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

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3 Work-related stress is associated with reduced parasympathetic activity and reduced HPA axis responsiveness*

Abstract

Background: Chronic work-related stress is a risk factor for cardiovascular disease (CVD). This relationship may be explained by stress related dysregulation of the sympathetic system, the parasympathetic system, and the hypothalamic-pituitary-adrenal (HPA) axis. *Method*: To test this hypothesis, 55 patients (34 males, 21 females) on sickness absence because of work-related stress and 40 healthy participants (16 males, 24 females) were exposed to a psychosocial stressor consisting of mental arithmetic and public speech. Pre-stressor levels of physiological variables and physiological reactivity and recovery were compared between groups. Blood pressure and heart rate were measured using a Finapres. Cardiac output, total peripheral resistance, and heart rate variability were estimated using the software Beatscope and Vsrrp. Concentrations of cortisol and alpha-amylase were determined in saliva. *Results*: In male patients, baseline systolic blood pressure was higher, whereas basal alpha-amylase and cortisol reactivity immediately after the acute stressor were lower than in healthy males. In female patients a tendency for lower basal cortisol was found as compared to healthy females. Furthermore, reduced basal heart rate variability and a trend for elevated basal cardiac output were observed in both male and female patients. *Conclusion*: Work-related stress is characterised by dysregulation of the sympathetic and parasympathetic system, and the HPA axis and this dysregulation was more pronounced in males than in females.

* de Vente, W., van Amsterdam, J.G.C., Olff, M., Kamphuis, J.H. & Emmelkamp, P.M.G. (*Accepted pending revision*) Work-related stress is associated with reduced parasympathetic activity and with reduced HPA axis responsiveness.

Work-related stress is a state that results from prolonged exposure to work-related stressors and is associated with burnout complaints and distress complaints (see Lindblom, Linton, Fedeli & Bryngelsson, 2006, for an overview; Schaufeli & Buunk, 2003). Burnout complaints include emotional exhaustion, negative attitudes towards work, and a sense of diminished competence to fulfil the demands posed by the job (Maslach, Schaufeli & Leiter, 2001; Schaufeli & Buunk, 2003). Burnout complaints can also include physical symptoms such as low energy and psychosomatic complaints (Schaufeli & Buunk, 2003). Distress complaints comprise symptoms of anxiety and depression such as irritability, depressed mood, fatigue, sleeping problems, and concentration problems. Chronic work related-stress is a risk factor for cardiovascular disease (CVD; Belkic, Landsbergis, Schnall, & Baker, 2004; Chandola et al., 2008; Melamed, Shirom, Toker, Berliner & Shapira, 2006). In this study we attempted to identify physiological stress mechanisms that may explain this relationship.

Various theories about stress, including the Transactional Theory of stress (Lazarus & Folkman, 1987) or the theory of allostatic load (McEwen & Wingfield, 2003), state that repeated or persistent exposure to stressors may result in emotional and physiological changes and health complaints. Accordingly, a clinical level of work-related stress is expected to be associated with changes in the physiological stress system. The physiological stress system includes central and peripheral parts of the nervous system. The main elements of the stress system are the sympathetic system including the Sympathetic Adrenal Medullary (SAM) axis, the parasympathetic system, and the Hypothalamic Pituitary Adrenal axis (Chrousos & Gold, 1992; Kaltsas & Chrousos, 2007). Generally, during an acute stress-response, the sympathetic system and the HPA axis become active, and the parasympathetic system becomes less active (Chrousos & Gold, 1992; Kaltsas & Chrousos, 2007). Sympathetic activation results in a release of catecholamines, which among others affects cardiovascular functioning (Kaltsas & Chrousos, 2007). In addition, the balance between sympathetic and parasympathetic activity also affects salivary alpha-amylase (Nater et al., 2006). Activation of the HPA axis results in a release of cortisol by the adrenal cortex.

Sustained, enhanced sympathetic activity is hypothesised to play a role in the association between stress and CVD through its influence on adverse health processes including the metabolic syndrome and atherosclerosis (Grassi, Arenare, Quarti-Trevano, Seravalle & Mancia, 2009; Melamed et al., 2006; Palatini & Julius, 2004). Since the parasympathetic, or vagal, system has regulatory influences on the sympathetic system, and is presumed to have cardioprotective properties (e.g., Osterziel & Dietz, 1996), reduced parasympathetic activity may also play a role in the association between stress and CVD. Indeed, reduced parasympathetic activity has shown to be a prognostic factor for CVD (Dekker et al., 2000). A shift in the balance between sympathetic and parasympathetic activity towards stronger sympathetic activation has also been demonstrated in association with work-related stress by observing elevated basal cardiovascular activity (e.g., Cacioppo et al., 2000; Steptoe, Siegrist, Kirschbaum & Marmot, 2004; Vrijkotte, van Doornen & de Geus, 2000) and elevated basal alpha-amylase levels (Nater, Rohleder, Schlotz, Ehlert & Kirschbaum, 2007). Furthermore, reduced parasympathetic activity has been observed in distress condi-

tions, including anxiety and depression (Friedman, 2007; Rottenberg, 2007), which are related to core complaints of work-related stress.

Enhanced sympathetic *reactivity* to stressors has been proposed as a mechanism explaining the development of enhanced sympathetic activity and adverse cardiovascular profiles (Manuck, 1994; Manuck, Kasprowicz & Muldoon, 1990). Support for this idea has indeed been reported (Gasperin, Netuveli, Dias-da-Costa & Patussi, 2009; Treiber et al., 2003). In addition to enhanced reactivity, delayed recovery is also hypothesised to be a risk factor for CVD (e.g., Schwartz et al., 2003), and can therefore be expected to play a role in the development of enhanced sympathetic activity. Support has indeed been found for the association between prolonged stress and enhanced sympathetic reactivity and delayed recovery as reflected in enhanced cardiovascular reactivity and delayed recovery (Gump & Matthews, 1999 for a review; Lepore, Miles, & Levy, 1997), or enhanced epinephrine reactivity (Pike et al., 1997). Hence, enhanced sympathetic reactivity and delayed recovery may be expected in association with prolonged work-related stress.

