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Sick and tired: psychological and physiological aspects of work-related stress

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4 Immune function and immune regulation in patients with work-related stress*

Abstract

Background: Prolonged stressor exposure can lead to changes in the physiological stress-system. We investigated whether work-related stress was associated with changes in immune functioning, Hypothalamic-Pituitary-Adrenal (HPA) axis functioning, and/or interrelations between these physiological stress-subsystems. *Method*: Sixty patients on sickness leave because of work-related stress and 43 healthy participants were compared on the immune measures immunoglobulin G (IgG) against Epstein-Barr virus (EBV) and C-reactive protein (CRP), and on the HPA axis indicators cortisol and dehydroepiandrosterone-sulphate (DHEAS). We assessed morning values and midday levels of cortisol and DHEAS. Neuroendocrine and immune measures were determined in saliva. *Results*: No between-group differences in immune and neuroendocrine measures were found. However, in patients, midday levels of DHEAS and the DHEAS awakening response were positively associated with anti EBV IgG levels, while no such associations were found in healthy individuals. In addition, in patients, no association was found between the cortisol awakening response (CAR) and the level of CRP, while in healthy individuals a positive association was observed between these measures. *Conclusion*: No evidence was obtained for changes in immune- or HPA axis-functioning in relation with work-related stress. However, a clinical level of work-related stress was characterised by altered immunomodulation by cortisol and DHEAS. The results suggested presence of glucocorticoid resistance in patients and involvement of DHEAS in the suppression of cellular immunity.

Work-related stress is a state that results from prolonged exposure to work-related stressors. Prolonged or chronic stress has been associated with various health complaints including common infections and rheumatoid arthritis (e.g., Davis et al., 2008; Mohren, et al., 2003; Heim, Ehlert & Hellhammer, 2000). Associations between chronic stress and these health complaints suggest the presence of immune dysregulation. Indeed, ample evidence supports the hypothesis for immune dysregulation in association with chronic stress (Brosschot et al., 1994; Kiecolt-Glaser, McGuire, Robles & Glaser, 2002; Segerström & Miller, 2004). For work-related stress, evidence for immune dysregulation is accumulating. For example, adverse psychosocial working conditions and burnout complaints have been demonstrated to be associated with immune changes (Bargellini et al., 2000; Grossi, Perski, Evengård, Blomkvist & Orth-Gomér, 2003; Lerman et al., 1999; Nakamura, Nagase, Yoshida & Ogino, 1999; Schnorpfeil et al., 2003; Toker, Shirom, Shapira, Berliner & Melamed, 2005).

The association between prolonged work-related stress and immune changes is expected since on the association between physiological stress systems and the immune system has been well established (Elenkov & Chrousos, 2006; Elenkov, Wilder, Chrousos & Vizi, 2000; Sapolsky, Romero & Munck, 2000; Uchino, Smith, Holt-Lunstad, Campo & Reblin, 2007). General stress theories, including the model of allostatic load (McEwen & Wingfield, 2003), state that prolonged exposure to stressors may result in changes in the physiological stress systems. These physiological stress systems consist of a) the sympathetic system, which includes the sympathetic-adrenal-medullary (SAM) axis, and b) the hypothalamic pituitary adreno-cortical (HPA) axis (McEwen & Wingfield, 2003; Sterling & Eyer, 1988). Prolonged exposure to stressors is considered to result in enhanced sympathetic and HPA axis activity, resulting in enhanced levels of catecholamines and cortisol (McEwen & Wingfield, 2003). Over time, a hyperactive sympathetic and HPA axis are hypothesised to undergo functional changes, resulting in normalised catecholamine levels (Julius, 1994) and reduced cortisol levels (Fries, Hesse, Hellhammer & Hellhammer, 2005). Thus, when exposed to prolonged stressors, the immune system is affected as a result of dysregulation of the sympathetic system and/or the HPA axis.

Generally, elevated levels of catecholamines and cortisol result in suppression of the inflammatory response and cellular immunity, and in enhancement of the anti-inflammatory response and humoral immunity (Elenkov et al., 2000; Elenkov & Chrousos, 2006; Segerström & Miller, 2004; Sapolsky et al., 2000). Conversely, a reduced level of cortisol is associated with enhanced inflammation and cellular immunity, and with reduced anti-inflammatory responses and humoral immunity (Elenkov et al., 2000; Elenkov & Chrousos, 2006; Sapolsky et al., 2000). Whether the effect of reduced cortisol on immune functioning is depending on catecholamine levels is not clear yet.

Several studies have documented results in accordance with immunosuppressive effects of cortisol and/or a shift towards more dominant humoral immunity in relatively healthy samples with work-related stress. For example, the burnout dimension Depersonalisation has been associated with lower cellular immunity, as indicated by reduced natural killer cell activity (Nakamura et al., 1999). Likewise, lower professional accomplishment was associated with lower cellular immunity, as reflected by lower numbers of total lymphocytes, T-cells, T-helper cells, and T-suppressor cells

(Bargellini et al., 2000). In addition, adverse psychosocial working conditions were associated with reduced cellular immunity and enhanced humoral immunity as indicated by a lower level of T-lymphocytes and T-helper lymphocytes, and a higher level of immunoglobulin G (IgG; Nakata et al., 2000).

Interestingly though, several findings suggest that burnout complaints in relatively healthy samples are associated with enhanced inflammatory activity, instead of the expected reduced inflammatory activity. For example, burnout complaints appeared to be associated with elevated leukocyte adhesiveness/aggregation (LAA; Lerman et al., 1999), elevated C-reactive protein (CRP; Toker, et al., 2005), or elevated tumor necrosis factor (TNF)-α (Grossi et al., 2003). Likewise, adverse psychosocial work-conditions were associated with elevated CRP (Schnorpfeil et al., 2003). Furthermore, among patients with coronary heart disease (CHD), vital exhaustion, a construct closely related to emotional exhaustion, was associated with elevated levels of the pro-inflammatory cytokines IL-1β and TNF-α (Appels, Bär, Bär, Bruggeman & de Baets, 2000). It has been proposed that this elevated inflammatory activity is due to mildly enhanced HPA axis activity (Schnorpfeil et al., 2003).

To date, only one study has investigated the immune function in patients with a clinical level of work-related stress (Mommersteeg et al., 2006a), as defined by presence of the burnout syndrome and impaired daily functioning. Mommersteeg et al. found enhanced anti-inflammatory activity as demonstrated by an increased production of interleukin-10 (IL-10), which supports a shift towards higher anti-inflammatory activity.

