Measuring Stress -

Assessment of cortisol as psychobiological marker for chronic work stress and burnout

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Chapter 1

Introduction and outline
1.1 Introduction

Chronic stress has since long been identified as risk factor for physical and mental health (Chrousos & Gold, 1992). In recent decades, however, public attention towards stress as serious hazard for human health and well-being has markedly increased. Especially work-related stress resulting from compressed work requirements, high competitive pressure, as well as job insecurity or unemployment is more and more often subject of public debate. Promoted by cases of prominent persons, also burnout is progressively recognized as severe condition associated with chronic stress exposure. This development is reflected in statistics describing still increasing prevalence rates of mental disorders (Ustun et al., 2004; Wittchen & Jacobi, 2005). Consequential health expenditures, but also increases in early retirement rates, inactive periods due to sick leave, productivity losses and stress-related work accidents clearly make stress a significant economic factor, too (Kalia, 2002). Thus, public awareness of stress-related issues is likely to ascend even further. Affected individuals, health experts and health policy makers need to meet the challenges connected with stress (Duhault, 2002).

In this regard and in order to design appropriate intervention and prevention strategies, a deeper understanding of how stress actually affects human health and physiology is essential. However, the biological mechanisms underlying the adverse effects of stress on health and well-being are not yet well understood. In general, stress can impact on physical and mental health both via changes in behaviour (e.g. health relevant behaviours such as dietary style, alcohol consumption, smoking, exercise, sleep behaviours, or adherence to medical regimens) or via direct alterations in diverse physiological systems. These direct stress-induced changes in human physiology are of interest in the field of psychobiological stress research. This research discipline strives to explore how stress-related psychological states and processes affect biological stress-responsive systems (e.g. central and autonomic nervous system, endocrine system, immune system) and how
psychological and biological factors interact to generate and regulate stress responses (Kemeny, 2003).

Within this area, the field of psychoneuroendocrinology specifically focuses on the role of the endocrine system in the stress process. Here, the hypothalamus-pituitary-adrenal (HPA) axis with its end-product cortisol is of major importance. Numerous investigations have addressed the role of cortisol in the development of stress-related disorders (e.g. Kudielka et al., 2006; Chrousos & Gold, 1992). The overall picture of results that emerged from this line of research, however, still is rather inconsistent. In particular, there is a significant divergence in results concerning a potential dysregulation of the HPA axis. While many studies agree that a general dysregulation of this axis seems to be involved in the pathogenesis of stress-related diseases and disorders, results are contradictory regarding the direction of this dysregulation. While some studies find a hyper-(re)activity of the axis, others report a hypo-(re)activity (Yehuda et al., 1993; Heim et al., 2000; Fries et al., 2005; Miller et al., 2007). This unsolved debate makes it difficult to interpret and draw conclusions from the existing cortisol literature. Especially for health professionals not well acquainted with this specific research area, the ambiguous findings on cortisol are of limited use regarding practical implications. Consistent and easily interpretable results from psychoneuroendocrinological research would potentially qualify cortisol as suitable and easy-to-measure biomarker for diagnostic and therapeutic purposes, but the current state of the literature still makes such applications difficult and there are only rare attempts in this direction (Nicolaidis, 2002).
1.2 Outline of thesis

One potential source for the existing divergence in the present literature might be of methodological origin. For instance, differences in study design, assessment techniques of cortisol and HPA axis regulation as well as assessments of confounding variables might have contributed to the described inconclusive pattern of results regarding the direction of HPA axis dysregulation. The present work thus aimed to identify factors that could help to shed some more light on this unresolved issue. Three independent empirical studies were conducted to this purpose. All studies were supervised by Prof. Dr. Brigitte M. Kudielka and submitted for publication in different peer-reviewed scientific journals. They are described in Chapters 3 to 5. All studies are presented in a self-contained way, each with an own introduction, methods section, results section, discussion and own references in order to allow the reader to easily access individual parts of this thesis.

While a general summery of theoretical stress concepts, stress physiology, stress research, and the existing literature on HPA axis dysregulation in individuals affected by chronic stress or burnout is given in Chapter 2, the first empirical study is presented in Chapter 3. It compares the cortisol awakening response (CAR), an established stress marker widely used especially in ambulatory research, across the four phases of the female menstrual cycle using an ambulatory, within-subject design. Evidence is provided that during the ovulatory period, the CAR is significantly increased. It is concluded that this time window should be omitted in assessment of the CAR to avoid additional, confounding variance. The second empirical study, presented in Chapter 4, also was an ambulatory study. Cortisol responses to a natural stressor, namely a demonstration lesson at school, were tested in a sample of healthy student teachers. Responses were compared to a non-stress control day and to responses to a laboratory stressor, the Trier Social Stress Test (TSST). Additionally, the CAR was assessed on the day of the lesson and on the control day and participants rated their levels of chronic work stress. Results showed that the comparison with a control
day yielded more realistic estimates of the stress-induced alterations in cortisol levels for both the laboratory stressor but especially also for the natural stressor. Additionally, the study showed that associations between the CAR and measures of chronic stress were observable only on the control day, but not on the day of the demonstration lesson. This finding suggests that associations between chronic stress and the CAR as indicator of basal HPA axis activity might be obscured by acute stress exposure. Both results underline the importance of including a control day in studies assessing cortisol response profiles and associations with chronic stress. Finally, there were no associations between cortisol responses to the demonstration lesson and the TSST suggesting that laboratory stressors do not seem to mirror natural stress responses. This finding highlights the importance for research in the natural environment, where stress processes can be observed with high ecological validity.

The third empirical study, presented in Chapter 4, was a laboratory study. Potential effects of chronic work stress and burnout on different functional levels of HPA axis regulation were tested by administering two different pharmacological stimulation tests in a sample of healthy school teachers: A low-dose ACTH\textsubscript{1-24} (Synacthen) test to assess sensitivity of the adrenal cortex and a dexamethasone-CRH (DEX-CRH) test to assess joined pituitary and adrenal cortex reactivity. Results showed that overcommitment to work (OC) and emotional exhaustion (EE) as core component of burnout were differentially related to HPA axis functioning as indexed by the two test procedures. While OC was associated with hyporeactive response profiles to DEX-CRH, EE was related to hyperreactive responses to Synacthen. These findings suggest a differential pattern of HPA axis dysregulation depending on both individual stress condition and tested functional level of the axis. The study thus might provide an approach to integrate apparently contradictory findings in terms of HPA axis hyper- and hypoactivity. Finally, Chapter 6 gives an overall discussion of the presented results from all three studies and outlines limitations and strengths as well as some ideas for future research.
1.3 References


Chapter 2

Theoretical background
2.1 Stress concepts and stress conditions

The first part of this chapter gives an overview of how stress is commonly defined and conceptualized in the literature. In the second part, the effort-reward-imbalance/overcommitment model as a model of work-related stress is described. Finally, the burnout syndrome as well as some common stress-related disorders are introduced.