The HPA axis may mediate the association between stress and CVD because of its effect on the immune function (Melamed et al., 2006). Melamed et al. (2006) argue that hyper- as well as hypo-activity of the HPA axis result in immunological changes that may facilitate atherosclerosis and CVD. Hyper- as well as hypo-activity of the HPA axis have been found in association with prolonged stress. For example, elevated basal levels of ACTH (Cacioppo et al., 2000), elevated basal cortisol levels (Evolathi, Hultcrantz & Collins, 2006; Netterstrøm & Hansen, 2000; Steptoe et al., 2004), or enhanced cortisol reactivity (Cacioppo et al., 2000) have been reported in association with prolonged stress, but also reduced basal cortisol levels and/or reduced cortisol reactivity (Fries, Hesse, Helhammer & Hellhammer, 2005; Heim, Ehlert & Hellhammer, 2000; Nicolson & van Diest, 2000). Hence, we expected to find dysregulation of the HPA axis in association with work-related stress.

Results on sympathetic and parasympathetic activity in association with work-related stress have been mixed (e.g., Gump & Matthews, 1999; van Doornen et al., 2009), which may be partly due to the *healthy worker effect* or restriction of range due to exclusion of severe cases. Therefore, we chose to include a clinical sample. To our knowledge, only two studies investigated sympathetic/-parasympathetic functioning in a clinical sample with work-related stress (de Vente, Olff, van Amsterdam, Kamphuis & Emmelkamp, 2003; Zanstra, Schellekens, Schaap & Kooistra, 2006). In our earlier study (de Vente et al., 2003), we found initial support for sympathetic predominance in the sympathetic-vagal balance as evidenced by elevated heart rate in rest and during a psychosocial stressor. Zanstra et al. (2006) also found support for sympathetic predominance in adaptation to repeated task performance, as they found no reduction of mean blood pressure and less vagal reactivity. In contrast to the mental performance task used by Zanstra et al. (2006) we chose for a psychosocial stressor, which may lead to more ecologically valid results.

With respect to HPA axis activity, results in samples with work-related stress have also been mixed (e.g., Bellingrath, Weigl & Kudielka, 2008, 2009; Kudielka, Bellingrath & Hellhammer, 2006; Langelaan, Bakker, Schaufeli, van Rhenen & van Doornen, 2006; Mommersteeg, Heijnen, Ver-

braak & van Doornen, 2006a; Mommersteeg, Keijsers, Heijnen, Verbraak, & van Doornen, 2006b; Sonnenschein et al., 2007), which may also be partly explained by inclusion of non-clinical cases. In our own previous study (de Vente et al., 2003), we have found support for an over-active HPA axis as we observed elevated cortisol levels at the moment of awakening in a clinical sample with work-related stress. Since the results on HPA axis responsiveness using a psychosocial stress procedure in our previous study were inconclusive, we decided to re-examine HPA axis responsiveness in a larger sample.

Thus, the current study replicates and extends our earlier work (de Vente et al., 2003) by including a larger sample and by including additional measures, with the aim to further elucidate the physiological mechanisms that may explain the association between work-related stress and CVD. The additional measures were: a) cardiac output, b) vascular resistance, c) heart rate variability, and d) alpha-amylase. Both cardiac output and vascular resistance reflect well the haemodynamic function and they are the main determinants of blood pressure. Cardiac output directly depends on heart rate, heart contractility, and vascular resistance (Berntson, Quigly & Lozano, 2007) and is an indicator of both sympathetic and parasympathetic activity (Mohrman, 2003). Vascular resistance is directly related to the peripheral vascular tone (Berntson et al., 2007) and primarily reflects sympathetic activity (Mohrman, 2003). Rapid, beat-to-beat, heart rate variability is an accepted measure of cardiac parasympathetic or vagal activation (Penttilä et al., 2001; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Alpha-amylase is a relatively new measure in stress research. Initially, an increase in the excretion of alpha-amylase in reaction to an acute stressor was suggested to be a relatively pure measure of sympathetic reactivity (e.g., Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996; Rohleder, Nater, Wolf, Ehlert & Kirschbaum, 2004; Speirs, Herring, Cooper, Hardy & Hind, 1974). Recent evidence, however, supports the notion that an increase in alpha-amylase excretion in reaction to a *psychosocial* stressor merely reflects the sympathetic tone, which is determined by both sympathetic and parasympathetic reactivity, rather than by sympathetic activation alone (Nater et al., 2006).

In sum, our aim was to examine the balance in sympathetic-parasympathetic activity and HPA axis activity, as mechanisms explaining the association between work-related stress and CVD. Therefore, we compared a clinical sample with work-related stress with a healthy reference sample on indices of sympathetic activity, parasympathetic activity, and HPA axis activity. Comparisons were made during rest situations as well as during a psychosocial stressor. We expected to find sympathetic predominance in the sympathetic-vagal balance in association with work-related stress. Furthermore, since our sample was recruited within weeks to a few months after having called themselves sick and Kudielka et al.'s (2006) suggested that ongoing chronic stress is generally associated with a hyperactive HPA axis, we expected to find support for a hyperactive rather than a hypo-active HPA axis.

Methods

Participants

Fifty-eight patients were recruited through occupational physicians (n = 43), general practitioners (n = 3), and by self-referral to our study (n = 12). Eligibility was based on a screening interview by telephone assessing the presence of work-related stress complaints which was administered by a clinical psychologist followed by an intake procedure. The intake procedure consisted of a semi-structured diagnostic interview conducted by a clinical psychologist and completion of the Beck Depression Inventory by the patient (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) endorsement of the symptoms of neurasthenia, that is, 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 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, all as assessed with 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 better account for the 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 psychological and physiological aspects of work-related stress (e.g., de Vente, Kamphuis, Emmelkamp & Blonk, 2008). For the current study, patients received refund of their travel expenses and a printed report of their baseline blood pressure and heart rate.

Forty healthy individuals were recruited by flyers in public places (e.g. libraries, supermarkets; n = 29) and among part-time working psychology students (n = 11). They were screened by telephone. Participants in good physical health and working for at least 16 hours a week were included in the study. Exclusion criteria were: 1) psychiatric illness as determined by the CIDI (WHO, 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 15 euro and received a printed report of their baseline blood pressure and heart rate values after attending the laboratory session and completion of the questionnaires.