In the present study, HPA axis activity and the immune function were investigated in association with a clinical level of work-related stress by comparing patients with work-related stress with a healthy reference group. Furthermore, group differences in immune regulation by the HPA axis were investigated. Parameters of the immune function examined were IgG against Epstein-Barr virus (EBV) and C-reactive protein (CRP). A higher level of anti-EBV IgG is a sign of poorer cellular immunity (Cacioppo et al., 2002) and a higher level of CRP is a marker of cytokine-mediated inflammation (Black, 2002). Indicators of HPA axis activity were cortisol and dehydrepiandrosterone-sulphate (DHEAS). DHEAS, the sulphated form of the steroid dehydrepiandrosterone (DHEA), is a relatively novel measure in stress research. DHEA is also released by the adrenal cortex (Kroboth, Salek, Pittenger, Fabia & Frye, 1999). DHEA has been proposed to have immunopotentiating effects (Chen & Parker, 2004; Kroboth et al., 1999). Indeed DHEA appears to be related to activity of the immune function (Bauer, 2008; Ledochowski, Murr, Jäger & Fuchs, 2001). Although the exact function and target tissue of DHEA in the stress-response is not clear yet, it has been suggested that DHEA levels provide a good index of adrenal steroidogenic capacity (Hucklebridge, Hussain, Evans & Clow, 2005). Since cortisol and DHEA appear to have opposite effects on the immune function, DHEA has been proposed as a functional cortisol antagonist (Chen & Parker, 2004; Goodyer, Park, Netherton & Herbert, 2001; Hechter, Grossman, & Chatterton, 1997). Studying DHEA in addition to cortisol could thus enhance insight in the immunoregulatory influence of the HPA axis.

Evidence so far regarding DHEA or DHEAS in association with prolonged stress is inconsistent. DHEAS levels appeared to be similar in relatively healthy samples with high- and low levels of burnout complaints (Grossi et al., 2003; Langelaan, Bakker, Schaufeli, van Rhenen & van Doornen, 2006; Moch, Panz, Joffe, Havlik & Moch, 2003). Chronic stress, though, has been associated with a reduced level of DHEA (Vedhara et al., 2002), while elevated DHEAS was found in association with post-traumatic stress disorder (Spivak et al., 2000) and a clinical level of work-related stress (Mommersteeg et al., 2006a).

Based on our earlier findings in morning cortisol (de Vente, Olff, van Amsterdam, Kamphuis & Emmelkamp, 2003), we predicted an elevated morning level of cortisol in the patient group. Furthermore, since reduced DHEAS is commonly associated with poorer health (Goodyer, Herbert, Altham, Pearson & Secher, 1996; Kroboth et al., 1999; van Niekerk, Huppert & Herbert, 2001), we predicted a reduced level of DHEAS in the patient group. The higher prevalence of common infections associated with work-related stress suggests suppressed of cellular immunity. In addition, we previously observed an elevated level of morning cortisol (de Vente et al., 2003), and found other indications for a hyperactive state of the sympathetic system in patients with work-related stress (Chapter 3; de Vente et al., 2003). We therefore anticipated observing suppression of cellular immunity and inflammatory responses resulting in elevated anti-EBV IgG and reduced C-reactive protein (CRP) in the patient group. In line with expected changes in HPA and immune parameters, we expected to find evidence for disturbed immune regulation by the HPA axis in patients. Therefore, group differences in the association between HPA axis activity and immune function were explored.

Method

Participants

Sixty patients with work-related stress were recruited through occupational health practitioners ($n = 44$), general practitioners ($n = 3$), and by self-referral ($n = 13$). Eligibility was based on a screening interview by telephone assessing presence of work-related stress complaints which was administered by a clinical psychologist. Subsequently, an intake procedure followed that consisted of a semi-structured diagnostic interview, which was conducted by a clinical psychologist and completion of the Beck Depression Inventory (BDI; Beck & Steer, 1987). During the semi-structured interview the complaint history was assessed and the Composite International Diagnostic Interview (CIDI; World Health Organisation [WHO], 1997) was administered. Inclusion criteria were: 1) fulfilment of the symptoms of neurasthenia, i.e., continuous mental and/or physical fatigue and increased fatigability, and at least two other stress complaints out of the following: dizziness, dyspepsia, muscular aches or pains, tension headaches, inability to relax, irritability, and sleep disturbance; 2) a primary role of (a) work-related stressor(s) in the development of complaints as judged by the patient, the referring clinician, and/or the clinical psychologist; and 3) presence of impaired daily functioning as indicated by (partial) sickness absence which had lasted at least two weeks but

less than six months. Exclusion criteria were: 1) a primary diagnosis of major depression, social phobia, panic disorder, somatoform disorder other than undifferentiated, posttraumatic stress disorder, obsessive-compulsive disorder, hypomania, or psychotic disorders, assessed with the short version of the CIDI (WHO, 1997); 2) severe depressive complaints (i.e., conservatively defined as ≥ 25 on the BDI); 3) a traumatic event in the past six months; and 4) a medical condition that could explain fatigue (e.g. diabetes); 5) excessive alcohol and/or drug use; and 6) pregnancy. The current physiological study was a part of a comprehensive study about treatment effects on work-related stress. Patients were again measured after a treatment phase of four months. Patients received refund of travel expenses for the laboratory visit.

Forty-three healthy individuals were recruited by flyers in public places (e.g. libraries, supermarkets; *n* = 31) and among part-time working psychology students (*n* = 12). They were screened by telephone. Participants in good physical health and working for at least sixteen hours a week were included in the study. Exclusion criteria were: 1) psychiatric illness as determined by the short version of the CIDI (World Health Organisation, 1997); 2) currently taking sick leave; 3) a traumatic event in the past six months; 4) a history of immune-, diabetic or other medical disease causing fatigue; 5) excessive alcohol and/or drug use; and 6) pregnancy. Healthy participants were paid 10 euro.

Procedure

The ethics committee of the Department of Psychology, University of Amsterdam, approved the research protocol and all participants gave written informed consent. Patients received the questionnaire-booklet and the salivettes for saliva collection after the intake interview. Healthy participants received the questionnaire booklet and the salivettes by mail. Morning saliva was collected on a weekday avoiding disturbing influences due to different sleeping habits in weekends. The method of saliva collection was explained in a detailed instruction form. Participants also reported the time of awakening and the time of collection of the four samples. Saliva for immune measures was collected during a laboratory visit, which took place the day after the morning saliva collection. During this laboratory visit, a psychosocial stress procedure was conducted, which has been documented elsewhere (de Vente et al., 2003).

Materials and Measures

Psychological measures and background variables
Burnout complaints were measured with the Maslach Burnout Inventory-General Survey (MBI-GS; Schaufeli & van Dierendonck, 2000), which consists of three subscales: Emotional Exhaustion, Depersonalisation, and Professional competence. Items are scored on 7-point Likert scales (0 = 'never' to 6 = 'always/daily') and mean subscale scores are calculated. Higher scores reflect higher levels of emotional exhaustion, distance/cynicism, and professional competence. Cronbach's alphas were .85 for Emotional exhaustion, .81 for Depersonalisation, and .75 for Professional competence in the patient sample. For healthy participants, Cronbach's alphas were .83, .73, and .68, respectively.