2.1.1 Stress

Although commonly used to describe diverse conditions in everyday language, a widely accepted scientific definition of stress is hard to be found. Historically, the term stress stems from the Indo-European root “str”, which refers to the exertion of pressure and which is the origin of the Latin word “strigere” (to tighten) or the modern English word “to strangle” (Chrousos, 2009).

At the beginning of the 20th century, Walter Cannon (1914, 1935) was the first to give a scientific account of the term stress, under which he subsumed both physical challenges associated for example with physical activity or hunger as well as emotional states such as fear. According to Cannon, the body strives to maintain a stable level of functioning, called equilibrium or homeostasis. Failure to reinstall homeostasis after challenge or threat will lead to damage. Cannon also coined the term fight-or-flight response, which describes responses to acute danger. A discharge of the sympathetic nervous system primes the individual for the fight-or-flight response.

Later, Hans Selye (1936), clinical endocrinologist and experimental biologist who is often called the “founding father of stress research”, conceptualized stress as an unspecific response of the body to any kind of foreign or noxious stimulus. He observed a release of
glucocorticoids as universal bodily response to various kinds of irritating or painful stimulation in his laboratory animal research and an equally universal set of symptoms following stress exposure, namely swelling of the adrenal cortex, atrophy of lymphatic structures as well as gastrointestinal ulcers. Selye termed these three symptom groups as stress triad. His findings led Selye to the conceptualization of the General Adaptation Syndrome (GAS). Here, Selye describes a triphasic stress process with an initial phase of alarm, a consequent phase of resistance and a final phase of exhaustion. The first alarm phase was thought to involve the fight-or-flight response coined by Cannon.

While Selye went so far as to regard the nature of a stimulus as irrelevant to the stress response, Mason (1968a, 1968b) took a contradictory perspective and suggested that there are specific characteristics that make a situation stressful for individuals. Thus, individual processing of a stressor would be more relevant for the determination of a stress response than the stressor itself. These typical situational stress criteria suggested by Mason include novelty, uncontrollability, ambiguity, and unpredictability of a situation as well as high ego-involvement and an anticipation of negative consequences.

A further step in the stress definition process was taken by Lazarus and Folkman (1984). Their cognitive-transactional model emphasizes the interactive processes between individual and situation. They distinguish between a first appraisal of a situation in terms of perceived demands or challenges (primary appraisal), a second appraisal in terms of own perceived resources and abilities to cope with these demands (secondary appraisal), and a final appraisal (reappraisal) of the situation as final evaluation. According to this model, stress would occur only when perceived demands exceed perceived resources and adaptive capacity.
This cognitive model emphasizing subjective evaluation of stressors is carried even further by approaches leaning on constructivistic theories (Biondi & Picardi, 1999). Such approaches suggest that every given stimulus has a specific, strictly personal and idiosyncratic meaning for an individual, depending for example on an individual’s cognitive patterns, life history and current life circumstances or interpersonal relationships. Thinking along these lines, it would be assumed that there is no objective external reality but that every individual perceives the world from his or her own and very personal perspective only. This would imply that every given stressor loses its “objective” characteristics. Thus, even in a carefully controlled laboratory setting, every individual would respond differently to the same external stress stimulus based on his or her personal meaningfulness of the presented stressor.

The idea of a general, universal response to stress is also contrasted by recent findings suggesting that different stressors yield specific “neurochemical markers” by activating different physiological pathways circuits (Pacak & Palkovits, 2001).

On a more technical or even meta-level, Ursin and Eriksen (2004) suggest to differentiate four dimensions of the stress process: stress stimuli or loads, the individual stress evaluation or stress experience, a general non-specific stress alarm response, and the experience of or feedback from the stress response. The last dimension, a subjective processing of the stress response, is thought to modify the “feeling of being stressed”. This model allows integrating different stress concepts by allocating them on the different dimensions.

Finally, Chrousos (2009) defines stress as a state in which the dynamic balance or homeostasis of the organism is threatened or perceived to be threatened. Whenever a given stimulus threatening this balance exceeds a certain threshold in terms of either
severity/magnitude or of temporal duration/chronicity, the organism reacts with the activation of adaptive homeostatic systems that generate compensatory responses to the stressor (Chrousos, 2009). It is further elaborated that these compensatory responses exert their effects according to an inverted U-shaped dose-response curve, in which an optimal balance is established in the central range of the curve and in which both insufficient and exaggerated responses lead to a state of disturbed homeostasis. Such a state might be harmful for the organism in the short term or in the long term. Besides the return to homeostasis or the fall into disturbed homeostasis, Chrousos (2009) also describes a state of “hyperstasis” as a third potential outcome in the interaction between stressor and counter-regulatory responses of the organism. Hyperstasis is conceptualized as a new state, in which the organism was able to gain and learn from the experience and attains improved homeostatic capacity.

2.1.2 Work stress

While the above presented theoretical accounts on stress aim to capture general features of the stress phenomenon, this section is dedicated to stress occurring specifically within the work domain. The work context plays a central role throughout most people’s adult lives. Most adults spent major parts of their daily time at the work place. Interestingly, many working individuals even introduce themselves to other people through the occupation they are engaged in. At work, individuals not only earn their livelihood, but also build social contacts, make experiences, develop skills, and eventually gain a sense of belonging and of meaning or purpose. Work creates opportunities to perform and contribute to joined working goals and consequently to receive positive feedback, appreciation or reward. In sum, work is a vital source of an individual’s self-esteem and identity. Going along with this quite dominant position of work in many people’s lives, chronic work stress can significantly impact on an individual’s development. Unsuccessful attempts to adapt to work-related demands can over
time create a burden that puts the individual at an increased risk for health hazards. It can also negatively impact on a person’s overall level of functioning including for example social functioning, cognitive functioning or even economic productivity. Taken together, work stress is a significant and relevant risk factor for personal development. Many different theoretical concepts of work stress have been proposed in the literature (e.g. the job-demand-control model by Karasek, 1979 or the job-demands-resources model by Bakker and Demerouti, 2006). However, in the following paragraphs only the effort-reward-imbalance/overcommitment model as model of work stress is described in detail, since this conceptualization has been applied in two of the three empirical studies of this dissertation to capture work-related stress.

