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## Exhaustion-related changes in cardiovascular and cortisol reactivity to acute psychosocial stress



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### HIGHLIGHTS

- High scorers on SMBQ showed a blunted HPA-axis response to stress provocation.
- The SAM-axis responses to stress provocation were unrelated to stress symptom scores.
- HPA- and HR-responses, and state anxiety habituated to the second V-TSST.
- Dysfunctional stress response flexibility was related to signs of exhaustion.

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### ABSTRACT

Prior findings indicate that individuals scoring high on vital exhaustion show a dysfunctional stress response (DSR), that is, reduced cortisol reactivity and habituation to psychosocial stressors. The main aim of the present study was to examine whether a DSR may be a vulnerability factor in exhaustion disorder (ED). We examined whether a DSR is present during the early stages of ED, and still is present after recovery. Three groups were studied: 1. Former ED patients ( $n = 14$ ); 2. persons who during the past 6 months had experienced stress at work and had a Shirom–Melamed Burnout Questionnaire (SMBQ) score over 3.75, considered to indicate a pre-stage of ED ( $n = 17$ ); 3. persons who had not experienced stress at work during the past 6 months and had a SMBQ score below 2.75 ( $n = 20$ ). The participants were exposed twice to a virtual version of the Trier Social Stress Test (V-TSST), during which salivary cortisol samples were collected. In addition, high frequency heart rate variability (HF-HRV), heart rate (HR), t-wave amplitude (TWA), and  $\alpha$ -amylase were assessed to examine stress reactivity and habituation in the autonomic nervous system (ANS).

The initial analyses showed clear hypothalamic–pituitary–adrenal (HPA) axis and sympathetic nervous system (SNS) activations in both V-TSST sessions, together with habituation of cortisol and heart rate in the second session, but without any significant group differences. However, the former ED patients showed considerable variation in self-reported signs of exhaustion (SMBQ). This led us to assign former ED patients with lower ratings into the low SMBQ group (LOWS) and those with higher ratings to the high SMBQ group (HIGHS). When repeating the analyses a different picture emerged; the HIGHS showed a lower cortisol response to the V-TSST than did the LOWS. Both groups' cortisol response habituated to the second V-TSST session. The ANS responses did not differ between the two groups.

Thus, persons in a pre-stage of ED and *unrecovered* former ED patients showed signs of DSR, in contrast to healthy controls and *recovered* former ED patients. The results may be interpreted as indicating that DSR in the HPA axis is present early on in the stress process, but subsides after successful recovery.

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### 1. Introduction

During the past decade, mental illnesses such as depression and burnout/exhaustion disorder (ED) have constituted a large part of

long-term sick leaves in many post-industrial countries. The major contributing factor suggested is the rapidly changing structure of work, with a shift from manual to mental demands [4]. The working conditions that may lead to mental illness are largely known: high job demands combined with low control and poor social support from colleagues and supervisors, imbalance between effort and reward, and organizational injustice [57]. Workplace programs for primary prevention of mental disorders are rare, though probably effective [58]. Besides preventive measures on the workplace or community level, it may also be important to identify vulnerable individuals. Although personality factors, for example “overcommitment” [54] and “performance-based self-esteem” [15], have been suggested to predispose the individual to accepting unreasonable levels of responsibility and workload, it remains doubtful whether personality factors fully explain individual susceptibility to ED. Apparently, a considerable part of the population has had a heavy workload for years without developing ED. It is possible that some kind of biological vulnerability is necessary to turn normal reactions to work stress into ED. Such biological vulnerability may explain the sleep disturbances and cognitive problems that are commonly encountered already in pre-stages of ED [9].

The responsiveness of the stress system to stressors is essential for a sense of wellbeing, adequate performance of tasks and positive social interactions [6]. The development and severity of conditions such as ED depend on the genetic, epigenetic and constitutional vulnerability or resilience of the individual to stress, the exposure to stressors during ‘critical periods’ of development, the presence of concurrent adverse or protective environmental factors, and the timing, magnitude and duration of stress [6]. Inappropriate basal activity and/or responsiveness of the stress system, in terms of both magnitude and duration, might impair growth, development and body composition, and might account for many behavioral, endocrine, metabolic, cardiovascular, autoimmune, and allergic disorders [6].

In work stress studies, salivary cortisol has often been used as a biomarker for hypothalamic–pituitary–adrenal (HPA) axis activation. However, in studies of ED, conflicting results showing increased, decreased or normal cortisol levels have been reported [41–43], and recent studies of well-defined ED patients have failed to show deviating diurnal cortisol patterns compared to controls [46,47,55]. One possibility is that the earlier stages of ED are characterized by increased levels of free cortisol, while later stages are associated with hypocortisolism, representing a breakdown of the endocrine feedback mechanisms [32, 38,40]. In support of this, a review of 62 articles found that the cortisol awakening response (CAR) co-varied positively with work stress and general life stress, while clinical burnout/exhaustion and fatigue were associated with reduced CAR [5]. The cumulative long-term effect of the physiological systems’ attempts to adapt to life demands has been labeled *allostatic load*, comprising four types: 1) too frequent “hits” of stress activation, 2) lack of adaptation to repeated stressors, 3) prolonged response, i.e. inability to shut off the response, and 4) inadequate response [37]. Thus, it is relevant to study the stress response in different stages of ED.

In this context, studies using experimental stress provocation have yielded interesting results. The Trier Social Stress Test (TSST) is the most widely used tool to induce stress in laboratory settings, and has been shown to reliably evoke a stress response with concurrent activation of the HPA axis and the two branches of the autonomic nervous system (ANS) (the sympathetic nervous system – SNS, and the parasympathetic nervous system – PNS) [1]. Teachers in active work but scoring high on vital exhaustion [32], and individuals scoring high on an allostatic load index (associated with burnout and chronic stress) [24] have been reported to respond with blunted cortisol responses to the TSST. Matthews et al. [36] found that higher levels of chronic stress were related to lower blood pressure and norepinephrine responses to an acute stress task similar to the

TSST. In a study by Kudielka et al. [32], vital exhaustion was also associated with reduced HPA-axis stress response habituation to repeated exposures to the TSST, habituation that in healthy persons normally occurs rapidly and most notably at the second stress exposure [26,51]. The results suggest that one pathway leading to ED may be an inability to respond adequately physiologically when confronted with new stressors (i.e., Scenario 4: inadequate response), as well as a reduced ability to adapt to repeated stress exposure (i.e., Scenario 2 above: lack of adaptation). The possible mechanisms behind an inadequate physiological response might be an up-regulated cortisol feedback inhibition of ACTH in the pituitary [65] or a down-regulation of pituitary CRF receptors after a period of CRF hypersecretion from the hypothalamus [21]. It is interesting to note that pharmacological blocking of the HPA and sympathetic-adreno-medullar (SAM) axes has been shown to impair cognitive functions [35]. Reduced flexibility in the HPA- and SAM-axis responses to acute stress may thus result in insufficient mastery of demanding situations due to lacking cognitive activation [60]. This means that increasing effort has to be invested in order to cope with attention- and energy-demanding work tasks, which in the long run may lead to a vicious cycle of deficient mastery and exhaustion, including cognitive complaints [48].

Deviances in the parasympathetic branch of the ANS, as measured by heart rate variability (HRV), have also been associated with stress. Low HRV has been related to poor attentional control, anxiety, post-traumatic stress disorder [12], vital exhaustion [63] and work stress [59]. It is possible that persons with ED show inadequate activation of the vagal system during stress provocation, although this has as yet been insufficiently studied.