Materials and Measures

Acute psychosocial stressor

To study physiological reactivity and recovery, participants were exposed to a psychosocial stressor. The stressor consisted of a speech preparation task, a mental arithmetic task, and a speech task (see Figure 1 for the complete procedure). The speech preparation task consisted of preparing a story about a dramatic social situation in which the participant was unfairly accused of causing damage to the property of others. The mental arithmetic task entailed continuous attention-demanding addition, subtraction, multiplication, and division. For the speech task, the prepared story had to be told in front of the camera and the participant was told that the tape would be analysed. Psychosocial stress procedures have demonstrated to enhance perceived stress and result in cardiovascular and neuroendocrine reactions (e.g., Kirschbaum, Pirke & Hellhammer, 1993; Childs & de Wit, 2009; Izawa et al., 2008; Nicholson & van Diest, 2000; Olff, Mulder, The, de Leij & Emmelkamp, 1999; Roy, Kirschbaum & Steptoe, 2001; de Vente et al., 2003).

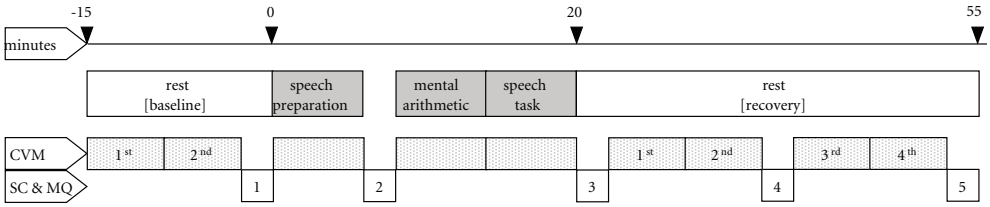


Figure 1: *Time diagram of the psychosocial stress procedure.*

Note: CVM: cardiovascular measurements; SC & MQ: saliva collection for endocrine measures and mood questionnaire.

Cardiovascular assessment

Heart rate (HR) and blood pressure (BP) were measured by continuous measurement of finger BP using a Finapres (Ohmeda Finapres type 2300^{E}, Blood Pressure Monitor) and the software Vsrrp98 (Molenkamp, 1998, University of Amsterdam, The Netherlands). Systolic blood pressure (SBP), diastolic blood pressure (DBP), HR, cardiac output (CO), and total peripheral resistance (TPR) were calculated using the software Beatscope (version 1.1; Finapres Medical Systems, 2002, the Netherlands). Heart rate variability was calculated using the root mean square of successive differences (RMSSD) of inter-beat intervals (IBI's): $\sqrt{(1/n\ \Sigma(IBI_i - IBI_{i-1})^2)}$, which is an adequate measure of the cardiac vagal tone (Penttilä et al., 2001; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). IBI's were defined as the number of milliseconds between peaks of subsequent systoles in the photo-plethysmographic signal, analysed with Vsrrp98 (version 5.4b). The photo-plethysmographic signal was inspected visually and artifacts and ectopic beats were removed. The obtained measure was

called estimated heart rate variability (EHRV). Mean values of cardiovascular measures were calculated per five minutes. The first five minutes of the pre-stressor baseline period were excluded from analyses, since cardiovascular activity during this period may have been affected by walking from the waiting room to the laboratory. Mean values during the last five minutes of the pre-stressor baseline phase (i.e., 2nd baseline, see Figure 1) were used as baseline values, indicative of basal functioning. For reactivity, mean values during the stress-inducing tasks were related to the pre-stressor baseline. For recovery, mean values of the first to third recovery phase were also related to the pre-stressor baseline.

Neuroendocrine measures and protocols

Alpha-amylase and cortisol were determined in saliva collected as described by Navazesh (1993). Accordingly, participants refrain from swallowing for a period of four minutes, allowing the saliva to accumulate in the floor of the mouth. The saliva is spitted out 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, participants rinse their mouths with water.

Saliva samples were stored on ice until the end of the experiment. Immediately after the session, that is, within 90 min after collection, saliva was homogenised by vigorous shaking using a vortex mixer and clarified by centrifugation (10.000 x g. for 4 min). Aliquots (0.5 ml) with clear supernatant were stored at -20°C until analysis.

Alpha-amylase activity was assayed photometrically (Roche, Almere, The Netherlands) after 500-fold dilution using 5 ethylidene-G_7PNP as substrate. The lower detection limit for amylase was 3 U/L. The amount of free cortisol was determined using enzyme-immuno assay (EIA). Kits were purchased from Diagnostic System Laboratories (DSL, Veghel, The Netherlands). The sensitivity of cortisol assay was 1 ng/ml. All samples were assayed in duplo. Intra-assay variability of alpha-amylase and cortisol was 0.4-2% and 2-10%, respectively.

Means of the first (-4 min. in relation to the start of the stressor) and second (+5 min.) saliva samples were used as resting values, indicative of basal functioning (see Figure 1). For alpha-amylase, the third (+20 min., i.e., immediately after cessation of the stressor) saliva sample indicated reactivity, and the fourth (+ 35 min.), and fifth (+50 min.) saliva samples recovery. For cortisol, the third (+20 min.) and fourth (+35 min.) saliva samples indicated reactivity, and the fifth (+ 50 min.) sample recovery.

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 (5 items), Depersonalisation (4 items), and Professional competence (6 items). Items were scored on 7-point Likert scales (0 = never to 6 = always/daily) and mean subscale scores were calculated. Higher scores reflect higher levels of emotional exhaustion, distant/cynical attitudes towards work, and professional competence. Cronbach's alphas were .85 for Emotional exhaustion, .81 for Deper-

sonalisation, 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 General fatigue consisted of eight items, which were scored on a 7-point Likert scale (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 subscales of 14 items each, 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. Cronbach's alphas were .93 for Depression, .80 for Anxiety, and .92 for Stress in the patient sample, and .83 for Depression, .72 for Anxiety, and .94 for Stress in the healthy sample.