The effort-reward-imbalance (ERI) model by Siegrist and co-workers (1996, 2004, 2005) conceptualizes stress as a perceived disturbance in the reciprocal social exchange between a worker and a given work setting. Based on the assumption that individuals strive for a fair ratio between costs and gains in their interpersonal relationships, the model suggests that emotional distress arises whenever a person perceives an imbalance between his/her work-related achievements or efforts and his/her rewards received in return. Here, efforts cover a variety of potential work-related investments made by an individual, including for instance tolerance of adverse working conditions such as time pressure, work overload, work disturbances or high physical demands. Rewards cover for example appropriate salary, respect from colleagues and superiors, social status, adequate support, fair treatment or job security. A work contract is thought to ensure reciprocal working conditions and mutual commitment, where investments made by one side are reciprocated by comparably high investments of the other side. In this way, a cooperative and well-balanced working alliance is established between employer and employee. When this norm or contract is violated, i.e. in situations of effort-reward-imbalance, dissatisfaction, arousal and distress are elicited. Such non-reciprocal working conditions are likely to occur in incomplete working contracts,
where work obligations and work benefits are not fully detailed, or when individuals intentionally or unintentionally accept unfair work contracts, for example for strategic reasons or to terminate a status of even less unfavourable working conditions or even unemployment. Finally, also intrinsic psychological characteristics of a person might increase the probability to meet non-reciprocal working conditions. Especially a maladaptive coping style termed overcommitment to work (OC) is thought to put an individual at increased risk for work settings characterized by effort-reward-imbalance (Siegrist, 2005).

Overcommitment to work (OC) refers to a cognitive-motivational pattern characteristic for people who tend to react with excessive efforts to high working demands. Such individuals have a high need for control and approval and are likely to exaggerate their work investments, overrun personal limits and overstress own resources. They might also encounter difficulties to withdraw from work obligations during leisure time (Siegrist, 2005).

Numerous cross-sectional as well as prospective studies have identified associations between ERI and/or OC and adverse health effects. Empirical evidence suggests that non-reciprocity at work increases the risk for development of cardiovascular disease (e.g. Siegrist et al., 1990; Bosma et al., 1998; Kivimäki et al., 2002; Kuper et al., 2002; Steptoe et al., 2004), diabetes (Kumari et al., 2004), psychiatric disorders (Stansfeld et al., 1999), or alcohol dependence (Head et al., 2004). In sum, existing research consistently has shown that work stress in terms of effort-reward-imbalance or an individual coping style characterized by overcommitment to work puts individuals at an increased risk for a variety of hazards to physical and mental health. In spite of the empirical evidence, which is supportive in terms of its validity, the ERI/OC model has also been criticized on a theoretical basis. For example, it was criticized that the model oversimplifies complex work realities and does not do justice to the wide variety of occupations that exists, or, more specifically, that it does not include
variables such as job autonomy, task identity or quality of relationship with supervisors (Bakker & Demerouti, 2006).

### 2.1.3 Burnout

In the International Statistical Classification of Diseases and Related Health Problems, 10<sup>th</sup> Revision (ICD-10; WHO, 1992), which is the standard classification system and diagnostic tool published by the World Health Organization, burnout is allocated in section Z on “Factors influencing health status and contact with health services”. It thus is not part of section F on “Mental and behavioural disorders”. This classification reflects that burnout is not formally accepted as self-contained diagnosis justifying treatment, but only as a “problem related to life-management difficulty” (Z73). Burnout (coded as Z73.0) is described as state of exhaustion and is listed alongside with conditions such as “lack of relaxation and leisure” (Z73.2) demonstrating that burnout is not yet formally recognized as pathological entity but rather as a special concomitant circumstance that can optionally be recorded in addition to primary mortality coding in the ICD-10.

This classification is in line with approaches that view burnout rather as a psychosocial condition than as a psychophysiological disorder. Thus, burnout would be more an interactional result of certain psychological characteristics (e.g. a high need for control or approval) and environmental stressors (e.g. job context, organization, societal influences etc.) than a result of biogenetic, psychological factors and the environment, which is the common etiological framework for mental disorders (Farber, 2000).

In contrast to this approach, which conceptualizes burnout as a psychosocial condition, some researchers and also clinical practitioners have argued that burnout represents a form of work-related or stress-induced depression (see e.g. Tennant, 2001; Rydmark et al., 2006).
and thus do accredit burnout with a status of a clinical disorder. For example, the psychiatrists Prof. Heuser (University Medical Center Charité Berlin) and Prof. Hegerl (University Medical Center Leipzig) recently emphasized in public media the similarities in symptomatology, aetiology as well as therapeutic interventions in depressed and burned out patients (Hegerl, 2011; Heuser, 2011). They pleaded to therefore substitute burnout diagnoses by diagnoses from the spectrum of depressive disorders. In line with this reasoning, Ahola et al. (2005) found in a large cross-sectional study comprising more than Finish 3000 employees that half of all burned out individuals also suffered from some depressive disorder. Thus, burnout and depression clearly are partly overlapping conditions.

However, many studies on the relationship between depression and burnout agree that while indeed there is a substantial overlap between the two constructs, they are still far from being strictly isomorphic or redundant (Glass & McKnight, 1996; Bakker et al., 2000). Aside from only occurring within an occupational context, burnout seems to differ in still some further aspects from clinical depression. When compared to depressed patients, burned out individuals seem to suffer less from symptoms such as weight changes, psychomotoric symptoms, suicidal tendencies, lack of emotional reactivity, feelings of guilt or feelings of inferiority or resignation (Brenninkmeyer et al., 2001).

Initially observed and introduced by Freudenberger (1974) for people-oriented occupations, burnout denotes a state of frustration, fatigue and exhaustion resulting from prolonged stress exposure in the context of excessive work obligations. Since 1974, the concept has triggered and received a lot of scientific attention. For some time, it was a general consensus that burnout does not yield much additional information to the existing literature on job dissatisfaction and work stress (Glass & McKnight, 1996). Later on, however, it was acknowledged that a part of the burnout picture is not reflected in this literature. The still nowadays most widely cited definition of burnout was then given by
Maslach and co-workers (Maslach & Jackson, 1986; Maslach et al., 2001). They distinguished three components of the burnout syndrome, namely emotional exhaustion, depersonalization and reduced personal work accomplishment. Emotional exhaustion represents the core component of burnout and refers to dysphoric feelings of being emotionally drained, frustrated or overextended and of having overtaxed one's mental and physical resources. Emotional exhaustion goes along with low energy or tiredness as well as lowered motivation to engage or invest in work. The depersonalization component is also referred to as work-related cynicism. It describes disillusioned, disaffected or even hostile responses to interpersonal aspects of the work situation. This behaviour is commonly regarded as an attempt to protect oneself from stress by avoiding emotional closeness and isolating oneself from interpersonal affect. It can therefore be seen as a form of maladaptive coping with work stress. Finally, the dimension of reduced work efficacy stands for a sense of decreased performance and a pessimistic evaluation of one’s own scope of work-related abilities.