Distress complaints were defined as fatigue, depression, anxiety, and stress complaints. Fatigue was measured with the subscale General fatigue of the Checklist Individual Strength (CIS; Beurskens et al., 2000). The subscale consists of eight items, which are scored on 7-point Likert scales (1 = 'false' to 7 = 'true'). Lower scores indicate lower levels of fatigue. Cronbach's alpha in the current sample was .90 in both the patient and healthy group. Depression, anxiety, and stress were measured with the Depression, Anxiety, and Stress Scales (DASS; de Beurs, van Dyck, Marquenie, Lange & Blonk, 2001). The DASS comprises three 14-item subscales referring to depressive, anxiety, and stress complaints. Severity of complaints during the past week is rated on 4-point Likert scales (0 = 'not at all/never applicable' to 3 = 'very much/most of the time applicable'). Higher scores represent higher levels of complaints. In the patient sample, Cronbach's alphas were .93 for Depression, .80 for Anxiety, and .92 for Stress. In the healthy sample they were .83 for Depression, .72 for Anxiety, and .94 for Stress.

Presence and duration of a cold during the past week, Body Mass Index (BMI), smoking, hours of sleep, and time of awakening were assessed by questionnaire. Time of awakening was dichotomised in before and after 9am, according to Evans et al. (2005). They demonstrated that morning cortisol levels were significantly lower for waking-up after 9am compared to waking-up before 9am. Women were also asked to report on menstrual phase, the use of oral contraceptives, and pre-/postmenopausal status.

Neuroendocrine parameters and protocol

Cortisol and DHEAS were measured in saliva during the first hour after awakening, i.e., at awakening, and at 30 and 60 minutes thereafter, and at midday. In addition, the cortisol awakening response (CAR) and two 'area under the curve' (AUC) measures were calculated. The CAR was defined as the highest cortisol-level in the hour after awakening (i.e., at 30 or 60 minutes after awakening) minus the cortisol level at awakening. The CAR is considered to indicate adrenal steroidogenic capacity (Schmidt-Reinwaldt et al., 1999). The AUC-measures were calculated according to Pruessner et al. (2003), i.e., the total amount of cortisol (cort-AUC ground) and the cortisol increase (cort-AUC rise). For calculation of the cort-AUC rise, we used the midday cortisol level as a reference instead of the level at awakening.

DHEAS demonstrated pronounced morning dynamics, which were characterised by a strong reduction after awakening. Therefore, the DHEAS awakening response (DAR) was calculated by subtracting the lowest DHEAS-level in the hour after awakening (i.e., at 30 or 60 minutes after awakening) from the level at the moment of awakening. This measure was used as another indicator of adrenal steroidogenic capacity. Furthermore, also for DHEAS, two 'area under the curve' (AUC) measures were calculated, i.e., DHEAS-AUC ground and DHEAS-AUC rise. The cortisol and DHEAS levels at midday (12.00am) were used as indices of basal HPA axis activity. The cortisol/DHEAS ratio was calculated for all morning samples, including the midday sample, as an indicator of hormonal (im)balance (Goodyer et al., 2001).

Saliva was collected using cotton swabs (non-coated Salivettes™, Sarstedt, Nümbrecht, Ger-

many). Participants were instructed to place it under the tongue or between cheek and teeth for about three minutes. If the swab was not saturated, it was permitted to slowly move it around in the mouth without chewing on it. Participants were instructed not to have breakfast or to brush their teeth within 15 minutes before a sample was collected. All samples were centrifuged (5000 x g, 5 min.), divided in 0.5 ml aliquots and stored at -20°C until analysis.

Immune measures and protocol

Specific immunoglobulin-G (IgG) antibody titers against the latent Epstein-Barr virus (EBV) and C-reactive protein (CRP) were determined in saliva collected in pre-stressor samples during the laboratory session. Saliva was collected as described by Navazesh (1993). Following this method, the participant refrains from swallowing for a period of four minutes, allowing the saliva to accumulate in the floor of the mouth. The saliva is spitted into a cup every 60 s. The collection starts with the instruction to void the mouth of saliva by swallowing. Fifteen minutes before the first saliva collection, the participant rinses the mouth with water. Saliva was homogenised by vigorous shaking using a vortex mixer and clarified by centrifugation (10.000 x g., 4 min). The clear supernatant was divided in 0.5 ml aliquots and stored at -20°C until analysis.

Assays

Amounts of free cortisol and DHEAS were determined using enzyme-immuno assay (EIA). CRP (ultrasensitive kit) and anti-EBV IgG (EBV VCA-IgG type) were examined by enzyme linked immunosorbent assay (ELISA). All kits were purchased from Diagnostic System Laboratories (DSL, Veghel, The Netherlands). Anti-EBV IgG and CRP were examined after 2-fold dilution of the saliva sample. The sensitivity of the cortisol, DHEAS, and CRP assay was 1 ng/ml, 0.08 ng/ml, and 1.6 ng/ml, respectively. All samples were assayed in duplo. Intra-assay variability of the cortisol, DHEAS, anti-EBV IgG, and CRP was 2-10%, 4-6%, 3%, and 1.7-3.6%, respectively.

Statistical analyses

Some physiological data were missing due to insufficient saliva (cortisol: 3%, DHEAS: 11%, anti-EBV IgG: 3%, CRP: 9%). Outliers (i.e., values ± >3 *SD*s of the mean, for skewed variables: ± >4 *SD*s of the mean) were removed for each measure separately (<2%). Pearson correlations were calculated between the CAR, the DAR, midday cortisol, and midday DHEAS. Group differences in neuroendocrine measures, including the CAR and the DAR, and immune measures were assessed by analysis of variance (ANOVA), using a one between-subjects factor (group) design. Group differences in neuroendocrine levels during the first hour after awakening were investigated by ANOVA for repeated measures, using a one within- (time), one between- (group) subjects factor design. When the assumption of sphericity was violated, Greenhouse-Geisser-adjusted results were presented. Group differences in associations between neuroendocrine and immune variables were investigated using regression analyses, by including the main effects of group and neuroendocrine variables and the group x neuroendocrine variable interaction in the equation. When the group x neuroendocrine

variable interaction was statistically significant, separate coefficients were reported for each group. All analyses were adjusted for age, gender, and BMI. Because of positively skewed data, DHEAS, cortisol/DHEAS, anti-EBV IgG, and CRP values were transformed ($\log_e$ for neuroendocrine measures and sqrt for immune measures). Two-sided tests were performed, applying a significance level of .05 for main effects and .10 for interaction effects. All analyses were conducted using SPSS 15.

Results

Sample characteristics

The patient- and healthy group differed with respect to gender distribution, $\chi^2(1) = 5.21$, $p = .023$. Furthermore, patients were four years older, $t(101) = 2.19$, $p = .031$, somewhat lower educated, $t(101) = -2.57$, $p = .012$, seven hours more employed, $t(66.4) = 4.14$, $p < .001$, and had a somewhat higher BMI than healthy participants $t(99) = 2.71$, $p = .008$ (see Table 1). Education did not appear to be a confounder in the analyses of group differences in neuroendocrine and immune measures. Hence, the presented results are not adjusted for education. Among patients, mean duration of sickness absence was 8.88 ($SD = 7.39$) weeks. None of the healthy participants was on sickness leave.