Mood during the psychosocial stress procedure was measured by the Profile Of Mood Scale (POMS; McNair, Lorr & Droppleman, 1971). The Vigor (5 items) and Fatigue (6 items) subscales were selected to measure 'energy'-aspects and the Tension (6 items) and Anger (7 items) subscales to measure aspects of negative affect. Items were scored on a five-point scale (0 = not at all to 4 = very much). Higher scores on the Vigor, Fatigue, Tension, and Anger subscales are indicative of more psychological activation, fatigue, tension, and anger, respectively. These mood-dimensions have been previously used to measure the subjective response during stress-inducing tasks (Roy et al., 2001; de Vente et al., 2003). Cronbach's alphas at the first administration during the psychosocial stress procedure (i.e., MQ1, see Figure 1) were 0.90 for Vigor, .92 for Fatigue, .82 for Tension, and .86 for Anger in patients, and .85 for Vigor, .91 for Fatigue, .74 for Tension, and .68 for Anger in healthy participants. Mood questionnaires were administered five times along with saliva collections (see Figure 1).

The relevant control variables for cardiovascular and neuroendocrine parameters such as smoking, hours of sleep, and Body Mass Index (BMI) were assessed by questionnaire. Women were also asked to report on menstrual phase, the use of oral contraceptives, and pre-/postmenopausal status.

Procedure

The ethics committee of the Department of Psychology, University of Amsterdam, approved the research protocol and all participants gave written informed consent. Questionnaires regarding biographical information and burnout and distress complaints were completed at home during the week before the psychosocial stress procedure in the laboratory. To control for time of the day effects, all psychosocial stress procedures took place between 14.00 and 16.30 hrs. Participants were asked to refrain from eating, smoking, and coffee and tea consumption for at least one hour before the start of the experiment. The blood pressure cuff was attached to the non-dominant arm and the

arm remained at approximately heart level throughout the session. To prevent pulse dampening, the Finapres was switched off for three minutes during the first and fourth saliva collection. During the whole experimental session, participants remained seated. The questionnaire regarding control variables such as smoking was completed at the start of the session.

Statistical analyses

Group-differences in baseline values of cardiovascular and neuroendocrine measures were assessed by analyses of variance (ANOVA), using a one between-subjects (group) factor design. Mood, cardiovascular, and neuroendocrine reactivity and recovery during the psychosocial stress procedure were examined with ANOVA for repeated measures, using a one within- (time), one between-subjects (group) factor design. When the assumption of sphericity was violated, Greenhouse-Geisser-corrected results were presented. When time-group interactions were statistically significant, simple contrasts were employed to explore differences in reactivity and recovery as compared to baseline. As gender differences in cardiovascular and cortisol activity and reactivity and in alpha-amylase activity have been reported previously (e.g., Grossi et al., 2005; Kajantie & Phillips, 2006; Kirschbaum, Wüst & Hellhammer, 1992; Kirschbaum, Kudielka, Gaab, Schommer & Hellhammer, 1999; Matthews, Gump & Owens, 2001; van Stegeren et al., 2008; Steptoe et al., 2004), effect-modification of gender was investigated. When effect-modification was found, stratified results were presented. As age, BMI, and gender are known to be related to physiological outcomes, they were added as covariates to all analyses concerning cardiovascular and neuroendocrine outcomes. In addition, menstrual phase, oral contraceptive use, and menopausal status are known to be related to cortisol (Kajantie & Phillips, 2006; Kirschbaum et al., 1999) and were therefore added as covariates when analysing cortisol.

Because of positively skewed data for Anger, Fatigue, EHRV, and alpha-amylase, square root transformed data were analysed. Two-sided test were performed, applying a significance level of .05. All analyses were carried out using SPSS 15. For cardiovascular and neuroendocrine measures, outliers (i.e., values ± >3 *SD*s of the mean) were removed; the number of outliers was < 5%. Some physiological data were missing due to equipment problems (CO, TPR, EHRV; <1%) or insufficient saliva (alpha-amylase, cortisol; 3 – 8.5%).

Results

Sample characteristics

Sample characteristics of the patient- and healthy group are presented in Table 1. Groups differed on gender distribution, $\chi^2(1, n = 95) = 4.42$, $p = .035$. Furthermore, patients were somewhat lower educated, $t(93) = -2.09$, $p = .039$, had a somewhat higher BMI, $t(93) = 2.42$, $p = .017$, and were 6.9 hours more employed, $t(58.0) = 4.39$, $p < .001$. Education did not appear to be a confounder in the analyses of group differences in neuroendocrine and immune measures. Hence, the presented results were not adjusted for education. Mean duration of sickness absence in patients was 8.58

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

	Patient (n = 55)	Healthy (n = 40)
Gender (male / female) [a]	34/21 (62/38)	16/24 (40/60)
Age (years) [a]	40.95 (9.70)	37.65 (9.72)
Education (1 = primary school, 6 = university) [a]	3.53 (1.53)	4.15 (1.29)
Employment (hrs/wk) [b]	36.35 (5.18)	29.45 (8.91)
Smoker (yes / no)	11/44 (20/80)	8/30 (21/79)
Sleep duration (hours)	7.50 (1.30)	7.90 (1.16)
Body mass index (kg/m^2) [a]	25.17 (4.12)	23.33 (2.88)
Emotional exhaustion (MBI-GS, range: 0-6) [b]	4.30 (1.29)	1.29 (0.82)
Depersonalisation (MBI-GS, range: 0-6) [b]	2.97 (1.48)	1.44 (0.93)
Professional competence (MBI-GS, range: 0-6)	3.76 (1.02)	3.85 (0.87)
General fatigue (CIS, range: 8-56) [b]	42.56 (9.37)	21.70 (9.95)
Anxiety (DASS, range: 0-42) [b]	7.66 (5.56)	2.45 (2.82)
Depression (DASS, range: 0-42) [b]	13.57 (7.89)	3.95 (3.60)
Stress (DASS, range: 0-42) [b]	19.31 (8.36)	7.13 (7.38)

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

(*SD* = 7.39) weeks. None of the healthy participants was on sickness leave. Three patients (5%) that were using beta-blocker anti-hypertensive medication were excluded from further analyses. Two patients (4%) used anti-depressive medication and four patients (7%) used an anxiolyticum. Five female patients (24%) were using oral contraceptives. Healthy participants, except for eight women (33%) who used oral contraceptives, were medication-free. Three women in the patient group (14%) reported to be in the menstrual phase (day 1-6), three (14%) in the follicular phase (day 7-14), and 10 (48%) in the luteal phase (day 15-28). In the healthy group (missing: n = 1), the numbers were eight (33%), four (17%), and seven (29%), respectively. Five patients (24%) and four healthy women (17%) reported having passed their menopause. No statistically significant differences were found in menstrual phase distribution or pre-post menopausal distribution.