A study on dysfunctional HPA-axis activation in exhausted teachers [32] is thus far the only one of its kind, and there is a need to further extend and deepen the research on decreased HPA- and SAM-axis flexibility as possible key factors in the onset and perpetuation of ED. It will probably remain an open issue whether this dysfunction develops during long-term stress or if it existed already beforehand, representing e.g. a genetically based vulnerability [8]. Irrespective of causality, there is a strong need to study the flexibility of the HPA-axis during repeated stress provocations at various stages of ED, together with possible deviations in the SAM-axis and the PNS, in order to gain new knowledge about possible physiological correlates of relevance for the development and perpetuation of ED.

Thus, the main aim of the present study was to examine whether dysfunctional flexibility of the stress response in the HPA and SAM axes is present during early stages of ED, and still present after recovery. In addition, we also assessed ANS reactivity. Saliva cortisol and  $\alpha$ -amylase were collected to measure HPA- and SAM-axis reactivity [45]. Cardiovascular reactivity was assessed using heart rate, T-wave amplitude as a proxy of sympathetic cardiac control [30,52], and high frequency heart rate variability (HF-HRV) as a measure of vagal cardiac control [3].

Three alternative outcome patterns were:

- Outcome type 1:* Dysfunctional stress response exists early on in the exhaustion process and also after essential recovery from ED. This may indicate that DSR is a predisposing factor or has developed early on in the stress process.
- Outcome type 2:* Dysfunctional stress response exists early on in the exhaustion process, but subsides after recovery. This may indicate that DSR is a sign of incipient exhaustion, but not a predisposing factor for ED.
- Outcome type 3:* Dysfunctional stress response evolves late in the exhaustion process and remains after essential recovery from ED. This may indicate that DSR does not develop until mental “breakdown,” and remains a chronic vulnerability, predisposing for relapse.

## 2. Methods

### 2.1. Participants

Three groups were included in the study:

1. To explore the flexibility of the stress response after substantial recovery from ED, we recruited a group of former patients who had essentially recovered from work-stress-related exhaustion, in that they were back at work. These former ED patients were well known to our research group after having participated in a previous intervention study [26]
2. To obtain participants representative of pre-stages of ED, we recruited persons who during the past 6 months had experienced stress at work, but had not been in contact with the healthcare system.
3. In addition, a control group was recruited, consisting of individuals who not had experienced work stress during the past 6 months.

#### 2.1.1. Recruitment of participants representative of pre-stages of ED and controls

The two latter groups (2 and 3) were recruited via advertisements in a local newspaper. In the advertisement it was emphasized that participation required the absence of any disorder affecting the function of the lungs or heart, asthma, hypertension or neurological disorder, and that medication with psychotropic drugs, betablockers, antifungal medicine, cortisone or other hormonal medications was not permitted. Persons that contacted us to announce their interest in participating were pre-checked on all these criteria in a telephone interview to assess their eligibility. Next, still eligible persons were screened with the Shirom–Melamed Burnout Questionnaire (SMBQ, see Section 2.4.2 below), in order to obtain a low stressed control group (CONTROLS) and a high stressed group considered to be in a pre-stage of ED (PRE-ED). The inclusion criterion for the PRE-ED was a SMBQ (global) score  $\geq 3.75$ , which has been suggested to represent a valid cut-off for burnout [14]. The eligibility criterion for the CONTROLS was a score  $\leq 2.75$  on the SMBQ (global) scale. Next, suitable participants were finally re-checked by an experienced occupational health physician, at a medical work-up a few weeks before the stress provocation, for any presence of a previous or present psychiatric or somatic disorder/condition/diagnosis (cardiovascular or pulmonary disorder, hypertension, asthma, thyroid disorder, diabetes, neurological disorder or pregnancy/breastfeeding) and for daily medication (psychotropic, betablockers, hormonal or antifungal treatments). Potential participants were asked by the doctor for any previous psychiatric problems and major life events that might compromise their well-being. Only participants that

**Table 1**  
Demographic characteristics of the participating former work-stress-related ED patients (n = 14), PRE-ED (n = 17), and CONTROLS (n = 20).

| Characteristic                 | Former ED  | PRE-ED     | CONTROLS   |
|--------------------------------|------------|------------|------------|
| Age                            |            |            |            |
| Mean (SD)                      | 52.0 (7.2) | 45.4 (6.7) | 49.2 (6.4) |
| Range                          | 33–61      | 35–58      | 38–60      |
| Gender (n)                     |            |            |            |
| Men                            | 8          | 7          | 10         |
| Women                          | 6          | 10         | 10         |
| Education (n)                  |            |            |            |
| Nine-year compulsory schooling | 3          | 0          | 0          |
| Upper secondary school         | 5          | 2          | 4          |
| University studies             | 6          | 15         | 16         |
| Employment (n)                 |            |            |            |
| Full-time work                 | 12         | 14         | 14         |
| Part-time work                 | 1          | 2          | 5          |
| Parental leave                 | 0          | 1          | 1          |
| Unemployed                     | 1          | 0          | 0          |

Abbreviations: ED = exhaustion disorder; PRE-ED = individuals considered to be in the pre-stage of ED; CONTROLS = individuals with no signs of ED.

were considered unaffected, anamnesticly and clinically, by any such problem were considered eligible.

Based on the demographic characteristics of the participating former ED patients, selection of participants to the PRE-ED and CONTROLS groups was carried out with the goal to optimize their similarity in age and gender. The aim was to obtain 20 participants in each group (10 men and 10 women). Late dropouts subsequently made these groups slightly smaller; see Table 1 for the demographic characteristics of the participants.

#### 2.1.2. Recruitment of former ED patients

Supposedly recovered former ED patients (FORMER-ED) were contacted by telephone. These participants were all well known to us from several previous studies and follow-ups [24,25,43–45]. Suitable participants were finally re-checked by a physician, at a medical work-up a few weeks before the stress provocation. In the FORMER-ED group, allowance was made for medication with antidepressants of the SSRI/SNRI types, since some FORMER-ED participants were on standing medication since around their debut of ED. One male participant had recently resigned from work but was accepted due to the shortage of male FORMER-ED participants. See Table 2 for an overview of the medical characteristics of the participants.

#### 2.1.3. Comparability of demographic characteristics and health parameters

Apart from trends toward shorter education in the FORMER-ED group and lower age in the PRE-ED group, demographic factors were rather similar across groups (Table 1). The mental and physical health characteristics of the final participants, being considered eligible after the medical screening, are shown in Table 2. None of the PRE-ED or CONTROLS participants reported any major traumatic life event, nor

**Table 2**  
Medical characteristics of the participating former work-stress-related ED patients (n = 14), PRE-ED (n = 17), and CONTROLS (n = 20).

| Characteristic                                                                 | Former ED | PRE-ED    | CONTROLS  |
|--------------------------------------------------------------------------------|-----------|-----------|-----------|
| Somatic disorders/complaints, total                                            | 5         | 0         | 1         |
| Hypertension                                                                   | 1         |           |           |
| Asthma                                                                         | 1         |           |           |
| Irritable bowel syndrome                                                       | 1         |           |           |
| Ankylosing spondylitis                                                         | 0         |           | 1         |
| Back pain                                                                      | 1         |           |           |
| Neck-shoulder pain                                                             | 1         |           |           |
| Mental problems                                                                |           |           |           |
| Mild anxiety attacks                                                           | 1         | 0         | 0         |
| Feeling well                                                                   | 13        | 17        | 20        |
| Medication                                                                     |           |           |           |
| Antidepressants; SSRI or SNRI*                                                 | 3         |           |           |
| Anxiolytics (hydroxyzine)                                                      | 1         |           |           |
| Medication for somatic conditions†                                             | 2         |           | 1         |
| Hormonal replacements/contraceptives                                           | 1         | 1         | 1         |
| No medication                                                                  | 9         | 16        | 18        |
| Menopausal status                                                              |           |           |           |
| Premenopause                                                                   | 3         | 6         | 5         |
| Postmenopause                                                                  | 3         | 4         | 5         |
| SCL-90 score at or above the 90th percentile on any of the three SCL subscales | 4         | 12        | 3         |
| Mean (SD)                                                                      |           |           |           |
| SCL-90 subscale means                                                          |           |           |           |
| Anxiety (10 items)                                                             | 0.5 (0.5) | 1.3 (0.6) | 0.3 (0.3) |
| Depression (13 items)                                                          | 0.6 (0.6) | 1.5 (0.9) | 0.2 (0.3) |
| Somatization (12 items)                                                        | 0.5 (0.6) | 0.6 (0.5) | 0.2 (0.2) |
| SMBQ global score at screening                                                 | –         | 5.0 (1.0) | 1.4 (0.3) |
| SMBQ global score in connection with the medical work-up                       | 3.3 (1.5) | 4.8 (1.0) | 1.6 (0.5) |

Abbreviations: SCL-90 = Symptom Checklist 90 [7]; SMBQ = Shirom–Melamed Burnout Questionnaire; ED = exhaustion disorder; PRE-ED = individuals considered to be in a pre-stage of ED; CONTROLS = individuals with no signs of ED.