Table 1: *Characteristics of patients and healthy participants [mean (SD)/frequency (%)].*

	Patient ($n = 60$)	Healthy ($n = 43$)
Gender (males/females) [a]	36/24 (60/40)	16/27 (37/63)
Age (years) [a]	41.23 (9.57)	37.02 (9.67)
Education (1-6) [a]	3.48 (1.55)	4.23 (1.32)
Employment (hrs/week) [b]	35.78 (5.67)	29.44 (8.82)
Smoker (yes/no)	11/49 (18/82)	8/32 (20/80)
Having a cold during past week (yes/no)	12/45 (21/79)	13/25 (34/66)
Duration of cold (days)	2.25 (0.75)	3.15 (2.03)
Sleep duration (hours)	7.51 (1.30)	7.88 (1.14)
Time of awakening (< / > 9am)	51/6 (89/11)	34/8 (81/19)
Body mass index (kg / mtrs²) [a]	25.11 (4.01)	23.14 (2.95)
Emotional exhaustion (MBI-GS, range: 0-6) [b]	4.26 (1.30)	1.26 (0.80)
Depersonalisation (MBI-GS, range: 0-6) [b]	2.91 (1.48)	1.41 (0.96)
Professional competence (MBI-GS, range: 0-6)	3.73 (1.06)	3.80 (0.86)
General fatigue (CIS, range: 8-56) [b]	41.88 (9.84)	21.12 (9.83)
Anxiety (DASS, range: 0-42) [b]	7.35 (5.45)	2.35 (2.77)
Depression (DASS, range: 0-42) [b]	13.09 (7.88)	3.84 (3.50)
Stress (DASS, range: 0-42) [b]	18.56 (8.63)	7.16 (7.17)

Note: MBI-GS: Maslach Burnout Inventory – General Survey; CIS: Checklist Individual Strength; DASS: Depression, Anxiety, and Stress Scales; [a] $p < .05$; [b] $p < .001$.

Two patients (3%) used anti-depressive medication, four patients (7%) used an anxiolyticum, and three patients (5%) used beta-blockers as anti-hypertensive medication. Five female patients (21%) were using oral contraceptives. Healthy participants, except for eight women (30%) who used oral contraceptives, were medication-free. Three women (13%) in the patient group (missing: $n = 1$) were in the menstrual phase (day 1-6), four (17%) in the follicular phase (day 7-14), and 11 (46%) in the luteal phase (day 15-28). In the healthy group (missing: $n = 4$), the numbers were five (19%), seven (26%), and seven (26%), respectively. Five patients (21%) and four healthy women (15%) reported having passed their menopause. No statistically significant differences were found in menstrual phase distributions or pre-post menopausal distributions.

Patients scored significantly higher on all complaints than healthy participants; effect sizes (i.e., Cohen's d) were between 1.14 and 2.63, all p-values were < .001. An exception was noted for Professional competence; the between-group effect was small (Cohen's d = 0.11) and not statistically significant.

Associations between indicators of the HPA axis

To the best of our knowledge, the DAR has not been used as an indicator of HPA axis activity before. As an initial step to validate this measure, it was related to conventional HPA axis indicators. Associations between the CAR, the DAR, midday cortisol, and midday DHEAS are presented in Table 2. The CAR is virtually unrelated to midday cortisol, which is consistent with previous research (Schmidt-Reinwald et al., 1999). The CAR is neither related to midday DHEAS. The DAR, however, correlates considerably with midday DHEAS. Midday cortisol and midday DHEAS are moderately related. Most interestingly, the CAR and the DAR are moderately associated, which supports the idea that the DAR also reflects steroidogenic adrenal capacity.

Table 2: *Pearson correlation coefficients between HPA axis indicators (N = 88).*

	1	2	3
1 CAR	-		
2 Midday cortisol	.13	-	
3 DAR	.31 [a]	.20	-
4 Midday DHEAS	.08	.37 [b]	.55 [b]

Note: CORT: cortisol; DHEAS: dehydroepiandrosterone-sulphate; CAR: cortisol awakening response; DAR: DHEAS awakening response; [a] $p < .05$; [b] $p < .001$.

Group differences in immune measures

Mean anti-EBV IgG values were 0.48 AU (S.E.M. = 0.06, n = 56) for patients and 0.49 AU (S.E.M. = 0.08, n = 42) for healthy participants. Mean values of CRP were 6.14 ng/ml (S.E.M. = 0.51, n = 55) for patients and 5.94 ng/ml (S.E.M. = 0.47, n = 38) for healthy participants. No group-differences emerged, anti-EBV IgG: $F(1,93)$ = 0.442, p = .508; CRP: $F(1,88)$ = 0.00, p = .961.

Group differences in HPA axis activity

Figures 1a-c depict mean values of cortisol, DHEAS, and cortisol/DHEAS during the morning. Morning cortisol, DHEAS, and cortisol/DHEAS values changed over time (p-values < .001). No group-differences were found for midday cortisol, midday DHEAS, and midday cortisol/DHEAS values, or for change during the morning in any of these measures (Table 3).

The mean CAR was 2.69 ng/ml, S.E.M.= 0.55 (7.43 nmol/L, S.E.M.= 1.51) for patients, and 3.78 ng/ml, S.E.M. = 0.72 (10.44 nmol/L, S.E.M. = 1.99) for healthy participants. The mean DAR was 5.17 ng/ml, S.E.M. = 0.90 (14.05 nmol/L, S.E.M. = 2.45) for patients, and 5.40 ng/ml, S.E.M. = 1.01
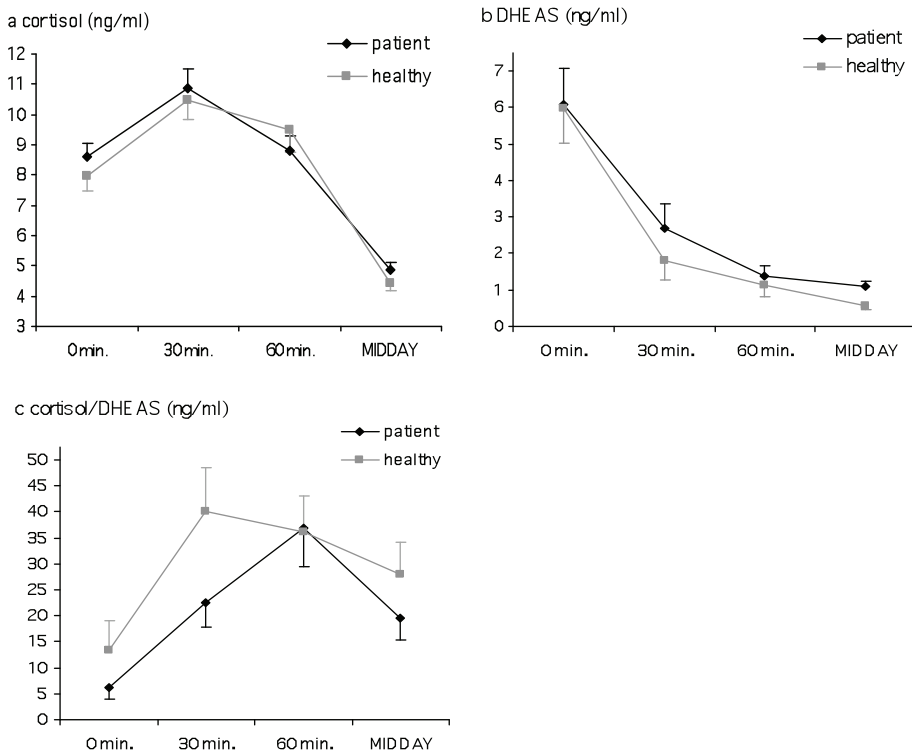
Figures 1a-c: *Means and standard errors of morning cortisol, DHEAS, and cortisol/DHEAS levels (0, 30, 60 minutes after awakening, midday).*