Patients had significantly higher mean scores on all complaints than healthy participants, effect sizes (i.e., Cohen's d) were between 1.20 and 2.73, all p-values < .001. An exception was noted for Professional competence, for which the between-group effect was small (Cohen's d = 0.10), and not statistically significant.

Mood during the psychosocial stress procedure

Anger, Vigour, Fatigue, and Tension changed over time (F-values > 6.60, p-values < .001); they increased during the stressors. These changes indicated stress-induction and enhanced effort to deal

with the situation. In accordance with their health status, healthy participants had a significantly higher mean Vigour score, a lower mean Fatigue score, and a lower mean Tension score than patients during the psychosocial stress procedure (F-values > 11.30, p-values < .001). Change of Vigour across time differed between patients and healthy participants ($F(2.8, 265.5) = 12.22$, $p < .001$). Vigour remained equal during the session in healthy participants, while it reduced in patients. Thus, patients reported loss of energy during the psychosocial stress procedure, indicating enhanced fatigability, while healthy participants did not. No between-group differences were found for Anger.

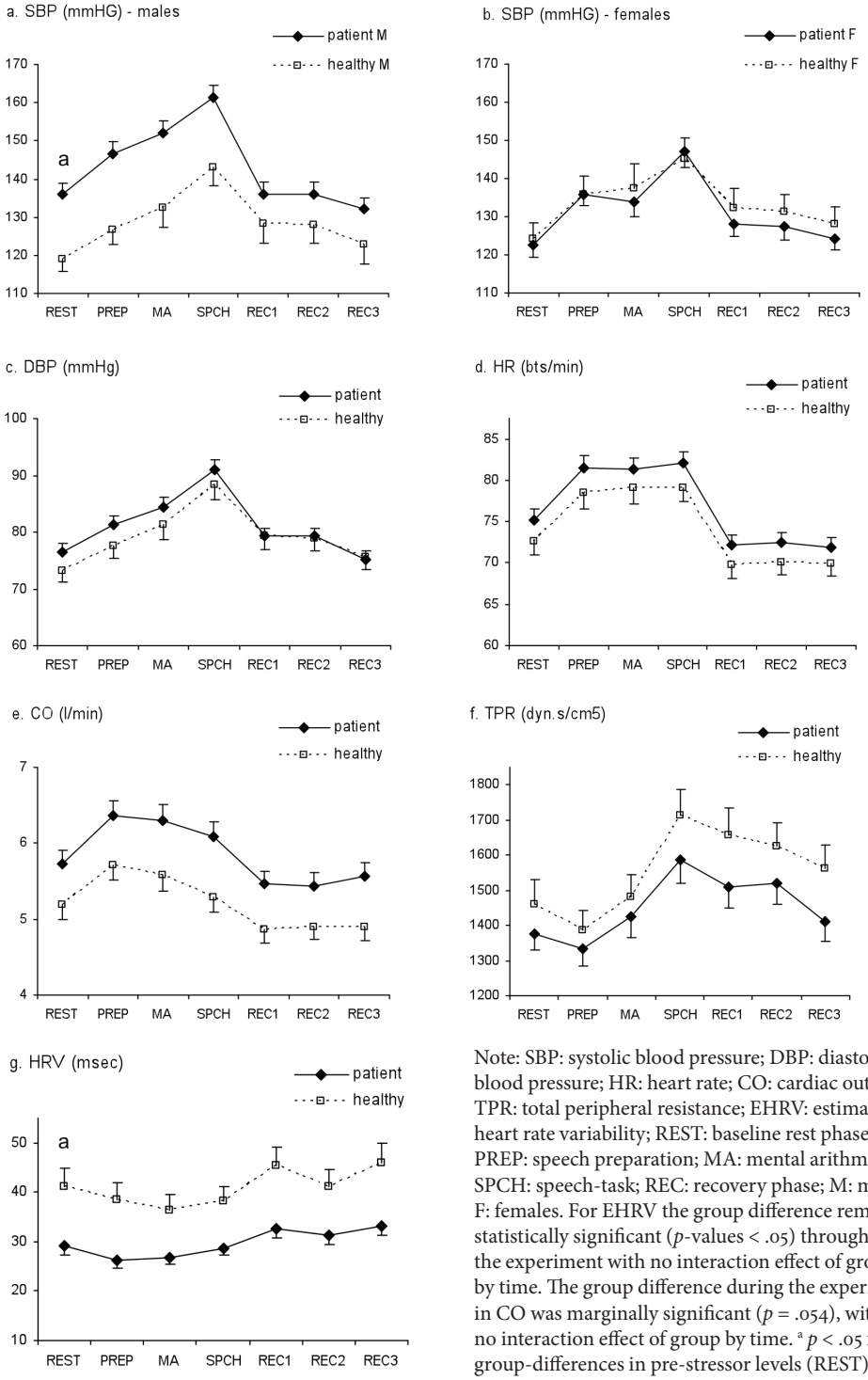
Sympathetic, parasympathetic, and neuroendocrine activity, reactivity, and recovery

Figures 2a-g show means and standard errors of cardiovascular variables, and Figures 3a-d demonstrate means and standard errors of neuroendocrine variables during the psychosocial stress procedure. Effect modification of gender was found for SBP, alpha-amylase, and cortisol, for which stratified results are presented.

In Table 2, test results of baseline values and reactivity and recovery during the psychosocial stress procedure relative to baseline values are listed. At baseline, patients demonstrated higher SBP (males only), lower EHRV, and lower alpha-amylase (males only) than healthy individuals. In addition, a trend was found for lower cortisol in female patients as compared to healthy females.