A recent review of the Deutsches Ärzteblatt (Kaschka et al., 2011) summarizes that the burnout syndrome already now is a widely-applied reason for medical certificates and exemptions from work and thus represents a highly relevant issue in health-related economics in Germany. However, the review criticizes that high-quality controlled studies on burnout are lacking and that future research will need to focus especially on aetiology, pathogenesis and possible neurobiological factors in the burnout process to further establish the scientific basis for this condition. It is concluded that the current literature does not justify a general use of the term burnout and it is therefore recommended not to use it as a medical diagnosis or as a basis for decisions regarding disability or other socioeconomic matters of the patient.
2.1.4 Stress-related disorders

While the previous section on the burnout syndrome showed that it is still under discussion whether burnout can be regarded as a clinical condition, the present section aims to present an overview on some physical as well as mental disorders that have been reliably associated with chronic stress exposure. Stress-related diseases are diseases for which consistent associations between stress exposure and likelihood of disease onset, but also reliable influences of stress on disease course and progression (e.g. duration, severity of symptoms, and likelihood of relapse or recurrence) have been found. Evidence from relevant prospective cohort studies and experimental studies is typically derived from individuals who are exposed to natural stressors such as war, bereavement, work-stress, unemployment, marital conflict, social isolation or care giving for chronically ill family members, because obviously ethical considerations prohibit exposing humans to severe or prolonged stress in experimental studies on the pathogenesis of serious diseases (Cohen et al., 2007).

Very consistent associations with chronic stress exposure have repeatedly been demonstrated for cardiovascular disease. There is strong empirical support from experimental and epidemiological studies for a reliable link between chronic psychological stress such as work stress or social isolation/loneliness and coronary heart disease or atherosclerosis (for a recent review see Steptoe & Kivimäki, 2012). Stress is thought to foster relevant pathogenic mechanisms such as systemic inflammatory processes, hypercoagulation or protracted activation of the autonomous nervous system and in this way to promote sclerotic processes, atherothrombotic and cardiac events (e.g. von Känel et al., 2009). A meta-analytical review of fourteen relevant prospective cohort studies including a total sample of more than eighty thousand employees yielded an estimate of an average 50% excess risk for coronary heart disease among employees experiencing chronic work stress, as indexed by different work-stress models, compared to individuals reporting little or no work stress (Kivimäki et al., 2006).
Further evidence for a relevant impact of stress resulting from different kinds of stressful life events has been found for a variety of functional gastrointestinal disorders (Suarez et al., 2010; Konturek et al., 2011). These are disorders affecting parts of the gastrointestinal tract, including for example the most prevalent condition Irritable Bowel Syndrome, which is characterized by chronic abdominal pain, discomfort, and bloating, or related conditions such as inflammatory bowel disease, food allergies, peptic ulcer disease or gastroesophageal reflux disease. Stress modifies the so-called “brain-gut-axis”, i.e. the intimate connection and communication between the central nervous system and the enteric nervous system, a subdivision of the autonomous nervous system that directly controls gastrointestinal functions. Via this system, stress affects various physiologic functions of the gastrointestinal system including gastric secretion (especially increased secretion of gastric acids), gut motility or visceral sensitivity (perception of pain). Stress also reduces the regenerative capacity of the gastrointestinal mucosa and mucosal blood flow and induces changes in the composition of gut microbiota (Konturek et al., 2011). Via bidirectional pathways, alterations in bacterial flora in turn profoundly impact on the brain-gut-axis and in this way modulate gastric functions such as gut motility, mucosal blood flow and gastric secretion. In addition, a significant cross talk has been shown between stress, the gastrointestinal system and the immune system (Konturek et al., 2011).

Also for a variety of skin disorders a significant effect of stress has been reported (Kimyai-Asadi & Usman, 2001; Magnavita et al., 2011). The skin represents the body’s largest organ, which separates our internal milieu from external insults. It is closely connected to other organs as well as to the central nervous system, immune system and the endocrine system. In this way, the skin enables an effective signal exchange and allows for rapid and selective responses to environmental stimuli in order to maintain local as well as systemic homeostasis (Zmijewski & Slominski, 2011). The skin is not only target of neuro-endocrine signalling, but it is also actively involved in synthesis of a variety of hormones.
(Zmijewski & Slominski, 2011). A dysregulation of this bidirectional skin neuro-endocrine signalling can lead to or be a marker of skin disorders. While the exact neurochemical mechanisms by which stress creates skin impairments are still poorly understood, they seem to include factors such as an altered release of neuromediators that regulate inflammatory and immune responses and a disruption of epidermal skin barrier function (Suarez et al., 2012). Kimyai-Asadi and Usman (2001) reviewed relevant research studies considering stress as etiologic factor in skin diseases such as psoriasis, urticaria, herpesvirus infections, atopic dermatitis, eczema, and pruritus. Here, stress comprised either exposure to stressful life events or high reported levels of psychological stress. They report that for all conditions, research could demonstrate that elevated levels of stress were associated with clinical occurrence of a skin disease, with disease recurrence/onset of new disease episodes (e.g. psoriatic flares), or with symptom exacerbation. In addition, symptoms seem to be readily responsive to relaxation or stress intervention techniques such as biofeedback, psychotherapy, relaxation therapy, meditation or hypnotherapy. Especially in the case of skin diseases, symptom manifestations in turn create psychological distress for affected individuals because of cosmetic and symptomatic effects which might trigger further symptom exacerbation and thus lead to a stress-disease cycle (Kimyai-Asadi & Usman, 2001).

While the associations between psychological stress and cardiovascular disease, gastrointestinal disease and skin disorders as well as some relevant respective mechanisms were briefly presented in the previous paragraphs, further physical diseases as for example cancer (Duijts et al., 2003; Heffner et al., 2003) or infectious diseases such as HIV (Capitanio et al., 1998; Leserman et al., 2002) have equally reliably been associated with stress.