\* Selective serotonin reuptake inhibitor (SSRI) or serotonin–norepinephrine reuptake inhibitor (SNRI).

† Antihypertensive and antiasthmatic drugs or mineral supplements.



did any of the FORMER-EDs due to the fact that PTSD-like exposures/symptoms was a basic exclusion criterion already in the early baseline studies on these participants [27,46]. The three subscales Somatization (12 items), Depression (13 items), and Anxiety (10 items) from the Symptom Checklist-90 (SCL-90, [7]) (see Section 2.4.1 below) were used to assess symptoms of possible psychiatric valence. As shown in Table 2, higher scores on the Anxiety and Depression scales were observed in the PRE-ED group compared to those of the FORMER-ED and CONTROLS, which was not unexpected due to their self-defined high stress level. However, none of the participants in the PRE-ED and CONTROLS groups showed any anamnestic or clinical signs of depression or anxiety disorder; all reported “feeling well”, which was also the case for most FORMER-ED participants.

Participants were paid SEK 600 (approx. 60€) for each TSST session, and were on request reimbursed for travel expenses and wage loss (max. SEK 600/session).

## 2.2. Stress provocation

The Trier Social Stress Test (TSST) is a widely used protocol for inducing social stress in laboratory settings [29]. The TSST has consistently been shown to reliably activate the hypothalamus–pituitary–adrenal (HPA) axis and the sympatho–adrenal–medullary (SAM) system with the corresponding endocrine and cardiovascular responses [1,33]. Briefly, the test participant is asked to hold a speech and to do an arithmetic task in front of a committee. The committee consists of three actors who show no emotional responses to the test participant, making the situation very stressful.

In the present study, a virtual reality version of the TSST (V-TSST) was used for stress provocation [26]. The V-TSST consists of a waiting room and a room where the evaluating committee is seated. Three virtual persons constitute the committee: a middle-aged man placed in the middle, a young woman to the left, and a young man to the right. Comments and instructions from the committee were given by prerecorded voices, in accordance with the standard TSST protocol [29]. Comments were activated by one of the test leaders with a remote keyboard invisible to the test participant. For example, if the participant had difficulties continuing the presentation, the middle-aged man told her/him that “you have time left,” or “please continue, I will tell you when your time is up.” The V-TSST has been shown to evoke reliable cortisol and cardiovascular responses in healthy men [2,10,26,62]. During stress induction the mean increase of saliva cortisol concentration were: 88% in the initial study by Jönsson et al. which validated the V-TSST ( $N = 10$ , mean age = 28.3,  $SD = 4.4$ ); 63% in the study by Annerstedt et al. ( $N = 30$ , mean age = 27.7,  $SD = 6.7$ ); and 146% in the Fich et al. study ( $N = 30$ , mean age = 24.4,  $SD = 2.8$ ). The corresponding increases in heart rate were: 20%, 18%, and 17%, respectively.

## 2.3. Procedure

The V-TSST was completed twice within one week, with at least one day in between. The lab sessions started at 14:00 h. Participants were told not to ingest food, caffeine, or tobacco during the 2 h before the experiment. Upon arrival to the lab, the test participant (TP) was placed in a comfortable chair and asked to fill in forms covering background data, the STAI-S form (see Section 2.4.3 below), and informed consent. Then the physiological recording equipment was attached. The TP was told that she/he was going to perform two stressful tasks in a virtual reality environment. Next, the experiment was carried out according to the following sequence of conditions:

1. BASE: The TP entered the virtual waiting room and a 5 min baseline was recorded.
2. The TP was then (virtually) let into the other room, facing the committee and told that, after preparation, she/he was going to give a presentation in front of the committee, pretending to apply for a

specific job. The TP was also told that, after the presentation, the committee would inform him/her about a second task.

3. PREP: The TP was transferred back to the waiting room to prepare the speech for 5 min. Taking notes was permitted during preparation, but not allowed to be used during the presentation.
4. SPEECH: The TP again entered the other room and delivered the presentation in front of the committee (5 min).
5. MATH: The TP performed the arithmetic task, which consisted of counting backwards from 1687 in steps of 13 (5 min).
6. RECOVERY: The TP returned to the waiting room and rested for 40 min.

During the second session, the job description was slightly changed [26,53]. Otherwise, the identical procedure was used on both days, with the addition that the TP was briefed about the purpose of the experiment after it had been concluded on Day 2.

Saliva samples were collected before the V-TSST (BASE and PREP), after the V-TSST, and 1 sample every 10 min during the 40-min recovery period, in accordance with earlier studies [2,10,26,62]. Pre-menopausal women were scheduled for participation when they were in the luteal phase of the menstrual cycle [28]. ECG and respiration was recorded continuously.

## 2.4. Questionnaires

### 2.4.1. Symptom Checklist-90

The SCL-90 is a widely used standardized inventory with 90 items expressing psychosomatic and emotional distress that are rated on a five-point scale (0–4), with labels ranging from ‘not at all’ to ‘extremely’, comprising nine main symptom scales. Definition of cases with possible mental disorders (SCL cases) is in the SCL manual [7] described as having scores in any two of the nine subscales at or above the 90th percentile. Since only three scales were used in the present study, a modified definition of caseness was used by requiring only one of the three subscales to have a score at or above the 90th percentile. The Swedish version of the SCL-90 has been thoroughly validated in a previous study, from which we also used Swedish age- and gender-corrected norms [11].

### 2.4.2. Shirom–Melamed Burnout Questionnaire

The SMBQ consists of 22 items that estimate 4 dimensions of burnout syndrome: burnout, tension, listlessness, and cognitive weariness [39]. The SMBQ-Global is represented by the mean of the four dimensions. A global score  $\leq 2.75$  indicates low burnout and scores  $\geq 3.75$  high burnout, as suggested by Grossi et al. [14]. The Swedish translation used has been previously validated by Grossi et al. [14] and Lundgren-Nilsson et al. [34]. Besides being administered to select participants and the CONTROLS and PRE-ED groups, a second SMBQ was administered to all participants before the medical work-up.

### 2.4.3. Spielberger state and trait anxiety inventory

The state scale of the STAI [56] was used to estimate participants' experiences of the V-TSST. The Swedish version of STAI was validated in previous studies [16,49,50]. Increased ANS and endocrine activity during TSST have been shown to concur with increased STAI-S [2,26]. The state scale was completed before the baseline recording (BASE) and after the post-stress 40-min recovery period. Before baseline, the TPs were instructed to answer the STAI-S based on how they felt at that moment, and after the post-V-TSST rest period, the TPs were instructed to answer based on how they felt during the V-TSST.

## 2.5. Endocrine and cardiovascular recordings and data reduction

### 2.5.1. Cortisol

Cortisol (and  $\alpha$ -amylase) was analyzed in saliva sampled with Salivette® tubes (Sarstedt Ltd., Leicester, UK) with synthetic swabs.