Table 3: *Test-results comparing midday values (ANOVA) and morning dynamics (ANOVA for repeated measures) of the neuroendocrine measures between the patient- and healthy group.*

| | midday [a] | | | morning dynamics [b] | | | | | |
| | group | | | group | | | group*time | | |
	df's	F	p	df's	F	p	df's	F	p
CORT	1,93	0.76	.386	1,90	0.38	.538	2.6,230.5	0.06	.972
DHEAS	1,89	1.53	.219	1,83	0.07	.787	2.8,231.6	1.31	.272
CORT/DHEAS	1,89	0.84	.361	1,80	0.01	.930	2.8,220.6	1.28	.284

Note: group: mean difference between the patient and the healthy group; group*time: interaction effects; CORT: cortisol; DHEAS: dehydroepiandrosterone-sulphate; CORT/DHEAS: Cortisol-DHEAS ratio; [a] values at 12.00am; [b] values at awakening, 30 min. and 60 min. after awakening, and at 12.00am.

(14.67 nmol/L, S.E.M.= 2.74) for healthy participants. These differences were not statistically significant, $F(1,92) = 0.57$, $p = .451$, and $F(1,81) = 0.10$, $p = .759$, respectively. No statistically significant differences were found for the AUC measures, all p-values > .30. Analyses excluding participants that failed to demonstrate a cortisol increase during the first hour after awakening (patients 29%, healthy individuals 23%), which might indicate non-compliance (Broderick, Arnold, Kudielka & Kirschbaum, 2004; Kudielka, Broderick & Kirschbaum, 2003), did not affect the statistical non-significance of the results.

Group differences in neuroendocrine immune modulation

Group differences in associations between neuroendocrine and immune measures were examined. To limit the number of analyses, a confined number of indices of neuroendocrine activity were selected consisting of basal and capacity indicators of HPA axis functioning. These selected indices were midday levels of cortisol, DHEAS, and the cortisol-DHEAS ratio, and the CAR and DAR. Regarding cellular immunity, statistically significant interaction effects were found for group x midday DHEAS on anti-EBV IgG and for group x DAR on anti-EBV IgG, indicative of group differences in the associations between basal DHEAS and anti-EBV IgG and between the DAR and anti-EBV IgG, respectively. Regarding inflammatory activity, a statistically significant interaction effect was found for group x CAR on CRP, which is indicative of a group difference in the association between the CAR and CRP. For the other associations, no interactions with group were observed, thus suggesting similar immunomodulatory effects among groups. For the significant group differences in associations between neuroendocrine measures and immune measures, stratified results are presented in Table 4. Regarding cellular immunity, a statistically significant positive association was found in patients between midday DHEAS and anti-EBV IgG, while this association was close to zero in healthy participants. Similarly, a statistically significant positive associa-

Table 4: *Stratified outcomes of the regression analyses associating neuroendocrine and immune measures.*

	group	*n*	B	CI	β	R²	*t*	*p*
Anti-EBV IgG								
midday DHEAS	patient	52	0.07	0.01 − 0.12	0.32	.10	2.20	.030
	healthy	41	-0.02	-0.09 − 0.05	-0.11	.01	-0.65	.517
DAR	patient	50	0.08	0.02 − 0.13	0.44	.19	2.86	.005
	healthy	35	0.01	-0.05 − 0.06	0.04	.00	0.25	.802
CRP								
CAR	patient	52	0.00	-0.04 − 0.04	0.01	.00	0.06	.956
	healthy	38	0.06	0.01 − 0.10	0.39	.15	2.60	.011

Note: DHEAS: dehydroepiandrosterone-sulphate; Cortisol/DHEAS: Cortisol-DHEAS ratio; anti-EBV IgG; anti-Epstein-Barr Virus immunoglobulin G; CRP: C-reactive protein; CAR: cortisol awakening response; DAR: DHEAS awakening response.

tion between the DAR (i.e., a larger DHEAS reduction) and anti-EBV IgG was found in patients, while this association was almost zero in healthy participants. Regarding inflammatory activity, a statistically significant positive association was found in healthy participants, between the CAR and CRP, while this association was absent in patients.

Discussion

The aim of this study was to investigate whether dysregulation of the immune function and the HPA axis were associated with a clinical level of work-related stress, and to reveal potential changes in neuroendocrine regulation of immune functioning. While no deviant levels of immune or neuroendocrine parameters were observed, support was obtained for immune dysregulation in patients with work-related stress. Regarding cellular immunity, basal DHEAS and the DAR were positively related with anti-EBV IgG in patients, but not in healthy individuals. These outcomes indicate that only in patients, higher basal DHEAS and a larger reduction of DHEAS after awakening, potentially indicative of larger adrenal steroidogenic capacity, were associated with reduced cellular immunity. Regarding inflammatory activity, the CAR was positively associated with CRP in healthy participants, but not in patients. These outcomes suggest that only in healthy participants, a stronger cortisol rise after awakening, indicative of larger adrenal steroidogenic capacity, is associated with enhanced inflammatory activity.

The positive association between basal DHEAS and the DAR with poorer cellular immunity (higher anti-EBV IgG) observed in patients may be counterintuitive in view of the presumed association between higher DHEAS levels and better health, and the supposed immunopotentiating

capacity of DHEAS. However, treatment of mice with DHEAS resulted in suppression of cellular immunity as evidenced by a higher IL-10 production (Cheng & Tseng, 2000), which suggests that high DHEAS levels may exert an immunosuppressive effect. Despite absence of between group-differences in absolute DHEAS levels in the current study, this significant association indicates a shift towards more anti-inflammatory activity and/or humoral immune activity in patients with work-related stress. A role for DHEAS in the shift of immune activity towards more anti-inflammatory activity is also in line with recent findings of Mommersteeg et al. (2006a).

The positive association between the CAR and more inflammatory activity observed in healthy participants may also seem surprising in view of the presumed anti-inflammatory effect of cortisol. It has been suggested, though, that under certain conditions, corticosteroids can also have immunostimulatory effects (e.g., Black, 2002). As noted previously, elevated cortisol has been observed in combination with enhanced inflammatory activity in a relatively healthy sample (Schnorpfeil et al., 2003). Our most remarkable finding is the observed regulatory effect of glucocorticoids on inflammatory activity in healthy participants, but not in patients with work-related stress. The absence of such an association suggests presence of glucocorticoid resistance of the immune system, which is in support of the glucocorticoid resistance model described by Miller, Cohen and Ritchey (2002). According to this model, chronic stress reduces the responsiveness of the immune systems to glucocorticoid hormones, such as cortisol, so that the inflammatory response is not well terminated.