All physiological measures changed over time during the psychosocial stress procedure (F-values > 3.40, p-values < .05). The observed patterns were consistent with expected activation and recovery due to stress-induction. An exception was the observed pattern in cortisol in healthy females, which demonstrated a reduction instead of an increase after baseline. Similar to differences in baseline values, group differences of mean values during the complete psychosocial stress procedure were found for SBP in males, EHRV, and alpha-amylase for males. A trend was found for CO, suggesting higher CO in patients. Since significant time x group interaction effects were absent, these main effects of group support differences in basal activation, independent of acute stress-induction.

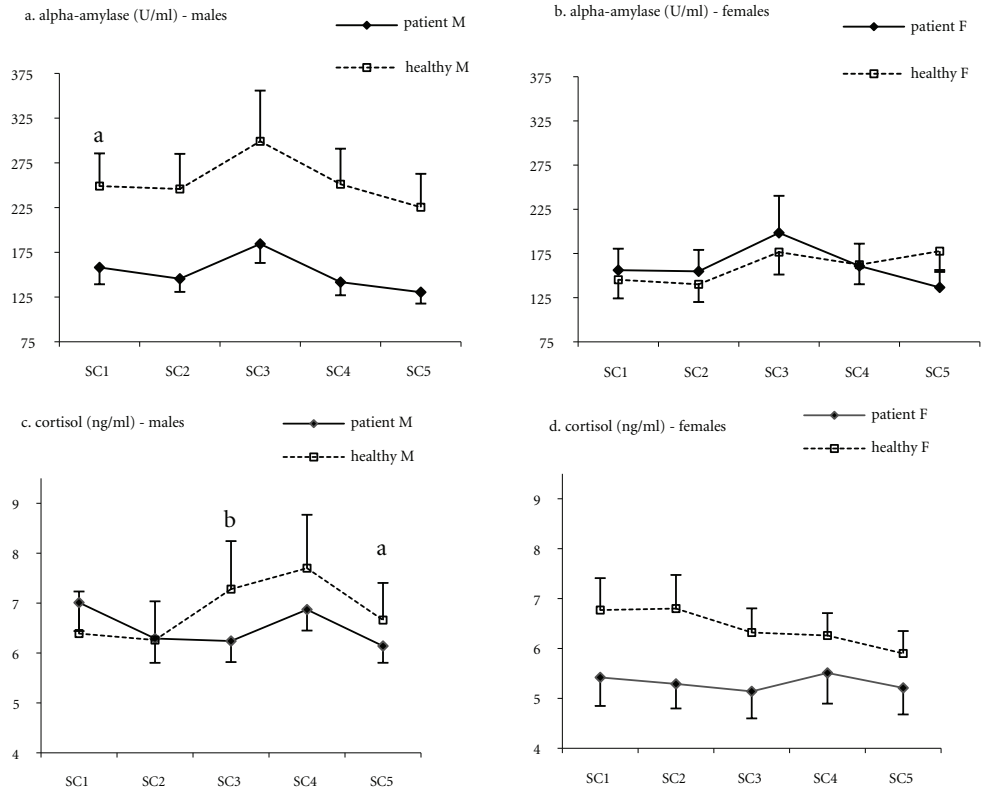
Differences in SBP-dynamics and the trends for DBP-dynamics, and alpha-amylase- dynamics (females) during the psychosocial stress procedure could not be attributed to either reactivity or recovery (i.e., none of the simple contrasts was statistically significant). For cortisol, healthy males showed earlier and stronger cortisol reactivity immediately after cessation of the stressor (+20 min.; $p = .008$) than male patients. Mean reactivity immediately after the stressor of healthy males was 0.91 ng/ml (2.51 nmol/L), which is almost equal to the operational guideline for cortisol-reactivity of 1 ng/ml (2.76 nmol/L; Kirschbaum & Hellhammer, 1989). A cortisol reaction could not be observed in male patients at this moment. A trend was found for a similar pattern at the fourth measurement (+35 min.; $p = .080$). Mean cortisol reactivity for healthy males at this point was 1.18 ng/ml (3.26 nmol/L), which clearly indicates a cortisol secretory response. Mean cortisol reactiv ity for male patients was 0.42 ng/ml (1.16 nmol/L), which does not cross the secretory threshold. In addition, at the fifth measurement (+50 min.), cortisol in healthy males had not returned to the baseline level, in contrast to cortisol in male patients ($p = .039$). The trend for different cortisol-dynamics (females) during the psychosocial stress procedure could not be attributed to either re-



Note: SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; CO: cardiac output; TPR: total peripheral resistance; EHRV: estimated heart rate variability; REST: baseline rest phase; PREP: speech preparation; MA: mental arithmetic; SPCH: speech-task; REC: recovery phase; M: males; F: females. For EHRV the group difference remained statistically significant (p-values < .05) throughout the experiment with no interaction effect of group by time. The group difference during the experiment in CO was marginally significant (p = .054), with no interaction effect of group by time. [a] p < .05 for group-differences in pre-stressor levels (REST).

Figures 2a-g: *Means and standard errors of cardiovascular measures during the psychosocial stress procedure.*

activity or recovery (i.e., none of the simple contrasts was statistically significant).



Figures 3a-d: *Means and standard errors of neuroendocrine measures during the psychosocial stress procedure.*

Note: SC: saliva collection; M: males; F: females. To convert salivary cortisol (ng/ml) to System International units (nmol/L), multiply by 2.76. For alpha-amylase (males), the group difference remained statistically significant (p-values < .05) throughout the experiment with no interaction effect of group by time. [a] $p < .05$ for group-differences in pre-stressor levels (REST) or recovery (SC5). [b] $p < .01$, for group-differences in reactivity (SC3).