On the day of analysis, the samples were left to thaw at room temperature for approximately 45 min and centrifuged at 3500 g for 10 min. Liquid–liquid extraction of 200  $\mu$ l saliva with 1 ml ethyl acetate, evaporated to dryness under nitrogen flow and re-dissolved in 200  $\mu$ l 10% methanol (MeOH), was carried out as described by Jensen et al. [23]. D-4-cortisol was used as the internal standard, and the transitions were:  $m/z$  363.2  $\rightarrow$   $m/z$  121.1 for cortisol and  $m/z$  367.2  $\rightarrow$   $m/z$  121.2 for D-4-cortisol. The calibration range was 0.5–90.0 nmol/l. For determination of cortisol, a volume of 25  $\mu$ l was injected into an Agilent 1200 HPLC (Agilent Technologies, Santa Clara, CA, USA), C18 2.1  $\times$  50 mm 2.6  $\mu$ m Kinetex column and a Krud-katcher ultra filter (Phenomenex, Torrance, CA). The mobile phase consisted of a 2 mM aquatic solution of ammonium acetate with 0.1% (v/v) formic acid and MeOH with 2 mM ammonium acetate and 0.1% (v/v) formic acid. Detection of cortisol was performed using a mass spectrometer, an Agilent 6460 QQQ (Agilent Technologies, Santa Clara, CA) equipped with a jet stream ESI ion source, and was operated in the positive ion mode, as described by Jensen et al. [23]. To show equivalence between different runs, natural saliva samples (2.5 nmol/l and 11.9 nmol/l) were used as control materials and analyzed together with the samples. Westgard control charts were used to document that the analytical method remained under analytical and statistical control – in other words, that the accuracy and the precision of the analytical methods remained stable [64].

### 2.5.2. $\alpha$ -Amylase

An enzymatic, colorimetric analysis for determination of  $\alpha$ -amylase was carried out with a COBAS Mira Plus (Roche Diagnostic Systems, Basel, Switzerland) and using an ABX Pentra Amylase CP reagent and ABX Pentra Multicalibrator from the Horiba Group. Samples were diluted 1:500 and run according to the manufacturer's instructions. Two urinary controls consisting of ABX Pentra N Control (55.6 U/L) and ABX Pentra P Control (165.3 U/L) were included in all runs, and plotted in Westgard control charts.

### 2.5.3. Heart rate

ECG and respiration were recorded at 1 kHz using the ML866 Power Lab data acquisition system and analyzed using its software Chart 5 (ADInstruments Pty Ltd.) and MATLAB (Math-Works, Inc., Natick, MA). ECG was assessed using disposable electrodes (Lead II Einthoven) and respiration using a strain gauge over the chest. Mean HR was analyzed for 5 min in each condition: BASE, PREP, SPEECH, MATH, and during the four following resting periods, i.e. 8 conditions. The same applies to TWA, HF-HRV and respiratory frequency below.

### 2.5.4. T-wave amplitude

The TWA is generally considered to be a proxy of cardiac sympathetic activity; its reliability, however, has been questioned by some researchers [13]. The TWA was computed as the difference in mV between the maximum 100–300 ms after the R-wave peak and the mean of the isoelectric period (40 ms) between the P- and the Q-waves [52] for each heart beat and averaged over 5 min.

### 2.5.5. High frequency heart rate variability

R–R intervals were transformed to a tachogram (ms) and linearly interpolated at 4 Hz. Data were linearly detrended and high-pass filtered (second order Butterworth filter, 0.10 Hz) to eliminate frequencies below the respiratory frequency. For each 5-min sequence, HRV power spectra were calculated, for 17 segments of 128 points (32 s) with 50% overlap, by means of fast Fourier transform (1024 points) following the application of multiple peak matched windows. The peak-matched multiple windows (PM MW) method optimizes the mean square error of the spectrum estimate when the spectrum can be expected to include peaks [17,18]. The PM MW method has been shown to give reliable results for the HRV spectrum [19,20]. The integral of the power spectrum was studied in the high frequency (HF) region (0.12–0.4 Hz) that is related to respiration [3]. The data were log-

transformed (ln) to approach a normal distribution. The respiration measures were used to ensure that the respiratory rate was within the HF range.

### 2.6. Ethics

The study protocol was approved by the Regional Ethical Review Board in Lund (reg. no. 2010/22), and conducted in accordance with the Declaration of Helsinki. All participants signed an informed consent form, which indicated specifically that participation was voluntary and could be terminated at any time.

### 2.7. Statistics

Repeated measures ANOVAs were used in all analyses for the physiological measures ( $p < 0.05$ ), with experimental CONDITION and DAY as repeated factors and GROUP as a between-subjects factor. Greenhouse–Geisser adjustments were used to correct for violation of the assumption of sphericity and were reported together with unadjusted degrees of freedom, adjusted  $p$ -values, and  $\eta^2_{\text{partial}}$ . Significant omnibus effects were followed up with polynomial contrast. Age and gender, and the three subscales of SCL 90 (Somatization, Depression and Anxiety) were used as covariates. When covariates significantly contributed to the model, the results were reported below, otherwise they were excluded from the model.

## 3. Results

### 3.1. Results based on the original three groups: FORMER ED, PRE-ED and CONTROLS

When analyzing the results of this initial group division, only a close-to-significant GROUP \* CONDITION interaction was found for cortisol. Compared to controls, a trend toward a lower response to the V-TSST was observed among persons in the pre-stage of ED, with the former ED patient group holding an intermediate position (see Appendix, Fig. A1). No other significant main effects of GROUP and no GROUP \* CONDITION interaction effects were found.

Detailed results from the initial analysis are presented in the Appendix.