Regarding morning cortisol, we did not replicate our finding of elevated cortisol at awakening in patients (de Vente et al, 2003), despite the similar conditions across the studies. Some indications for a reduced contrast between the current patient and healthy sample may serve as a partial explanation. Reduced contrast appeared from a marginally significant lower level of anxiety complaints in the current patient group (i.e., the previous patient group had a higher level), and statistically significant lower professional competence and more depressive symptoms in the current healthy sample as compared to the previous healthy sample. The absence of cortisol changes in the patient group is in line with other studies that used clinical samples with work-related stress (Mommersteeg et al., 2006a; Mommersteeg, Heijnen, Verbraak & van Doornen, 2006b).

We observed no deviant DHEAS values in patients with work-related stress, which is in line with previous studies in non-clinical samples with burnout complaints (Grossi, et al., 2003; Moch, et al., 2003). As depression has been associated before with higher levels of DHEA during the day (Heuser et al., 1998), the finding of enhanced DHEAS by Mommersteeg et al. (2006a) may be due to a higher level of depressive complaints in their sample. As different measures were used in our and their study, this hypothesis could not be examined.

Immune changes have been observed in absence of HPA axis dysregulation before (e.g., Grossi et al., 2003; Mommersteeg et al., 2006a). These observations may imply that immune changes can also occur without mediation of the HPA axis. Otherwise, they may indicate that the HPA axis needs to be examined under different conditions, for example at a different time of the day or by examining reactivity to an acute stressor, to reveal dysregulation. Regarding the former, Goodyer

et al. (1996) for example, found that only morning and not evening DHEA, and evening and not morning cortisol differed between children and adolescents with and without major depression. Regarding the latter, to the best of our knowledge, no studies have included HPA axis variables and immune measures when studying reactivity to an acute stressor in clinical samples with work-related stress. However, in relatively healthy samples associations have been demonstrated between sympathetic (Owen & Steptoe, 2003) and HPA axis (Kunz-Ebrecht, Mohamed-Ali, Feldman, Kirschbaum & Steptoe, 2003) reactivity to an acute mental stressor and pro-inflammatory cytokine concentrations, specifically IL-6. Also in breast cancer survivors, an association has been observed between reduced HPA axis reactivity and enhanced IL-6 production (Bower et al., 2007). Hence, investigating reactivity to an acute stressor may be a promising path for further research about the HPA axis and immune functioning in samples with a clinical level of work-related as well.

The absence of differences in immune measures between the healthy and patient group was unexpected, particularly because we previously observed group-differences in cardiovascular and cortisol basal activity and reactivity in similar samples (Chapter 3; de Vente et al., 2003). The absence of finding changes may be due to the selection of immune measures in this study. Segerström and Miller (2004) observed that immune changes associated with chronic stress were only found in functional immune measures, which were not included in this study. Therefore, on the basis of this limited number of immune parameters in this study, it cannot be concluded that immune changes are absent in patients with work-related stress.

This study adds to the proposed explanation by another research group (Mommersteeg et al., 2006a) of the upregulated IL-10 production observed in a clinical sample with work-related stress. Mommersteeg et al. (2006a) hypothesised that the upregulated IL-10 production may be a consequence of subclinical infections caused by reactivation of the latent Epstein-Barr virus or the common rhinovirus. However, our results do not indicate reactivation of the latent Epstein-Barr virus. Regarding the rhinovirus causing common colds, we can add that, similar to the findings of Mommersteeg et al., no significant difference in reported colds between the healthy and patient sample were observed in our study. However, this does not preclude presence of subclinical infections.

The following methodological issue merits further reflection. In contrast to most studies, we took repeated DHEAS measures during the morning. Since DHEAS is considered to demonstrate little diurnal variation (Kroboth et al., 1999), most studies take a single DHEAS measure. However, when examining DHEAS in the first hour after awakening we found evidence for morning dynamics. Similar to DHEA (i.e., the unsulphated hormone; Hucklebridge et al., 2005), DHEAS demonstrated the highest morning level at the moment of awakening. Our findings further suggest that an elevation of DHEAS in saliva takes place before a rise in cortisol occurs. Interestingly, a similar reaction pattern has been observed for DHEA and cortisol in reaction to a psychosocial laboratory stressor (Izawa et al., 2008). In parallel with the cortisol awakening response as an indicator of adrenal steroidogenic capacity, we constructed a DHEAS measure that reflected the maximum dynamics during the morning. The pattern of associations of the DAR with the cortisol measures, more specifically, presence of a moderate association of the DAR with the CAR and absence of an association

with basal cortisol, supports the notion that the DAR is an indicator of adrenal steroidogenic capacity. To use this measure as an indicator of adrenal steroidogenic capacity can be disputed, though. Carlström et al. (2002) for example have stated that DHEAS variation in serum is highly dependent on alterations in its main binding protein, albumin, instead of on changes in adrenocortical steroid secretion. Hence, the value of DHEAS morning dynamics in saliva awaits further research.

This study highlights the immunomodulatory effect of DHEAS. The finding that in some studies psychopathology was associated with changes in DHEAS levels rather than in cortisol levels (Mommersteeg et al., 2006a; Assies, et al., 2004) illustrates DHEAS' potential relevance for future stress research. Furthermore, the fact that DHEAS can be adequately determined in saliva implies a considerably reduction of practical obstacles to measure it.

In conclusion, although absolute levels of immune and HPA axis parameters appeared unchanged in patients with work-related stress, support was obtained for different immune modulation by the HPA axis in this group, involving both inflammatory activity and cellular immunity. The results supported presence of glucocorticoid resistance in patients and a role for DHEAS in the suppression of cellular immunity.

References

American Psychiatric Association (1994). *Diagnostic and statistical manual of mental disorders: 4th ed. (DSM-IV)*. Washington DC: American Psychiatric Association.

Appels, A., Bar, F.W., Bar, J., Bruggeman, C. & de Baets, M. (2000). Inflammation, depressive symptomatology, and coronary artery disease. *Psychosomatic Medicine*, *62*, 601-605.

Assies, J.,Visser, I., Nicolson, N.A., Eggelte, T.A., Wekking, E.M., Huyser, J., Lieverse, R. & Schene, A.H. (2004). Elevated salivary dehydroepiandrosterone-sulphate but normal cortisol levels in medicated depressed patients: preliminary findings. *Psychiatry Research*, *128*, 117-122.

Bauer, M.E. (2008). Chronic stress and immunosenescence: A review. *Neuroimmunomodulation*, *15*, 241-250.

Bargellini, A. Barbieri, A., Rovesti, S., Vivoli, R., Roncaglia, R. & Borella, P. (2000). Relation between immune variables and burnout in a sample of physicians. *Occupational and Environmental Medicine*, *57*, 453-457.

Beck, A.T. & Steer, R.A. (1987). *Beck Depression Inventory. Manual*. New York: Harcourt Brace.

Beurskens, A., Bültmann, U., Kant, IJ., Vercoulen, J.H.M., Bleijenberg, G. & Swaen, G.M.H. (2000). Fatigue among working people: validity of a questionnaire measure. *Occupational and Environmental Medicine*, *57*, 353-357.