Even more obvious, however, is the role of severe and/or prolonged psychological stress in some mental disorders. Most evident is the role of stress in the aetiology of
posttraumatic stress disorder (PTSD), which describes a pathological reaction to a severe stressor persisting longer than one month after stress exposure, or for acute stress disorder, which according to the diagnostic guidelines of the ICD-10 is diagnosed if the respective symptoms are present immediately and up to one month after stress exposure (WHO, 1992). A highly researched area is also the association between psychological or psychosocial stress and depression. In a review article, Hammen (2005) summarizes that by now there are numerous empirical affirmations for the link between life stress and depression, including also twin studies and studies on “natural experiments”. For example, some studies showed that the occurrence of critical life events were 2.5 more likely in depressed patients compared to healthy controls or that 80% of depressed individuals experienced major stressful life events before disease onset. However, Hammen (2005) also outlines methodological shortcomings of this line of research. For instance, respective research typically assesses acute episodic, circumscribed stressors, especially loss events. However, it was found that chronic stress (defined as ongoing stress for more than twelve months) was a stronger predictor of depression than such acute and episodic stress events. Hammen (2005) also recapitulates that research interest in clinical or developmental psychology and related fields tends to move away from the above outlined unidirectional models of the associations between stress and a given disease (here: depression) and that in future research, increasing awareness will be given to more dynamic and more individualized relationships between different kinds of stressors and disease. For depression, a sensitization process for repeated depressive episodes is suggested, which makes a person more vulnerable to spontaneous, i.e. stressor-independent episodes of depression over time. Interestingly, however, this principle only seems to hold for individuals with a low genetic predisposition to depression. Furthermore, depressed individuals seem to be more likely to experience or even “generate” stressful life events after disease onset, especially interpersonal events, suggesting a bidirectional link between stressful events and depressive symptomatology that might even lead to a self-perpetuating cycle of depression and stress.
Finally, Hammen (2005) highlights that also developmental influences including effects of childhood and lifetime stress exposure must be taken into account when investigating stress reactivity and the development of depression.

An approach taking an even earlier perspective into account is the theory on fetal programming (e.g. Kajantie, 2006). This research shows that factors during the fetal period may have lifelong programming effects on different physiological systems and may in this way significantly impact on disease vulnerability later in life. Respective studies demonstrated effects of this early life period on stress-related adult diseases such as cardiovascular disease, the metabolic syndrome, but also depressive disorders. Programming of the hypothalamus-pituitary-adrenal axis has been identified as one key factor mediating associations with these disorders (Kajantie, 2006).

As the previous paragraphs show, the occurrences of many stressors, especially of those that are serious and of prolonged duration, are associated with illnesses of different kinds. However, the majority of individuals exposed to stress remain healthy. Therefore, much interest has been and will be given to factors and individual differences determining vulnerability versus resilience to potential adverse effects of stress on physical and mental health, including genetic, constitutional, epigenetic as well as psychological factors (Cohen et al., 2007).
2.2 Stress physiology

The following section of Chapter 2 describes central and peripheral functions of the stress response as well as relevant physiological structures of the stress system that generate the biological stress response.

2.2.1 Physiological functions of the stress response

Within the central nervous system, the biological stress response comprises the facilitation of arousal, alertness, vigilance, attention and aggression as well as the suppression of sleep. All of these functions prepare the organism for a ready fight-or-flight response. In the periphery, blood oxygenation, respiration, heart rate, arterial blood pressure, blood coagulation, cardiovascular and muscular tone as well as glycogenolysis and lipolysis are increased (Chrousos, 2009; von Känel et al., 2009). At the same time, gastrointestinal functions are inhibited at the level of the stomach and reproduction and growth are inhibited (Chrousos, 2009). These processes help to meet the increased metabolic demands that can be associated with a number of stressors and challenges.

There are complex effects of stress on the immune system with influences on both innate and acquired immunity (Chrousos, 2009). Innate or natural immunity is activated under acute stress. It refers to an immune response that does not provide defence against any particular pathogen but rather attacks a number of different pathogens in an all-purpose fashion. Components of natural immunity (e.g. natural killer cells, macrophages, granulocytes) can react within a relatively short time frame (minutes to hours) when challenged and is relatively low in energy consumption. Activation of natural immunity under conditions of acute stress prepares the organism for a fight-or-flight response and allows the organism to readily defend itself against pathogens that it might encounter in the case of potential injuries or infections. Under chronic stress, natural immunity is inhibited, because
with increasing chronicity of the stressor, the potential adaptiveness of the immune response decreases. Adaptive or acquired immunity is characterized by greater specificity, i.e. its components (mainly lymphocytes and different kinds of immunoglobulin) respond to one and only one pathogen or invader. Adaptive immunity is slow (reaction in days to weeks) and energy intensive and thus reacts only when a stressor persists for a longer time period. Most chronic stressors, however, are associated with a global immunosuppression (Segerstrom & Miller, 2004).

2.2.2 The hypothalamus-pituitary-adrenal axis

The hypothalamus-pituitary-adrenal (HPA) axis is a principal control and regulatory system and the body’s major stress responsive axis. It links the hormone system and the central nervous system (Kudielka et al., 2012). HPA axis components within the central nervous system comprise corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP) producing neurons within the paraventricular nuclei (PVN) of the hypothalamus. CRH is released into the hypophyseal portal system, a system of blood vessels that links the hypothalamus with the anterior pituitary glands. Here, CRH triggers the secretion of adrenocorticotrophic hormone (ACTH). While AVP has only a limited direct effect on ACTH secretion by itself, it indirectly stimulates ACTH release by acting synergistically with CRH and by stimulating further CRH production at the level of the hypothalamus. The adrenal cortex is the primary target tissue of ACTH released from the pituitary glands. Here, the glucocorticoid cortisol as end-product and final effector of the HPA axis is secreted from the zona fasciculata within the adrenal cortex (Chrousos, 1998).

Under normal and non-stressful conditions, both CRH and AVP are secreted according to an circadian rhythm and in a highly concordant pulsatile fashion in the PVN of the hypothalamus. The circadian rhythm leads to a marked fluctuation of basal cortisol levels observable throughout the day. Basal diurnal cortisol levels typically reach a minimum during
the first half of the night and rise towards the morning following a gradual increase in amplitude and frequency of secretory episodes in the cascade of CRH and AVP, ACTH and cortisol. Superimposed on this basal circadian increase in the morning hours, cortisol levels sharply peak after awakening; this peak is referred to as cortisol awakening response (CAR) (Wilhelm et al., 2007). In the following, elevated levels are observed during the morning hours until levels decline again in the afternoon and towards evening and night time. The circadian rhythm is mainly controlled by the body's endogenous pacemaker, the suprachiasmatic nucleus (SCN) in the anterior part of the hypothalamus, which is also referred to as the “biological clock” (Buijs et al., 2003). The described diurnal fluctuations can be perturbed or disrupted by external factors such as exposure to light, food intake, and physical activity or by stress exposure. Under these conditions, amplitude and frequency of secretory bursts of CRH/AVP, ACTH and cortisol are increased (Chrousos, 1998).