### 3.2. Results based on two groups: participants with low versus high SMBQ scores

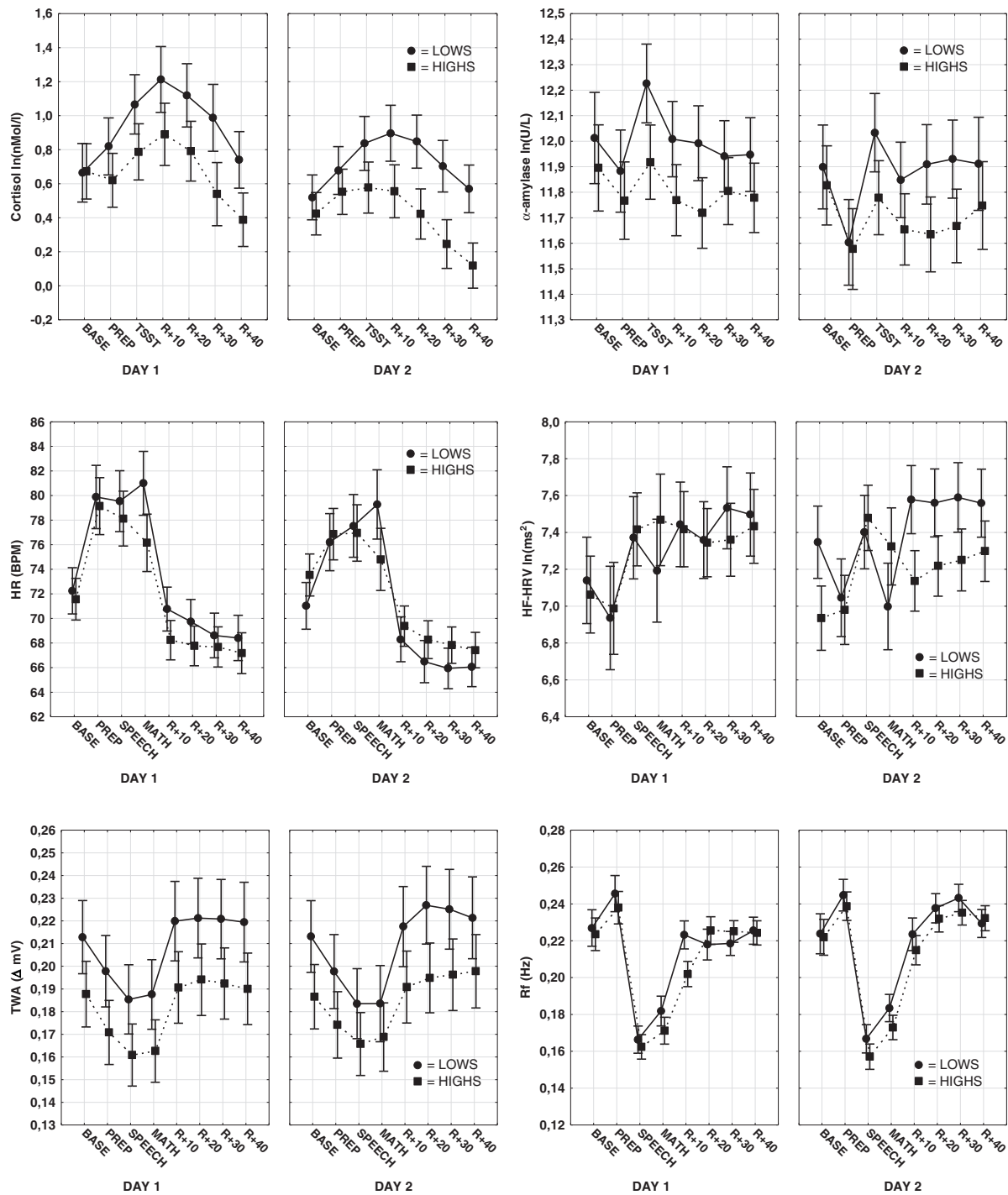
The former ED patients, who were supposed to be essentially recovered from ED, displayed considerable variation in self-reported signs of exhaustion, as measured using the SMBQ (range: 1.4–5.6). This led us to move participants in the former ED group with lower scores ( $M = 1.79$ ,  $SD = 0.35$ ,  $n = 5$ ) into the CONTROL group, and those with higher SMBQ scores ( $M = 4.08$ ,  $SD = 1.14$ ,  $n = 9$ ) into the PRE-ED groups. Thus, one group with low SMBQ scores (LOWS) and one group with high SMBQ scores (HIGHS) were examined.

#### 3.2.1. SMBQ

The LOWS ( $M = 1.56$ ,  $SD = 0.33$ ) had significantly lower SMBQ scores than those of the HIGHS ( $M = 4.47$ ,  $SD = 1.06$ ),  $t(49) = 13.54$ ,  $p < .0001$ .

#### 3.2.2. STAI-S

A main effect of GROUP showed that the LOWS ( $M = 27.71$ ,  $SD = 6.10$ ) generally had lower state anxiety than the HIGHS ( $M = 36.78$ ,  $SD = 9.73$ ):  $F(1, 49) = 24.93$ ,  $p < .0001$ ,  $\eta^2 = .34$ . Participants experienced the situation before stress induction as less anxiety-provoking than that during stress induction,  $F(1, 49) = 21.22$ ,  $p < .0001$ ,  $\eta^2 = .30$ , indicating that stress induction was successful. There was also a



**Fig. 1.** Endocrine and cardiovascular reactivity to repeated V-TSST. Values are means ( $\pm$ SE). HIGHS = individuals in the pre-stage of ED together with former ED patients scoring high on SMBQ; LOWS = CONTROLS together with former ED patients scoring low on SMBQ. R+10–40 = recovery during the first 10 to 40 min after stress induction. HF-HRV = high frequency heart rate variability, TWA = T-wave amplitude and Rf = respiratory frequency.

main effect of DAY:  $F(1, 49) = 13.86, p < .01, \eta^2 = .22$ . Finally a CONDITION \* DAY interaction showed that participants rated the V-TSST as more anxiety-provoking during the first session ( $M = 38.00, SD = 6.62$ ) compared to the second one ( $M = 32.57, SD = 9.56$ ). Corresponding STAI-S ratings before stress induction were for Day 1 ( $M = 29.63, SD = 6.62$ ) and for Day 2 ( $M = 29.84, SD = 9.56$ ). Thus, participants habituated to the stress task the second day.

### 3.2.3. Endocrine and cardiovascular measures

The endocrine and cardiovascular results presented below are depicted in Fig. 1.

**3.2.3.1. Cortisol.** A main effect of CONDITION showed that cortisol increased during the V-TSST and then decreased during recovery:  $F(6, 282) = 15.30, p < .0001, \eta^2 = .25, \epsilon = .29, F_{\text{quadratic}}(1, 47) = 49.99$ ,

$p < .0001$ ,  $\eta^2 = .52$ . There was also a main effect of DAY:  $F(1, 47) = 8.21$ ,  $p < .01$ ,  $\eta^2 = .15$ . The cortisol response was lower during the second session.

A GROUP \* CONDITION interaction revealed that participants with lower SMBQ ratings responded with stronger cortisol responses than did participants with higher ratings:  $F(6, 282) = 3.38$ ,  $p < .05$ ,  $\eta^2 = .07$ ,  $\epsilon = .29$ ,  $F_{\text{linear}}(1, 47) = 5.23$ ,  $p < .05$ ,  $\eta^2 = .10$ .

Men responded to the V-TSST with higher cortisol magnitudes compared to those of women, as shown by a GENDER \* CONDITION interaction:  $F(6, 282) = 8.47$ ,  $p < .001$ ,  $\eta^2 = .15$ ,  $\epsilon = .29$ ,  $F_{\text{linear}}(1, 47) = 4.62$ ,  $p < .05$ ,  $\eta^2 = .09$ , and  $F_{\text{quadratic}}(1, 47) = 17.42$ ,  $p < .001$ ,  $\eta^2 = .27$ . Also a main effect of GENDER was found:  $F(1, 47) = 6.10$ ,  $p < .05$ ,  $\eta^2 = .12$ ; men generally had higher cortisol levels than women did. Note that there was no significant GENDER \* GROUP interaction effect,  $F(1, 47) = 1.17$ , *n.s.*, or GENDER \* GROUP \* CONDITION interaction effect,  $F(6, 282) = 1.75$ , *n.s.*

**3.2.3.2.  $\alpha$ -Amylase.** A main effect of CONDITION was found:  $F(6, 294) = 6.23$ ,  $p < .001$ ,  $\eta^2 = .11$ ,  $\epsilon = .56$ . A contrast (order 4) showed that  $\alpha$ -amylase concentration decreased during preparation and increased during stress induction, and then decreased and stabilized during recovery:  $F(1, 49) = 10.66$ ,  $p < .01$ ,  $\eta^2 = .18$ .

**3.2.3.3. TWA.** A significant DAY \* CONDITION interaction showed that TWA magnitude was lower (increased SNS activity) during the last three recovery conditions at the first session as compared to the second one:  $F(7, 308) = 4.42$ ,  $p < .001$ ,  $\eta^2 = .09$ ,  $\epsilon = .59$ ,  $F_{\text{linear/linear}}(1, 44) = 14.31$ ,  $p < .001$ ,  $\eta^2 = .25$ .

An interaction effect of AGE \* DAY \* CONDITION was also found:  $F(7, 308) = 4.81$ ,  $p < .001$ ,  $\eta^2 = .10$ ,  $\epsilon = .59$ ,  $F_{\text{linear/linear}}(1, 44) = 16.06$ ,  $p < .001$ ,  $\eta^2 = .27$ . Younger individuals responded with a more pronounced decrease in TWA to the V-TSST during the first session and older individuals with a greater TWA decrease during the second session (not depicted).