Black, P.H. (2002). Stress and the inflammatory response: A review of neurogenic inflammation. *Brain, Behavior, and Immunity*, *16*, 622-653.

Bower, J.E., Ganz, P.A., Aziz, N., Olmstead, R., Irwin, M.R. & Cole, S.W. (2007). Inflammatory responses to psychological stress in fatigues breast cancer survivors: relationship to glucocorticoids. *Brain, Behavior, and Immunity*, *21*, 251-258.

Broderick, J.E., Arnold, D., Kudielka, B.M. & Kirschbaum, C. (2004). Salivary cortisol sampling compliance: comparison of patients and healthy volunteers. *Psychoneuroendocrinology*, *29*, 636-650.

Brosschot, J.F., Benschop, R.J., Godaert, G.L., Olff, M., de Smet, M., Heijnen, C.J. & Ballieux, R.E. (1994). Influence of life stress on immunological reactivity to mild psychological stress. *Psychosomatic Medicine*, *56*, 216-242.

Cacioppo, J.T., Kiecolt-Glaser, J.K., Malarkey, W.B., Laskowski, B.F., Rozlog, L.A., Poehlman, K.M., Burleson, M.H. & Glaser, R. (2002). Autonomic and glucocorticoid associations with the steady-state expression of latent Epstein-Barr virus. *Hormones and Behavior*, *42*, 32-41.

Carlström, K., Karlsson, R. & Von Schoultz, B. (2002). Diurnal rhythm and effects of oral contraceptives on serum dehydroepiandrosterone-sulphate (DHEAS) are related to altered serum albumine rather than to changes in adrenocortocal steroid secretion. *Scandinavian Journal of Clinical and Laboratory Investigation*, *62*, 361-368.

Chen, C.C. & Parker, C.R. Jr. (2004). Adrenal androgens and the immune system. *Seminars in Reproductive Medicine*, *22*, 369-377.

Cheng, G.F. & Tseng, J. (2000). Regulation of murine interleukin-10 production by dehydroepiandrosterone. *Journal of Interferon and Cytokine Research*, *20*, 471-478.

Davis, M.C., Zautra, A.J., Younger, J., Motivala, S.J., Attrep, J. & Irwin, M.R. (2008). Chronic stress and regulation of cellular markers of inflammation in rheumatoid arthritis: Implications for fatigue. *Brain, Behavior, and Immunity*, *22*, 24-32.

de Beurs, E., van Dyck, R., Marquenie, L.A., Lange, A. & Blonk, R.W.B. (2001). De DASS: Een vragenlijst voor het meten van depressie, angst en stress [The DASS: a questionnaire for the measurement of Depression, Anxiety and Stress]. *Gedragstherapie*, *34*, 35-53.

de Vente, W., Olff, M., van Amsterdam, J.G.C., Kamphuis, J.H. & Emmelkamp, P.M.G. (2003). Physiological differences between burnout patients and healthy controls: blood pressure, heart rate and cortisol responses. *Occupational and Environmental Medicine*, *60*, 54i- 61i.

Elenkov, I.J. & Chrousos, G.P. (2006). Stress system – Organization, physiology and immunoregulation. *Neuroimmunomodulation*, *13*, 257-267.

Elenkov, I.J., Wilder, R.L., Chrousos, G.P. & Vizi, E.S. (2000). The sympathetic nerve – An integrative interface between two supersystems: The brain and the immune system. *Pharmacological Reviews*, *52*, 595-638.

Evans, P.D., Clow, A., Hucklebridge, F.H. & Lai, J. (2005). A meta-analysis of the awakening cortisol response and its relationship to time of waking. (abstract) *Psychosomatic Medicine*, *67*, A93.

Fries, E., Hesse, J., Helhammer, J. & Hellhammer, D.H. (2005). A new view on hypocortisolism. *Psychoneuroendocrinology*, *30*, 1010-1016.

Goodyer, I.M., Herbert, J., Altham, P.M.E., Pearson, J. & Secher, H.M. (1996). Adrenal secretion during major depression in 8- to 16- year old: 1. Altered diurnal rhythms in salivary cortisol and dehydroepiandrosterone (DHEA) at presentation. *Psychological Medicine*, *26*, 245-256.

Goodyer, I.M., Park, R.J., Netherton, C.M. & Herbert, J. (2001). Possible role of cortisol and dehydroepiandrosterone in human development and psychopathology. *British Journal of Psychiatry*, *179*, 243-249.

Grossi, G., Perski, A., Evengard, B., Blomkvist, V. & Orth-Gomer, K. (2003). Physiological correlates of burnout among women. *Journal of Psychosomatic Research*, *55*, 309-316.

Hechter, O., Grossman, A. & Chatterton, R.T. Jr. (1997). Relationship of dehydroepiandrosterone and cortisol in disease. *Medical Hypotheses*, *49*, 85-91.

Heim, C., Ehlert, U. & Hellhammer, D.H. (2000). The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. *Psychoneuroendocrinology*, *25*, 1-35.

Heuser, I., Deuschle, M., Luppa, P., Schweiger, U., Standhardt, H. & Weber, B. (1998). Increased diurnal plasma concentration of dehydroepiandrosterone in depressed patients. *Journal of Clinical Endocrinology and Metabolism*. 83, 3130-3133.

Hucklebridge, F., Hussain, T., Evans P. & Clow, A. (2005). The diurnal patterns of the adrenal steroids cortisol and dehydroepiandrosterone (DHEA) in relation to awakening. *Psychoneuroendocrinology*, *30*, 51-57.

Izawa, S., Sugaya, N., Shirotsuki, K., Yamada, K.C., Ogawa, N., Ouchi, Y., Nagano, Y., Suzuki, K. & Nomura, S. (2008). Salivary dehydroepiandrosterone secretion in response to acute psychosocial stress and its correlations with biological and psychological changes. *Biological Psychology*, *79*, 294-298.

Julius, S. (1994). Abnormalities of autonomic nervous control in human hypertension. *Cardiovascular Drugs and Therapy*, *8*, 11-20.

Kiecolt-Glaser, J.K., McGuire, L., Robles, T.F. & Glaser, R. (2002). Psychoneuroimmunology: psychological influences on immune function and health. *Journal of Consulting and Clinical Psychology*, *70*, 537-547.

Kroboth, P.D., Salek, F.S., Pittenger, AL., Fabia, T.J. & Frye, R.F. (1999). DHEA and DHEA-S: A review. *Journal of Clinical Pharmacology*, *39*, 327-348.

Kudielka, B.M., Broderick, J.E. & Kirschbaum, C. (2003). Compliance with saliva sampling protocols: electronic monitoring reveals invalid cortisol daytime profiles in non-complaint subjects. *Psychosomatic Medicine*, *65*, 313-319.

Kunz-Ebrecht, S.R., Mohamed-Ali, V., Feldman, P.J., Kirschbaum, C. & Steptoe, A. (2003). Cortisol responses to mild psychological stress are inversely associated with pro-inflammatory cytokines. *Brain, Behavior, and Immunity*, *17*, 373-383.