Cortisol exerts its various effects described in the previous section via an ubiquitously distributed binary receptor system including glucocorticoid receptors (GRs) as well as mineralocorticoid receptors (MRs) (de Kloet et al., 2005). In addition to these effects, cortisol also dampens primary stress, immune and inflammatory reactions in order to prevent them from overshooting (“glucocorticoids contain the water damage caused by the fire brigade”, Chrousos, 1998). Accordingly, responses of the HPA axis are time-lagged with peak responses occurring approximately 20 minutes after stressor onset (Schlotz et al., 2008). This is why the HPA axis is also called “the slow arm” of the stress system. MRs and GRs significantly differ in terms of their affinity to cortisol with a six to tenfold higher affinity of the MR compared to the GR. Therefore, MR activity is sustained even under basal conditions while GRs are activated only under conditions of acute stress or in phases of high circadian activity (de Kloet et al., 2005).

Finally, cortisol as end-product of the HPA axis also plays a key role in the termination of the stress response. Via a negative feedback control system, higher circulating cortisol
levels lead to inhibitory effects at the level of the PVN in the hypothalamus. In this way, the stress system itself limits the time duration of exposure to glucocorticoids in the various target tissues (Chrousos, 1998).

According to the free hormone hypothesis, a major proportion of cortisol circulating in blood plasma is bound to the plasma protein corticosteroid binding globulin (CBG) and thus can be regarded as biologically inactive (Ekins, 1990; Hammond, 1990). Consequently, only the small free, unbound fraction of cortisol that is biologically active (about 5-10%) can exert the above described effects in the target tissues. Thus, CBG levels determine the amount of cortisol that is actually available to the organism (Kumsta et al., 2007). In blood, typically total cortisol concentrations are measured. Salivary cortisol, by contrast, reflects only the biologically active fraction of cortisol, because only free cortisol can enter saliva. Unbound cortisol enters saliva by passive diffusion through the cells of the salivary glands (Kudielka et al., 2012).

2.2.3 The autonomic nervous system

The second arm of the stress system encompasses the autonomous (sympathetic) nervous system. Because it responds immediately to stress or threat, it is also termed the “fast arm” of the stress system. Relevant components of this system are the locus coeruleus (LC) located within the brain stem as well as other mostly noradrenergic (NE) cell groups within the medulla and pons (Chrousos, 1998). For this reason, this part of the stress system is commonly referred to as locus coeruleus/noradrenaline or locus coeruleus/norepinephrine (LC/NE) system, which releases catecholamines (adrenaline and noradrenaline) under activation. Specifically, stress leads to the peripheral release of adrenaline from the adrenal medulla and to a discharge of noradrenaline from sympathetic nerve endings throughout the remainder of the sympathetic nervous system (Kudielka & Kirschbaum, 2007).
Interestingly, a dissociation between the HPA axis and the LC/NE system can be observed under repeated exposure to stress. While HPA axis responses habituate after repeated exposure, the LC/NE system shows a continued activation in response to repeated exposure to a given stressor (Schommer et al., 2003).
2.3 Stress research: Assessments of HPA axis functioning

The following sections will give an overview over commonly employed approaches in psychobiological stress research to assess various aspects of HPA axis functioning.

2.3.1 Basal HPA axis activity: The CAR

Basal HPA axis activity refers to activity of the axis that can be found in the absence of external stimulation or perturbation. As briefly described above, due to a rhythmic release of CRH, the HPA axis is subject to an circadian rhythm with marked variations in cortisol levels observable in the course of a day. These basal, free-cycling cortisol day profiles as well as the CAR as discrete entity that is superimposed on the basal rhythm have been described and investigated by a considerable body of psychoneuroendocrinological research. The CAR as well-established marker of basal HPA axis activity will be described in the following.

The CAR was first described in the mid 1990s by Pruessner et al. (1997) as a 50-75% increase in cortisol levels within the first 30 minutes after awakening. Then, the CAR was also introduced as reliable biological marker for the assessment of adrenocortical activity and as superior to single assessments at fixed time points. Since then, research has broadened our knowledge on the CAR, including knowledge on aspects such as normal values of the CAR, potential confounders and other relevant methodological considerations, influences of interindividual differences such as age, gender, health or psychosocial stressors on the CAR, relevant environmental factors such as light exposure or underlying regulatory physiological mechanisms.

In a highly controlled sleep laboratory study, Wilhelm et al. (2007) collected repeated blood and saliva samples during night sleep and after awakening and found that ACTH as
well as cortisol levels rise significantly steeper in the transitory phase between sleep and wakefulness than during sleep hours before awakening. It can be concluded from this study that the transition from sleep to the waking state is necessary for the occurrence of a CAR and that the CAR is a distinct entity that is not a mere part of the underlying circadian rhythm of the HPA axis. Thus, the cortisol awakening response truly is “a response to awakening”.

Regarding normative values, evidence shows that a regular CAR can be observed in more than 75% of subjects (Wüst et al., 2000). One study found that only a small proportion of subjects (approximately 15%) does not exhibit a typical increase after awakening in spite of strict methodological procedures (“CAR non-responders”) (Dockray et al., 2008). Furthermore, a medium to high intraindividual stability across days was shown along with a high interindividual variability (Wüst et al., 2000). It is important to consider the different sources of variation in order to avoid additional variance that is not related to the original research question at hand. Interindividual difference measures that have been studied in association with the CAR include factors such as age and gender, female reproductive factors such as menstrual cycle phase and hormonal contraception, physical and mental health status including mainly cardiovascular, autoimmune or psychiatric diseases, health behaviours such as smoking, sleep-related aspects such as sleep duration, sleep quality or time of awakening, as well as stress-related factors (Fries et al., 2009). While evidence for a potential impact of age, gender, smoking or use of hormonal contraceptives so far remains inconclusive or only very small effects could be demonstrated (Pruessner et al., 1997; Kirschbaum et al., 1999; Wüst et al., 2000; Kudielka & Kirschbaum, 2003), more consistent associations were shown for health status (Kudielka & Kirschbaum, 2003; Fries et al., 2009) and (habitual) time of awakening (Kudielka & Kirschbaum, 2003; Kudielka et al., 2006b; Fries et al., 2009). Also light exposure was linked to a higher CAR (Thorn et al., 2004). Regarding female menstrual cycle phase, so far only two cross-sectional studies have investigated potential influences on the CAR (Kudielka & Kirschbaum, 2003; Bouma et al., 2009).
Comparing women in the follicular and in the luteal phase, both studies found no differences in the CAR between these two groups.

Regarding psychosocial stressors, a recent meta-analysis related job stress and general life stress to a higher CAR, but fatigue, burnout, or exhaustion to a smaller CAR (Chida & Steptoe, 2009). However, research findings are not consistent on this issue (Kudielka et al., 2006a; Chida & Steptoe, 2009). A higher CAR was also found on days with an upcoming stressor and on work days as opposed to non-stress control days or weekends (Kunz-Ebrecht et al., 2004; Rohleder et al., 2007). In line with these observations, Fries et al. (2009) speculate that the CAR has the function to activate prospective memory representations and in this way to enable the individual’s orientation about the self in time and space as well as anticipation about the upcoming demands of the present day.