**3.2.3.4. HF-HRV.** Only a main effect of CONDITION was found for HF-HRV as a dependent variable:  $F(7, 315) = 6.20$ ,  $p < .01$ ,  $\eta^2 = .12$ ,  $\epsilon = .29$ ;  $F_{\text{linear}}(1, 45) = 17.30$ ,  $p < .001$ ,  $\eta^2 = .28$ . However, after including AGE as a covariate, this effect completely disappeared. The effect of AGE was significant:  $F(1, 44) = 23.99$ ,  $p < .0001$ ,  $\eta^2 = .35$ . The overall HF-HRV magnitude decreased as a function of increasing age. Finally, also the effect of the SCL-90 subscale anxiety showed that anxiety covaried negatively with HF-HRV magnitude:  $F(1, 42) = 6.35$ ,  $p < .05$ ,  $\eta^2 = .13$ . No other significant effects were found.

**3.2.3.5. HR.** A main effect of CONDITION showed that HR increased during the V-TSST, decreased to a level slightly below baseline and then stabilized:  $F(7, 315) = 56.63$ ,  $p < .0001$ ,  $\eta^2 = .56$ ,  $\epsilon = .22$ ;  $F_{\text{linear}}(1, 45) = 138.44$ ,  $p < .0001$ ,  $\eta^2 = .76$ ;  $F_{\text{quadratic}}(1, 45) = 19.77$ ,  $p < .001$ ,  $\eta^2 = .31$ ; and  $F_{\text{cubic}}(1, 45) = 53.53$ ,  $p < .0001$ ,  $\eta^2 = .54$ . A CONDITION \* DAY interaction was also found,  $F(7, 315) = 4.94$ ,  $p < .001$ ,  $\eta^2 = .10$ ,  $\epsilon = .63$ ;  $F_{\text{linear/cubic}}(1, 45) = 10.97$ ,  $p < .01$ ,  $\eta^2 = .20$ , showing that the HR response was lower during the second V-TSST session. No other significant effects were found.

**3.2.3.6. Respiratory frequency.** The repeated measures ANOVA revealed a main effect of CONDITION:  $F(7, 315) = 57.94$ ,  $p < .0001$ ,  $\eta^2 = .56$ ,  $\epsilon = .71$ ;  $F_{\text{linear}}(1, 45) = 13.11$ ,  $p < .001$ ,  $\eta^2 = .23$ ;  $F_{\text{quadratic}}(1, 45) = 112.01$ ,  $p < .0001$ ,  $\eta^2 = .71$ ; and  $F_{\text{cubic}}(1, 45) = 70.10$ ,  $p < .0001$ ,  $\eta^2 = .61$ . Respiratory frequency decreased during SPEECH and MATH and then returned and stabilized to values similar to baseline.

A main effect of DAY was also found,  $F(1, 45) = 4.01$ ,  $p < .05$ ,  $\eta^2 = .08$ , showing slightly higher magnitude on the second day. Finally, the CONDITION \* DAY interaction was significant:  $F(7, 315) = 2.23$ ,  $p < .05$ ,  $\eta^2 = .05$ ,  $\epsilon = .84$ ;  $F_{\text{linear/linear}}(1, 45) = 7.13$ ,  $p < .05$ ,  $\eta^2 = .14$ .

During the second session, respiratory frequency was higher during recovery after the V-TSST compared to the first session. No other significant effects were found.

## 4. Discussion

### 4.1. General discussion

Subjective ratings of state anxiety showed that stress induction was successful. In accord with this finding, the main effects of CONDITION showed that cortisol,  $\alpha$ -amylase, and HR increased, and that respiratory frequency decreased during the V-TSST, which is in line with prior studies [2,10,26,44]. The participants estimated the stress task to be less anxiety-provoking during the second session as compared to the first one. Also indicating habituation, and in line with prior findings [26,51,53], cortisol and HR responses were larger to the first V-TSST session than to the second session. Replicating the study by Kudielka et al. [32], the high SMBQ group showed a blunted cortisol response to the V-TSST as compared to the low SMBQ group. Not replicating that study, however, both LOWS and HIGHS showed HPA-axis stress response habituation to the repeated stress task.

The men responded with larger salivary cortisol reactivity to stress induction than did the women, a result that is rather consistent in the TSST literature. However, this difference in HPA reactivity generally concerns studies in which menstrual cycle and oral contraceptives are not controlled for. Women have been shown to respond similar to men when they are in the luteal phase [28]. Because this was an inclusion criterion in the present study, the larger cortisol reactivity in men is a bit surprising. Note, however, that there were no significant GENDER \* GROUP or GENDER \* GROUP \* CONDITION interaction effects.

Concerning the cardiovascular measures and  $\alpha$ -amylase, we found no group differences during stress provocation. Nor did we find a generally reduced HF-HRV in the HIGH group, something that has been reported in a few studies on work-related stress and vital exhaustion [59,63].

In essence, the sum of the present findings indicate that HIGHS, i.e. persons in a pre-stage of exhaustion disorder together with *unrecovered* former ED patients, do show signs of reduced flexibility in stress response, in contrast to LOWS, i.e. healthy controls and *recovered* former ED patients. Taken together, this pattern of results most closely resembles the Outcome type 2 described at the end of the Introduction section: Dysfunctional flexibility in stress response is present early on in the stress process, but subsides after successful recovery. This pattern has several implications for our understanding of ED.

1. Because dysfunctional flexibility in stress response was not found among *recovered* former ED patients, there is no support for the notion that dysfunctional stress response is a predisposing factor representing, for example, genetically based vulnerability. Having recovered well from ED thus seems to be associated, not unexpectedly, with regaining normal stress response flexibility. This results does however not concord with the conclusions from the study by Wahlberg et al. [61] who at a 12 month follow-up of women with job-stress related sick leave found signs of persistent HPA axis hyporeactivity despite substantial clinical improvement.
2. The fact that dysfunctional flexibility in stress response is associated with pre-stages of ED and with lacking recovery from ED would seem to indicate that the dysfunction is somehow related to ongoing (acute or perpetuated) exhaustion symptomatology. This finding is in accord with the allostatic load aspect "inadequate response" suggested by McEwen and Seaman [37]. The results are also in accordance with the study by Juster



et al. [24], who found a blunted response to the TSST and higher self-ratings of burnout symptoms in a group with a high allostatic load index. The blunted cortisol response seen in the present study is also in line with the one previous experimental study of the cortisol response to TSST in states of self-rated vital exhaustion (see [32]).

The observation of dysfunctional flexibility in the cortisol stress response as an early sign of ED may suggest that individuals in this stage have to cope not only with everyday stressors per se, but also with inadequate energy resources. The inability to mobilize sufficient cognitive activation [60] in many everyday situations may be frustrating and pave the way for feelings of failure, leading to extraordinary work efforts in order to cope. Pushing oneself at work without attending to the need for restoration is likely to impair sleep quality and have a negative impact on social relations and family life. It is possible that these combined factors, with time, will lead to a “vicious cycle” ending in ED and long-term sick leave [31]. A recent study of a heterogeneous group of persons on long-term sick leave showed that this group displayed a blunted response to the TSST [22].

To sum up the present results, we observed a blunted cortisol response among persons in the pre-stage of ED and among unrecovered former ED patients. The results could inspire the development of new diagnostic methods to characterize the degree of ED and provide valuable information about the degree of restoration after rehabilitation. Good recovery should thus be associated with a fully normal endocrine response to acute stress. Considering the rapid development of IT and virtual reality applications, in the future it may be possible to transform the basic core of the V-TSST method of stress provocation and response assessment into a rather simple diagnostic tool to assess physiological stress response flexibility. In the meantime, it is worth noting that even a short questionnaire such as the SMBQ seems to give a fair indication of incipient ED – and the absence of recovery from ED.

