independent stimuli which are able to compensate for a diminished potency of ACTH to induce adrenal steroid genesis (Bornstein and Chrousos, 1999; Bornstein et al., 2008). Their involvement in the induction of FMS patient's post-algesimetric adrenocortical activation is suggested by coincidence of elevated norepinephrine levels with first cortisol peak 10 min and increased IL-6 levels with second cortisol peak 240 min after PPT measurement in FMS patients. There is further evidence implying the involvement of norepinephrine and IL-6 in the induction of an ACTH-independent adrenocortical activation. For instance, Alzheimer Disease patients were found to exhibit an early cortisol response following painful stimulation which was not preceded by elevated ACTH levels, but accompanied by increased norepinephrine levels (Pascualy et al., 2000). Furthermore, healthy participants have been reported to display an early cortisol response, but delayed IL-6 response after painful stimulation (Edwards et al., 2008).

As compared with FMS patients controls did exhibit significantly elevated ACTH levels 30 min before PPT measurement indicative of an anticipation effect. ACTH increase 30 min pre-testing was not accompanied by a pre-algesimetric cortisol

response in controls. Increased cortisol levels have been shown to be dependent on preceding elevation of ACTH levels (Kirschbaum et al., 1993). Decline in control's ACTH levels from 30 min to 10 min pre-testing may account for the lack in their pre-algesimetric cortisol response.

Several limitations of our study should be noted. First, the study is limited by a small sample size. Second, we did not measure total cortisol levels and DEX levels following late-night administration of DEX. As FMS patients' circulating monocytes were found to be less sensitive to the immunosuppressive actions of DEX it is conceivable that their post-DEX cortisol values following DEX administration have been modulated by their reduced GC sensitivity (Lowy and Meltzer, 1987).

To summarize, the present findings do not support the hypothesis that FMS core symptoms pain and fatigue are promoted by reduced cortisol levels. Instead, they indicate that deficiencies in the GR signaling pathway in circulating monocytes may contribute to the maintenance of FMS core symptoms pain and fatigue by facilitating an enhanced IL-6 reactivity.

Conflict of interest

All authors declare that there are no conflicts of interest.

Acknowledgements

We especially thank Prof. Dr. Alexander L. Gerlach, Institute for Clinical Psychology and Psychotherapy, University of Cologne, for his advice in screening for presence of major depressive disorder (MDD) in patients with fibromyalgia syndrome (FMS). We extend our gratitude to Renate Engel and Nicole Reinert for the performance of the cytokine measurements. This research was supported in part by grants from the Center for Psychobiological and Psychosomatic Research, University of Trier and Centre de Recherche Public de la Sante, Luxembourg, Luxembourg.

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