Concerning methodological procedures, compliance to sampling times has been identified as a crucial factor in the valid assessment of the CAR. A delayed collection especially of the first sample directly after awakening has been linked to a flattened or absent CAR and different methods have been introduced to control for and increase subjects’ adherence to sampling protocols (Kudielka et al., 2003; Dockray et al., 2008; Desantis et al., 2009; Okun et al., 2009).

A recent review by Clow et al. (2010) and co-workers gives some ideas on the underlying physiological mechanisms that regulate the CAR. The authors describe that the adrenal glands appear to be relatively desensitized to the stimulatory effects of rising levels of ACTH immediately before awakening. This mechanism seems to be mediated by the central pacemaker or biological clock, the SCN. The SCN is also functionally linked with the hippocampus, a structure that has consistently been associated with modulation of the CAR. The authors speculate that the described pre-awakening decrease in adrenal
sensitivity may provide the capacity which allows the marked increase in cortisol secretion in
the immediate post-awakening period.

As the cortisol awakening response can easily be assessed from saliva samples, it is
considered a useful and easy-to-measure marker of HPA axis activity (Kudielka et al., 2012).
It is non-invasive, inexpensive and especially suitable for ambulatory stress research,
because it requires little sampling time and effort. Samples can be self-administered by study
participants at home and can then be stored in a regular refrigerator. Because salivary
cortisol has a high temporal stability even at room temperature, samples can be sent by post
and no specialized storage or processing is required as for other biological parameters. Four
samples collected with strict reference to awakening (directly as well as 30, 45, and 60
minutes after awakening) are sufficient to cover the post-awakening period in the
assessment of the CAR.

2.3.2 HPA axis reactivity to acute stress: The TSST and natural stressors

The acute response of the HPA axis to stress or challenge can be regarded as an
important marker of individual stress regulation. Observing HPA reactivity to acute stressors
thus is a central approach in psychobiological stress research. It can help to identify healthy
and pathological stress response patterns and thus to understand how stress relates to
health and disease (Chrousos & Gold, 1992; McEwen, 1998).

Generally, the stress system can be challenged with laboratory stress protocols or
HPA axis responses to stressors occurring in real-life can be traced. Laboratory stress
protocols take place under highly controlled conditions and thus allow avoiding the influence
of additional confounding factors that might occur in field settings. However, findings derived
from laboratory research also lack ecological validity and thus are restricted to the laboratory
setting. It is unclear whether commonly employed stress protocols indeed mirror natural
stress processes. This is why ambulatory research is needed to complement findings derived from the laboratory. Ambulatory assessment traces stress process occurring in real-life and therefore yields more generalizable evidence on individual stress regulation.

One standard stress protocol for the laboratory setting that is widely used for the induction of moderate psychosocial stress is the so-called Trier Social Stress Test (TSST). It was introduced almost twenty years ago by Kirschbaum et al. (1993). This protocol comprises a simulated job interview in front of a trained audience. The participant is led by the experimenter into a room with a video camera, a microphone and two confidants of the experimenter who are introduced as members of the job committee. The participant is then instructed to prepare for the job interview and is given the opportunity to take some notes. After this brief preparatory period (3 minutes), the participant is asked to step in front of the microphone and deliver a free speech in order to introduce himself to the committee. Some questions of the committee members may follow in case the participant's speech does not fill the time designated to this task (5 minutes). Here, the two confidants especially question the candidate about his personal strengths or weaknesses that might qualify or disqualify him for the open position. After this task, the participant is asked to perform a mental arithmetic task counting backwards from 2023 in steps of 17 as fast and as accurately as possible (again 5 minutes). At the end of the test session and after collection of all post-stress samples, the subject is fully debriefed about the goal of the study and the simulated nature of the stress test and is also informed that no analyses will be conducted relying on video or microphone recordings.

According to a meta-analysis by Dickerson & Kemeny (2004), the TSST is one of only few available laboratory stress protocols satisfying both required criteria of an adequate motivated performance task for the induction of acute psychosocial stress, namely elements provoking feelings of uncontrollability and of social evaluative threat in the subject. As research over the last two decades shows, the TSST is a potent stimulator of the stress
system and induces significant and reliable changes in cardiovascular and endocrine parameters in the majority of subjects tested. Samples should be collected before onset and after cessation of the TSST protocol and in time intervals that are adjusted to the dynamic of the selected stress outcome parameter. For salivary cortisol, twofold to threefold (Kudielka, 2007) or fourfold (Kirschbaum et al., 1993) elevations over pre-stress levels have commonly been observed in most subjects. As mentioned earlier, repeated exposure to the TSST has been shown to result in habituated response profiles regarding HPA axis parameters, but uniform responses of the sympathetic nervous system (Schommer et al., 2003).

The TSST has been applied in different populations such as younger and older adults as well as clinical populations (Kudielka, 2007). Today, modifications of the TSST protocol are available that allow applying the test in children (Buske-Kirschbaum et al., 2003), in a group format (von Dawans et al., 2011), as a placebo condition (Het et al., 2009) or as a virtual reality version (Jonsson et al., 2010).

2.3.3 HPA axis regulation: Pharmacological approaches

Finally, pharmacological tests can be used to assess certain aspects of HPA axis regulation. These tests obviously have very different characteristics than assessments via the CAR or via laboratory stress protocols such as the TSST that aim to induce psychosocial stress in participants. Here, a synthetic version of an endogenous endocrine signals is administered to the organism. Different levels of the HPA axis can in this way be stimulated. The effect of this signal can be traced via different outcome parameters. For this approach, medical supervision is needed and subjects with certain health conditions might not be eligible for this approach. Furthermore, pharmacological approaches are more expensive and more laborious compared to the other assessment techniques.
One widely-used pharmacological provocation test is the so-called dexamethasone-suppression test (DST). Here, a dose of dexamethasone is administered to the body, usually orally via a pill, which mimics the effects of endogenous cortisol in the organism. As described earlier, the HPA axis is regulated by a negative feedback loop that ensures the termination of the stress response. Higher levels of circulating cortisol thus result in the inhibition of further cortisol production. During the DST, dexamethasone leads to a suppression of endogenous cortisol production, primarily through binding of dexamethasone to receptors at the level of the pituitary. In this way, the integrity of the negative feedback system can be tested. More specifically, the amount of dexamethasone-induced inhibition of endogenous cortisol production is thought to reflect the sensitivity of glucocorticoid receptors in mediating feedback inhibition of the HPA axis. Dexamethasone medication usually takes place the night before the actual test samples are collected. Subjects are instructed to take in the pill before they go to bed. On the subsequent day, markedly suppressed cortisol levels in collected test samples can be observed in healthy subjects. Deviances in the amount of suppression in both directions, i.e. either hyper-suppression or hypo-suppression (in the extreme case: non-suppression), have been associated with HPA axis malfunction and disease.