#### 4.2. Study limitations

The present study has several limitations that warrant use of caution in interpreting the results. One issue is the fact that clear-cut group differences in stress response could not be shown using the original definition of groups. It is likely that the study had too little statistical power to detect group differences across three groups, partly due to an unfortunate attrition of participants in the HIGHS and former ED groups, participants that could not be replaced within the given frame of the study. Still, we did observe a tendency toward group differences in the cortisol response, with the PRE-ED group having the lowest response and the CONTROLS having the highest response, and with the former ED patients falling in between. It is possible that larger group sizes would have provided statistically significant group differences in cortisol responses. Moreover, the fact that some participants in the supposedly recovered former ED group were insufficiently recovered should preferably have led to their replacement with suitably recovered ED patients. We had, however, exhausted this possibility by (finally) inviting all former ED patients from our previous study [27] who met the inclusion criteria and had a medical history well known to us. Another issue concerns the absence of an additional group of participants in an acute state of ED; such a group could have provided a characterization of an important supplementary stage of ED. For ethical and methodological reasons, we decided not to include such participants; there is a risk that they would be in a state of crisis, thus rendering stress provocation inhumane/unethical and any results difficult to interpret. Concerning the stress provocation technique, which was a computerized virtual reality version of the original TSST, it might be questioned whether this artificial environment

was in fact as effective as the original “real” TSST. The V-TSST has, however, been shown to evoke reliable cortisol and cardiovascular responses in several previous studies [2,10,26,62]. Finally, we cannot rule out the possibility that the etiology of the development of ED in our two sub-groups of former ED patients – the recovered group that showed no signs of dysfunctional flexibility of the stress response and the unrecovered group that did – was different. It is not conceivable that the unrecovered subgroup comprised individuals with a specific vulnerability (predisposition) for ED that perpetuated their symptomatology [8]. On this point, the results from the study by Wahlberg et al. [61], suggesting a trait-like vulnerability among persons falling ill with exhaustion disorder, must also be taken into account. Interestingly, Wahlberg et al. found a reduced response to CRH at both the pituitary level (ACTH) and the adrenal level (cortisol), which suggests that the hyporeactivity is of central origin. In future studies of the neuroendocrine changes associated with ED, it may be fruitful to explore whether it is possible to define subgroups of individuals in which different combinations of vulnerability factors (e.g., genetic, personality, HPA-axis responsivity) interact in the development of ED and, if so, to study what impact such combined factors have on the likelihood of successful rehabilitation, return to work and regained quality of life. Irrespective of causality, we assume that the present results have to some extent contributed to our knowledge about the physiological correlates of relevance to the development and perpetuation of ED.

#### 5. Conclusions

- During a twice-repeated stress provocation using a virtual reality version of the Trier Social Stress Test, participants with high scores on a stress symptom rating scale, and participants with insufficient long-term recovery after exhaustion disorder, showed a blunted HPA-axis (salivary cortisol) response compared to controls with low scores on a stress symptom rating scale and well-recovered exhaustion disorder patients.
- No group differences in response to the stress provocation were seen in the ANS measures: heart rate, T-wave amplitude, respiratory sinus arrhythmia, high frequency heart rate variability and salivary  $\alpha$ -amylase.
- Cortisol and HR responses to the stress provocation were lower during the second session compared to the first, suggesting a general habituation to the stressor, corresponding with decreased self-ratings of state anxiety.
- The results may be taken to support the view that dysfunctional flexibility in stress response is present early on in the stress process, but subsides after recovery. This may indicate that dysfunctional flexibility is a sign of incipient exhaustion, but not a predisposing factor for exhaustion disorder.

#### Role of funding source

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#### Contributors

Peter Jönsson, Kai Österberg, Mattias Wallergård, Gerd Johansson, Åse Marie Hansen and Björn Karlson were responsible for the study design. Peter Jönsson and Mattias Wallergård performed the experiment

in the virtual reality lab and collected all data. Peter Jönsson and Kai Österberg chiefly wrote the manuscript. Åse Marie Hansen and Anne Helene Garde were responsible for the cortisol and  $\alpha$ -amylase analyses. Peter Jönsson conducted the cardiovascular analyses and the statistical analyses. All authors read the manuscript and approved the final version.

### Conflict of interest

All authors declare that they have no conflicts of interest.

### Acknowledgments

We would like to thank Lisbeth Prahl and Birgitta Pålsson for helping us contact the former exhaustion disorder patients, and for administrating the scheduling to the lab. We also thank Birgitta Malmberg, Carl Aronsson, Åsa Almqvist, and Kristin Mattson who carried out the medical examinations. Finally, without Joakim Eriksson, who was responsible for the virtual reality lab, it would have been impossible to complete the study.

This paper is dedicated to our beloved colleague Björn Karlson, who sadly past away during the end of the project.

### Appendix A. Results based on the initial division of participants into CONTROLS, persons in a pre-stage of ED (PRE-ED) and former ED patients

#### A.1. Questionnaire ratings

##### A.1.1. SMBQ

Subjective burnout ratings significantly differed between the three groups:  $F(2, 48) = 47.3, p < .0001$ . Pairwise Bonferroni corrected comparisons showed that the CONTROLS group ( $M = 1.58, SD = 0.50$ ), the former ED patients ( $M = 3.26, SD = 1.46$ ), and the PRE-ED group ( $M = 4.75, SD = 0.96$ ) all differed significantly from each other, all  $ps < .0001$ .

##### A.1.2. STAI-S

The repeated measures ANOVA with TIME (before and after V-TSST) and SESSION (first and second) as repeated factors, and GROUP (CONTROLS, PRE-ED, FORMER ED) as a between-subjects factor, showed a main effect of TIME:  $F(1, 48) = 23.10, p < .0001, \eta^2 = .33$ . That is, overall subjective ratings of state anxiety suggest that stress induction was successful. Also a main effect of SESSION was found:  $F(1, 48) = 15.57, p < .001, \eta^2 = .25$ ; state anxiety as a result of V-TSST was lower at the second session. The analyses also revealed a main effect of GROUP:  $F(2, 48) = 10.35, p < .001, \eta^2 = .30$ . Bonferroni pairwise tests indicated that CONTROLS had lower STAI-S scores than the PRE-ED group and the former ED patients,  $p < .001$  and  $p < .05$ , respectively. The latter two groups did not differ significantly.

Finally, there was a significant TIME \* SESSION interaction:  $F(1, 48) = 16.01, p < .001, \eta^2 = .25$ . Stress reactivity to the first session was higher to the first session than to the second one.

#### A.2. Endocrine and cardiovascular measures

##### A.2.1. Cortisol

There was a main effect of CONDITION:  $F(6, 270) = 15.52, p < .0001, \eta^2 = .26, \epsilon = .30$ , together with a quadratic contrast,  $F(1, 45) = 50.82, p < .0001, \eta^2 = .53$ , showing that cortisol increased during the V-TSST and then decreased during recovery. A main effect of DAY was also found,  $F(1, 45) = 7.56, p < .01, \eta^2 = .14$ , indicating that the cortisol response was lower during the second session.

The CONDITION \* GROUP interaction approached significance:  $F(12, 270) = 2.30, p = .072, \eta^2 = .09, \epsilon = .30$ . Compared to controls, a trend toward a lower response to the V-TSST was observed among PRE-ED participants, with the former ED patient group holding an intermediate position.

The women responded with lower cortisol reactivity to the V-TSST than the men did, as shown by a CONDITION \* GENDER interaction:  $F(6, 270) = 7.78, p < .01, \eta^2 = .15, \epsilon = .30$ ;  $F_{\text{linear}}(1, 45) = 4.36, p < .05, \eta^2 = .09$ , and  $F_{\text{quadratic}}(1, 45) = 15.99, p < .001, \eta^2 = .26$ . Moreover, a main effect of GENDER was found:  $F(1, 45) = 5.84, p < .05, \eta^2 = .12$ .

##### A.2.2. $\alpha$ -Amylase

A main effect of CONDITION:  $F(6, 288) = 5.62, p < .01, \eta^2 = .27, \epsilon = .56$ , together with a contrast (order 4),  $F(1, 48) = 9.96, p < .01, \eta^2 = .17$ ,  $\alpha$ -amylase concentration decreased during preparation and increased during stress induction, and then decreased and stabilized during recovery.