Table 2: *Test-results comparing pre-stressor resting values (ANOVA), mean values during the psychosocial stress procedure, and reactivity and recovery (ANOVA for repeated measures) between the patient- and healthy group.*

		resting [a] group			mean during session [a] group			reactivity & recovery [a] group x phase		
		df's	*F*	*p*	df's	*F*	*p*	df's	*F*	*p*
SBP	M	1,46	6.57	**.014**	1,46	4.87	**.032**	3.3,153.6	3.11	**.024**
	F	1,41	0.18	.676	1,41	0.23	.636	3.7,151.4	0.68	.596
DBP		1,90	0.44	.507	1,90	1.13	.291	3.5,310.6	2.08	.094
HR		1,90	0.97	.328	1,90	1.39	.242	3.0,268.8	0.44	.723
CO		1,89	1.71	.195	1,89	3.81	.054	3.7,327.9	0.61	.644
TPR		1,89	0.85	.360	1,89	1.31	.256	3.4,298.2	1.16	.327
EHRV		1,89	4.60	**.035**	1,89	5.83	**.018**	3.7,332.8	0.50	.726
AA	M	1,41	7.14	**.011**	1,41	6.60	**.014**	2.0,83.0	0.21	.813
	F	1,39	0.23	.634	1,39	<0.01	.998	2.5,98.8	2.47	.077
CORT	M	1,45	1.38	.246	1,45	<0.01	.997	2.0,89.5	3.32	**.041**
	F	1,34	2.92	.097	1,34	1.22	.278	2.1,72.5	2.61	.077

Note: Group: mean difference between the patient and the healthy group; group*phase: interaction effect of group x phase of the psychosocial stress procedure; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; CO: cardiac output; TPR: total peripheral resistance; EHRV: estimated heart rate variability; AA: alpha-amylase; CORT: cortisol; M: males; F: females. [a] All analyses were adjusted for the covariates age, BMI, and gender. Cortisol analyses for females were also adjusted for menstrual phase, oral contraceptive use, and menopausal status. Statistically significant differences are presented in bold.

Discussion

This study assessed whether work-related stress is characterised by dysregulation of the sympathetic-vagal balance and the HPA axis that may explain the association between work-related stress and CVD. The observed elevated basal systolic blood pressure (males only), reduced basal heart rate variability, and a trend for elevated cardiac output indeed indicated a predominance of the sympathetic system in the patient group with work-related stress. The reduction in basal alpha-amylase in males is apparently inconsistent with the predicted sympathetic predominance. The latter result is discussed in further detail below. In contrast to prediction, reduced cortisol reactivity to an acute psychosocial stressor was observed in male patients, which suggests hypo-reactivity of the HPA axis, rather than hyperactivity.

The simultaneous predominance of the sympathetic system and hypo-reactivity of the HPA axis was an unexpected finding. Sympathetic predominance characterised by high blood pressure and elevated cardiac output probably reflects the initial stage of the stress-process, as such changes are typical for the initial development of hypertension (Palatini & Julius, 2009). Hypo-activity of the

HPA axis, in contrast, has been suggested to occur after a period of chronic stress (Fries et al., 2005) and thus represents an index of a progressed stress state. However, the time frame for structural and/or functional transitions of both systems is rather unclear. Our findings may imply that these transitions do not occur simultaneously.

The unexpected finding of reduced basal alpha-amylase-level in male patients deserves further discussion, as it seems to be inconsistent with the other indicators of a basal sympathetic predominance in the male patient group. One may argue that alpha-amylase values were confounded by salivary flow rate. To rule out this possibility of confounding, we tested whether flow rate differed between groups and we reanalysed the data by adjusting for flow rate. Indeed, in line with other studies (e.g., Bosch et al., 1996; Rohleder, Wolf, Maldonado & Kirschbaum, 2006), no support for confounding was obtained as no differences in flow rate between healthy males and male patients were observed (data not shown), and similar outcomes were obtained when analyses were adjusted for flow-rate (results not shown). A more plausible explanation is that negative affect may have affected basal alpha-amylase levels, as negative associations have been found between resting alpha-amylase and negative affect as well as with avoidance behaviour (e.g., Fortunato, Dribin, Granger & Buss, 2008). Hence, the group-difference in alpha-amylase that we observed may reflect a group-difference in a, potentially disease state related, basal affective state. It is also possible that in a resting condition, alpha-amylase reflects a different balance between sympathetic and parasympathetic activation than during a stress reaction. Alpha-amylase in resting conditions may be more strongly influenced by parasympathetic activation than by sympathetic activation. Indeed, animal research has demonstrated that alpha-amylase also increases under parasympathetic activation (see Nater et al., 2005, for an overview). Hence, the higher alpha-amylase level during rest in males in the healthy group may be a reflection of their higher basal parasympathetic activation. This reasoning then sustains that the observation of simultaneous elevated basal cardiovascular activity, reduced basal heart rate variability, and reduced basal alpha-amylase in patients is a result of reduced parasympathetic activity. The stressor, in its turn then, evokes an additional sympathetic response that results in a similar alpha-amylase reaction in both groups. Whether reduced alpha-amylase in a resting condition should be interpreted more as an indication of reduced parasympathetic activation rather than reduced sympathetic activation remains to be elucidated in future research. It is interesting to note, though, that chronic stress in children has also been associated with reduced alpha-amylase in rest (Wolf, Nichols & Chen, 2008).

Our study further supports previously reported gender differences in cardiovascular functioning and in cortisol reactivity to a psychosocial stressor (e.g., Grossi et al., 2005; Kajantie & Phillips, 2006; Kirschbaum et al., 1992, 1999; Matthews et al., 2001; Stroud, Salovey & Epel, 2002). The association between gender and alpha-amylase has been seldom examined. Our study supports the gender-dependency of basal alpha-amylase levels reported by van Stegeren et al. (2008), whereas others (Kivlighan & Granger, 2006) found no gender difference in either basal alpha-amylase levels or alpha-amylase reactivity. Gender differences in physiological measures have been ascribed to biological (e.g., hormonal) and psychological differences (e.g., appraisal and coping; Kajantie &

Phillips, 2006; Kirschbaum et al., 1992; Rohleder, Wolf & Kirschbaum, 2003). Hormonal differences may have contributed to the observed lower cortisol reactivity in females, because some women used oral contraceptives, which is generally associated with slightly lower cortisol levels (Kirschbaum et al., 1999). In addition, the measurements for females were not specifically scheduled in the luteal phase in which women demonstrate similar cortisol levels to men (Kirschbaum et al., 1999). With respect to psychological aspects, no indications for gender differences were found that could have otherwise accounted for gender differences in cortisol. More specifically, we observed no differences in appraisal of the tasks during the psychosocial stress procedure as indicated by self-reported anger, tension, fatigue, and vigor (data not shown). However, a discrepancy between self-reported measures of affect and physiological reactivity to stressors has been reported previously (e.g., Kudielka et al., 1998; Stroud et al., 2002). Stroud et al. found that gender differences in cortisol reactivity depended on the type of stressor (i.e., achievement task vs. social rejection task), suggesting that gender differences in appraisal and coping processes seem to be present, despite the fact that they are not detected by measuring self-reported affect. More specifically, similar to our results, Stroud et al. found that women demonstrated no cortisol reaction to the achievement task that consisted of mental arithmetic and public speech. Hence, our psychosocial stressor may have been suboptimal to study acute physiological stress reactivity in women.