Langelaan, S., Bakker, A.B., Schaufeli, W.B., van Rhenen, W. & van Doornen, L.J.P. (2006). Do burned-out and work-engaged employees differ in the functioning of the hypothalamic-pituitary-adrenal axis? *Scandinavian Journal of Work and Environmental Health*, *32*, 339-348.

Ledochowski, M., Murr, C., Jäger, M. & Fuchs, D. (2001). Dehydroepiandrosterone, ageing, and immune activation. *Experimental Gerontology*, *36*, 1739-1747.

Lerman, Y., Melamed, S., Shragin, Y., Kushnir, T., Rotgoltz, Y., Shirom, A. & Aronson, M. (1999). Association between burnout at work and leukocyte adhesiveness/aggregation. *Psychosomatic Medicine*, *61*, 828-833.

Lindblom, K.M., Linton, S.J., Fedeli, C., & Bryngelsson, I.L. (2006). Burnout in the working population: relations to psychosocial work-factors. *International Journal of Behavioral Medicine*, *13*, 51-59.

Maslach C., Schaufeli, W.B. & Leiter M.P. (2001). Job Burnout. *Annual Review of Psychology*, *52*, 397-422.

McEwen, B.S. & Wingfield, J.C. (2003). The concept of allostasis in biology and biomedicine. *Hormones and Behavior*, *43*, 2-15.

Miller, G.E., Cohen, S. & Ritchey, A.K. (2002). Chronic psychological stress and the regulation of pro-inflammatory cytokines: A glucocorticoid-resistance model. *Health Psychology*, *21*, 531-541.

Moch, S.L., Panz, V.R., Joffe, B.I., Havlik, I. & Moch, J.D. (2003). Longitudinal changes in pituitary adrenal hormones in South African women with burnout. *Endocrine*, *21*, 267-272.

Mohren, D.C., Swaen, G., Kant, IJ, van Amelsvoort, L.G., Borm, P.G. & Galama, J.M. (2003). Common infections and the role of burnout in a Dutch working population. *Journal of Psychosomatic Research*, *55*, 201-208.

Mommersteeg, P.M., Heijnen, C.J. Kavelaars, A. & van Doornen, L.J. (2006a). Immune and endocrine function in burnout syndrome. *Psychosomatic Medicine*, *68*, 879-886.

Mommersteeg, P.M.C., Heijnen, C.J., Verbraak, M.J.P.M. & van Doornen, L.J.P. (2006b). Clinical burnout is not reflected in the cortisol awakening response, the day curve or the response to a low-dose dexamethasone suppression test. *Psychoneuroendocrinology*, *31*, 216-225.

Nakamura, H., Nagase, H., Yoshida, M. & Ogino, K. (1999). Natural killer (NK) cell activity and NK subsets in workers with a tendency of burnout. *Journal of Psychosomatic Research*, *46*, 569-578.

Nakata, A., Araki, S., Tanigawa, T., Miki, A., Sakurai, S., Kawakami, N., Yokoyama, K. & Yokoyama, M. (2000). Decrease of suppressor-inducer (CD4+CD45RA) T lymphocytes and increase of serum immunoglobulin G due to perceived job stress in Japanese nuclear electric power plant workers. *Journal of Occupational and Environmental Medicine*, *42*, 143-150.

Navazesh, M. (1993). Methods for collecting saliva. *Annals of the New York Academy of Sciences*, *694*, 73-77.

Owen, N. & Steptoe, A. (2003). Natural killer cell and proinflammatory cytokine responses to mental stress: associations with heart rate and heart rate variability. *Biological Psychology*, *63*, 101-115.

Pruessner, J.C., Kirschbaum, C., Meinlschmid, G. & Helhammer, D.H. (2003). Two formulas for computation

of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology*, *28*, 916-931.

Sapolsky, R.M., Romero, L.M. & Munck, A.U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory and preparative actions. *Endocrine Reviews*, *21*, 55-89.

Schaufeli, W.B. & Buunk, B.P. (2003). Burnout: An overview of 25 years of research and theorizing. In: M.J. Schabracq, J.A.M. Winnubst, C.C. Cooper (Eds.), *The handbook of work and health psychology* (2nd ed., pp. 383–429). Wiley, West Sussex, England.

Schaufeli, W. & van Dierendonck, D. (2000). *UBOS, Utrechtse Burnout Schaal. Handleiding.* [MBI-GS, Maslach Burnout Inventory – General Survey. Manual]. Swets & Zeitlinger B.V., Lisse, The Netherlands.

Schmidt-Reinwald, A., Pruessner J.C., Hellhammer, D.H., Federenko, I., Rohleder, N., Schurmeyer, T.H. & Kirschbaum, C. (1999). The cortisol response to awakening in relation to different challenge tests and a 12-hour cortisol rhythm. *Life Sciences*, *64*, 1653-1660.

Schnorpfeil, P., Noll, A., Schulze, R., Ehlert, U., Frey, K., Fischer, J.E. (2003). Allostatic load and work-conditions. *Social Science and Medicine*, *57*, 647-656.

Segerström, S.C. & Miller, G.E. (2004). Psychological stress and the human immune system: A meta-analytic study of 30 years of inquiry. *Psychological Bulletin*, *130*, 601-630.

Spivak, B., Maayan, R., Kotler, M., Mester, R., Gil-Ad, I., Shtaif, B. & Weizman, A. (2000). Elevated circulatory level of GABA(A)-antagonistic neurosteroids in patients with combat-related post-traumatic stress disorder. *Psychological Medicine*, *30*, 1227-1231.

Sterling, P. & Eyer, J. (1988). Allostasis: a new paradigm to explain arousal pathology. In: S. Fischer, J. Reason (Eds.), *Handbook of life stress, cognition and health* (pp. 629-649). Wiley, New York, United States of America.

Toker, S., Shirom, A., Shapira, I., Berliner, S. & Melamed, S. (2005). The association between burnout, depression, anxiety, and inflammation biomarkers: C-reactive protein and fibrinogen in men and women. *Journal of Occupational Health Psychology*, *10*, 344-362.

Uchino, B.N., Smith, T.W., Holt-Lunstad, J., Campo, R. & Reblin, M. (2007). Stress and illness. In: J.T. Cacioppo, L.G., Tassinary, G.G., Berntson (Eds.), *Handbook of psychophysiology* (3rd ed., pp. 608-632). Cambridge University Press, New York.

van Niekerk, J.K., Huppert, F.A. & Herbert, J. (2001). Salivary cortisol and DHEA: association with measures of cognition and well-being in normal and older men, effects of three months of DHEA supplementation. *Psychoneuroendocrinology*, *26*, 591-612.

Vedhara, K., McDermott, M.P., Evans, T.G., Treanor, J.J., Plummer, S., Tallon, D., Cruttenden, K.A. & Schiffito, G. (2002). Chronic stress in non-elderly caregivers. Psychological, neuroendocrine and immune implications. *Journal of Psychosomatic Research*, *53*, 1153-1161.

World Health Organisation. (1997). *Composite International Diagnostic Interview (CIDI) Manual, ed* 2.1. Geneva, World Health Organisation.

World Health Organisation (1992). *International Classification of Diseases, ed* 10. *(ICD-10)*. Geneva, World Health Organisation.