##### A.2.3. TWA

The results showed a significant CONDITION \* DAY interaction:  $F(7, 14) = 5.43, p < .001, \eta^2 = .11, \epsilon = .58$ ;  $F_{\text{linear/linear}}(1, 43) = 14.23, p < .001, \eta^2 = .26$ , and  $F_{\text{linear/cubic}}(1, 43) = 5.49, p < .05, \eta^2 = .11$ . There was also a CONDITION \* DAY \* AGE interaction,  $F(1, 43) = 5.78, p < .001, \eta^2 = .12, \epsilon = .58$ , with linear by linear  $F_{\text{linear/linear}}(1, 43) = 16.91, p < .001, \eta^2 = .28$ , and linear by cubic  $F_{\text{linear/cubic}}(1, 43) = 5.23, p < .05, \eta^2 = .11$ , contrasts. As shown in Fig. A1, younger participants responded with increased TWA (decreased SNS activity) at the second session, while older participants responded with slightly decreased TWA (increased SNS activity) to stress induction and decreased TWA during recovery at the second session as compared to the first one.

##### A.2.4. HF-HRV

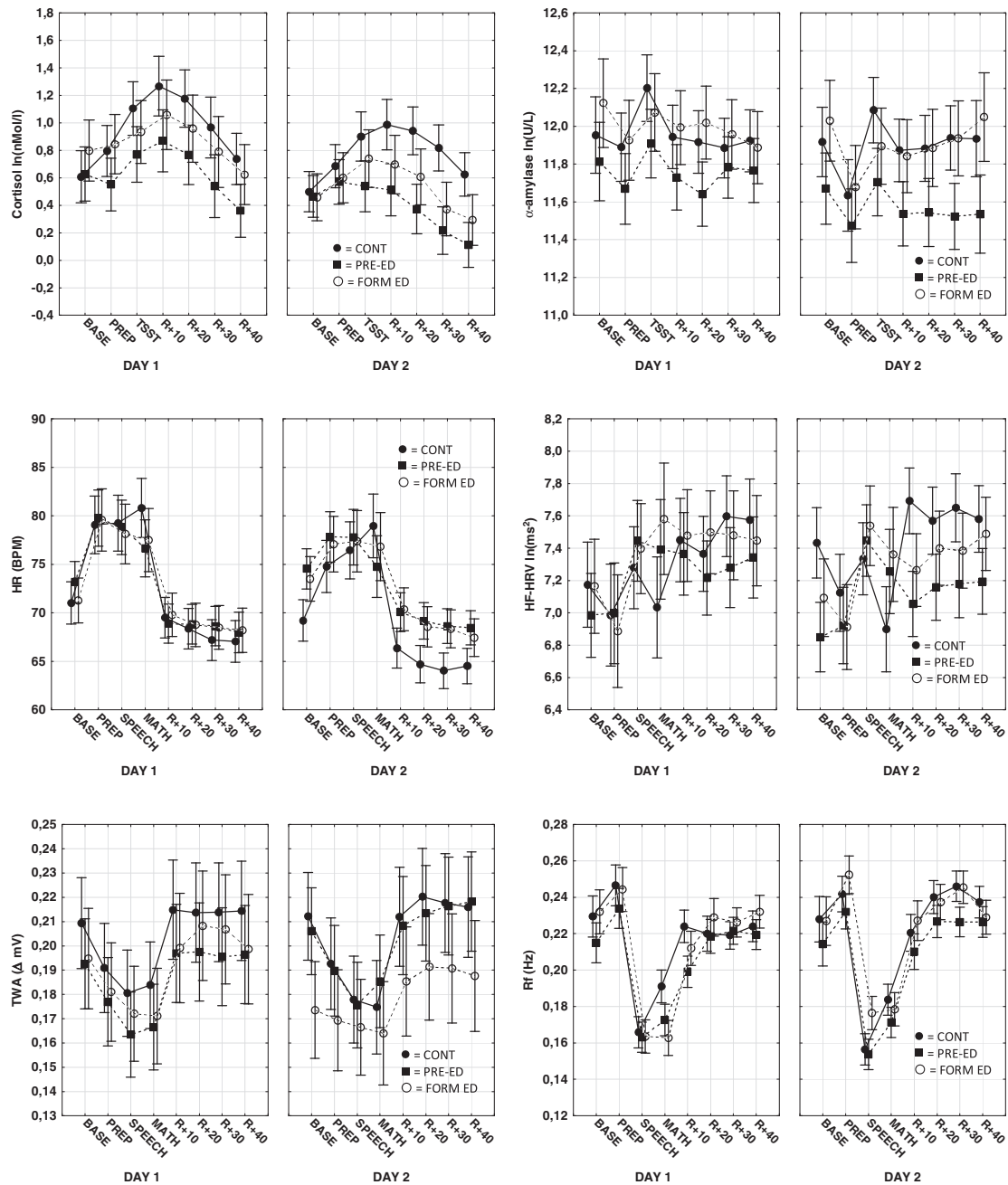
Regarding HF-HRV, an effect of AGE was found,  $F(1, 43) = 22.53, p < .0001, \eta^2 = .34$ , indicating that younger participants had higher HF-HRV magnitude compared to older participants (see Fig. A1). Also an effect of depression was found,  $F(1, 41) = 6.04, p < .05, \eta^2 = .13$ . Lower ratings of depression co-varied with higher HF-HRV magnitudes. Using anxiety as a co-variate showed a similar negative relationship, the lower ratings of anxiety, the higher HF-HRV magnitude,  $F(1, 41) = 5.45, p < .05, \eta^2 = .12$ .

##### A.2.5. HR

During the two sessions, HR increased during the V-TSST and then decreased and stabilized:  $F(7, 308) = 55.66, p < .0001, \eta^2 = .56, \epsilon = .22$ ,  $F_{\text{linear}}(1, 44) = 135.99, p < .0001, \eta^2 = .76$ ,  $F_{\text{quadratic}}(1, 44) = 18.66, p < .001, \eta^2 = .76$ , and  $F_{\text{cubic}}(1, 44) = 52.44, p < .0001, \eta^2 = .54$ . Furthermore, a CONDITION \* DAY interaction was significant,  $F(7, 308) = 5.32, p < .0001, \eta^2 = .11, \epsilon = .62$ ,  $F_{\text{linear/quadratic}}(1, 44) = 4.23, p < .05, \eta^2 = .09$ , and  $F_{\text{linear/cubic}}(1, 44) = 12.67, p < .001, \eta^2 = .22$ , indicating that HR reactivity was less pronounced during the second session.

##### A.2.6. Respiratory frequency

A main effect of CONDITION was found:  $F(7, 308) = 58.24, p < .0001, \eta^2 = .57, \epsilon = .71$ ,  $F_{\text{linear}}(1, 44) = 13.09, p < .001, \eta^2 = .24$ ,  $F_{\text{quadratic}}(1, 44) = 110.51, p < .0001, \eta^2 = .72$ , and  $F_{\text{cubic}}(1, 44) = 70.49, p < .0001, \eta^2 = .62$ . Rf decreased during SPEECH and MATH, thereafter returning to about the same level as during baseline. A main effect of DAY was also significant,  $F(1, 44) = 4.35, p < .05, \eta^2 = .09$  showing that overall Rf was slightly higher during the second session.



**Fig. A1.** Endocrine and cardiovascular reactivity to repeated V-TSST. Values are means ( $\pm$ SE). PRE-ED = individuals in the pre-stage of ED. CONT = controls scoring low on SMBQ, FORM ED = former ED patients. R+ 10–40 = recovery during the first 10 to 40 min after stress induction. HF-HRV = high frequency heart rate variability, TWA = T-wave amplitude and Rf = respiratory frequency.

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