As a clinical implication, this study suggests to carefully monitor signs of (pre)hypertension in patients with work-related stress, as we found elevated SBP among males and a tendency for elevated CO in the group as a whole. Moreover, DBP was 80 mmHg in male patients, which is nowadays considered 'prehypertension' (American Heart Association, 2004), and mean DBP during the psychosocial stress procedure was significantly higher in male patients than in healthy males (results not shown). As mentioned, elevated cardiac output in association with enhanced blood pressure has been proposed as characteristic of the initial phase in the development of hypertension (e.g., Palatini & Julius, 2009). In this phase, elevation in blood pressure is suggested to be more easily reversible than in more progressed stages in developing hypertension (Julius, 1993). As hypertension poses a main risk factor for cardiovascular disease and mortality (e.g., Yusuf, Reddy, Oûnpuu & Anand, 2001), preventing its development is of pre-eminent importance. Our data suggest that particular attention is indicated for males, as they exhibited more marked adverse cardiovascular profiles.

Treatment implications for reduced cortisol responsiveness (in contrast to reduced basal values) are not available. In particular with respect to work-related stress, insufficient information about the defects in cortisol responsiveness and their potential spontaneous normalisation is available. However, HPA axis hypo-activity as characterised by reduced basal cortisol levels is found in chronic fatigue syndrome (Cleare, 2003). Treatment studies for chronic fatigue syndrome have demonstrated positive effects of a low dose of hydrocortisone on the cortisol level and on complaints (Cleare, 2003). Furthermore, there is also evidence that psychotherapy results in increases of basal cortisol in PTSD (Olff, de Vries, Güzelcan, Assies & Gersons, 2007) and in chronic fatigue syndrome (Roberts, Papadopoulos, Wessely, Chalder & Cleare, 2009). Future studies may investi-

gate whether pharmacotherapy or psychotherapy normalises cortisol *responsiveness* in work-related stress as well.

Some methodological issues and limitations of this study deserve consideration. Firstly, as discussed above, the stress response of healthy females was below the criterion for HPA axis activation, which may have hindered detection of differences in women with and without work-related stress. Secondly, instead of using heart rate variability based on the electrical heart signal as a measure of vagal parasympathetic activity, the photo-plethysmographic blood pressure signal was analysed. This is an indirect, and potentially confounded, measure of actual heart rate variability. Some support for estimated heart rate variability as a measure of parasympathetic activity was obtained, though. First, the between-group findings, i.e., elevated systolic blood pressure and cardiac output in patients, are in line with the estimated heart rate variability outcomes, that is, they suggest a reduction of cardiac parasympathetic activation. In addition, the estimated variability outcomes demonstrated the expected pattern over time (i.e., reduction under stress-conditions, increase under recovery), with comparable differences between stress and recovery periods as reported by other groups (e.g., Bosch, de Geus, Veerman, Hoogstraten & Nieuw Amerongen, 2003; Kunz-Ebrecht, Mohamed-Ali, Feldman, Kirschbaum & Steptoe, 2003; Vrijkotte et al., 2000). Despite this support, interpretation of the estimated variability as a measure of parasympathetic activity should be done with caution. Thirdly, we studied recovery of cardiovascular and neuroendocrine variables until 35 minutes after cessation of the acute psychosocial stressor. For cortisol, this period is too short to expect complete recovery; return to the baseline level usually takes at least one hour. However, the information derived from the cortisol measurement 35 minutes after termination of the stressor still provides insight in the process of recovery.

Future research may focus on the longitudinal course of sympathetic and parasympathetic adaptation and HPA axis adaptation to prolonged stress conditions such a work-related stress. Concerning sympathetic and parasympathetic functioning and the development of hypertension, the time frame for developing enhanced vascular resistance after an initially elevated sympathetic cardiac drive as described by Palatini and Julius (2009) in association with exposure to stressors is not clear yet. Clarifying the time-course in association with work-related stress requires longitudinal research. Regarding the HPA axis, in particular the development of hypo-activity is less clear (Fries et al., 2005; Heim et al., 2000; de Kloet, Vreugdenhil, Oitzl, & Joels, 1998). The change is hypothesised to result from a progressive reduction of sensitivity of the pituitary gland to corticotrophin releasing hormone. The exact time-course of this change is not clear yet. Longitudinal research investigating structural and functional HPA axis changes could enhance knowledge about the development of HPA axis hypo-activity and its time-course. The finding that hypo-activity of HPA axis in rats developed after cessation of prolonged exposure to various stressors (Ostrander, Ulrich-Lai, Choi, Richtand & Herman, 2006), and the observation of Kudielka et al. (2006) in their review of the recent literature that ongoing chronic stress is generally associated with HPA axis hyperactivity, while HPA axis hypo-activity is more often found after a period of chronic stress, promotes studying the HPA axis in humans also after cessation of the stressor.

In summary, a clinical level of work-related stress is associated with adverse physiological changes that most likely play a role in the increased risk for CVD associated with chronic work-related stress. More specifically, we found reduced parasympathetic activity and a tendency for elevated cardiac output in males as well as females, which points towards predominance of sympathetic activity in the sympathetic-vagal balance. As basal systolic blood pressure was also elevated in males, further support was found for a sympathetic predominance in males. In addition, in males, reduced cortisol reactivity to a psychosocial stressor was found, which suggests hypo-reactivity of the HPA axis. Further longitudinal research is needed in order to assess the developmental trajectory of sympathetic and parasympathetic changes and HPA axis hypo-activity as a consequence of work-related stress and the associations with cardiovascular diseases.

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