The DST is a standard tool in the diagnosis of the so-called Cushing-Syndrome, a medical condition characterized by excessively high cortisol levels, in which the described negative feedback loop of the HPA axis is impaired. The DST can also be used in the diagnosis of major depression, which typically is associated with non-suppression (Holsboer, 2001). This pattern was found to normalize during clinical remission of the depressive disorder, while persistence of the abnormal DST profile has been associated with an ominous prognostic outcome (Heuser et al., 1994). In patients with post-traumatic stress disorder (PTST), by contrast, hyper-suppression in the DST has typically been observed (Yehuda et al., 1993).
As the DST has also been criticized for its low sensitivity, the combined dexamethasone-CRH (DEX-CRH) test has been introduced as an alternative procedure with superior sensitivity in the assessment of psychiatric conditions (Holsboer et al., 1987; Heuser et al., 1994). Here, a dose of 100 µg human CRH is administered via an intravenous catheter after pre-medication with 1.5 mg dexamethasone as described above. Thus, the release of ACTH and cortisol is stimulated after prior suppression. This approach is suitable to assess joined pituitary and adrenal cortex reactivity. As for the DST, a normalization of an initially aberrant response profile in patients with remitted depression has been found to be predictive in terms of a positive prognostic outlook, while a persisting abnormality has been shown to indicate adverse treatment responses and to correlate with chronicity or relapse (Zobel et al., 1999). An increased response to the DEX-CRH procedure has not only been described for psychiatric patients, especially depressed individuals, but also for healthy subjects who are at a high genetic risk for affective disorders (Holsboer et al., 1995). Vasopressin seems to be critically involved in the described HPA axis disturbance uncovered by the DEX-CRH test. Keck et al. (2001) could show that pre-treatment with a selective vasopressin receptor antagonist abolished the described aberrant response to DEX-CRH in a line of hyper-anxious rats. This evidence supports the idea that under conditions of long-term HPA axis activation, including chronic stress exposure or psychiatric illness such as anxiety or depression, a gradual shift in the synergistic interplay of CRH and vasopressin in HPA axis regulation can be observed: While under normal conditions, CRH gives the dominating signals to stimulate ACTH secretion, an enhanced vasopressinergic drive, i.e. a more vasopressin-dominated signal for ACTH secretion can be found under the described pathological conditions. Taken together, a hyper-drive of the vasopressinergic system might mediate the excessive response to DEX-CRH described in psychiatric patients (Keck et al., 2001).

Finally, the HPA axis can also be stimulated at the level of the adrenal cortex. This level of stimulation is targeted by an ACTH stimulation test design. Here, a dose of synthetic
ACTH<sub>1-24</sub> (Synacthen) is administered via an intravenous catheter to stimulate the release of cortisol at the adrenal cortex. In the standard dose, 250 µg of ACTH is administered to test the capacity of the adrenal glands to produce cortisol in response to a strong signal. In the low-dose form of this test, by contrast, only 1 µg is applied in order to prevent supra-physiological stimulation of the adrenal glands and to assess the sensitivity of the adrenal cortex to a small incoming endocrine signal. The ACTH stimulation test has been used to assess impairments or alterations of adrenal functioning under diverse conditions including various physical or mental diseases, in high-risk groups, or under chronic stress exposure (van Waas et al., 2012; Heather et al., in press; Maes et al., 1993).
2.4 Stress and HPA axis dysregulation

This chapter aims to first give an overview of the research literature that is available on the association between chronic stress and alterations of the HPA axis as major stress responsive system and assumed mediator between stress and disease. It will be shown that findings so far are relatively inconsistent regarding the direction of dysregulation with reported hyper- or hypo(re)activity of HPA axis indicators. Next, some theoretical accounts on both hyper- and hypoactivity of the HPA axis will be discussed. Finally, some methodological considerations will be presented that might contribute to this inconsistency. At this point, also the contributions of the three empirical research studies of this thesis that are presented in Chapters 3 to 5 will be introduced.

2.4.1 Findings on HPA axis alterations under chronic stress

Findings will be presented according to the three dimensions of HPA axis functioning described in the previous paragraph: Firstly, associations of chronic stress or burnout with the CAR will be described, secondly, associations with reactivity to acute stress, and thirdly, associations with response profiles to pharmacological stimulation.

Concerning the CAR, the already above mentioned recent meta-analysis comprising 147 empirical studies from 62 research papers related job stress (in terms of e.g. effort-reward-imbalance, overcommitment to work, high demand/low control, work overload) and general life stress (e.g. financial strain, loneliness, poor marital quality) to a higher CAR, but fatigue, burnout, or exhaustion to a smaller CAR (Chida & Steptoe, 2009). However, there are several studies on the CAR that are not in line with this meta-analytically derived pattern (Kudielka et al., 2006a; Chida & Steptoe, 2009). For example, Bellingrath et al. (2008) observed no association between measures of chronic work stress, burnout or exhaustion
and basal cortisol levels including the CAR in a large sample comprising 135 healthy school teachers. Similarly, Mommersteeg et al. (2006) found that clinical burnout was not reflected in the CAR in their sample of 74 burned out individuals on partial or full sick-leave who were compared to 35 healthy controls. Grossi and co-workers (2005) reported elevated awakening values and a higher CAR volume (area under the curve, AUC) in 22 patients on sick-leave due to burnout compared to 22 working control subjects. Similarly, De Vente et al. (2003) found higher salivary cortisol responses to awakening in a sample of patients diagnosed with burnout.

Concerning HPA axis reactivity to acute stress or challenge, Bellingrath and Kudielka (2008) found that job stress in terms of overcommitment to work was marginally associated with lower response profiles to the TSST in a sample of 53 healthy school teachers. This association was observed for ACTH, total plasma as well as free salivary cortisol and it became significant when only the subgroup of responders of the total sample was analysed. In addition, a higher imbalance between efforts and rewards was related to a higher increase in total plasma cortisol concentrations in this study; however, this association disappeared when depressive symptoms were controlled for. In a previous report, Siegrist et al. (1997) observed reduced reactivity in terms of heart rate, adrenaline and cortisol output in response to a stressful Stroop task in managers suffering from high effort-reward-imbalance conditions at work. Finally, Wirtz et al. (2008) reported overcommitment to work to be associated with lower salivary cortisol as well as noradrenaline levels after the TSST in a sample of 58 male subjects. A meta-analysis on associations between acute stress reactivity and various chronic psychosocial exposures that included 729 studies from 161 articles (Chida & Hamer, 2008) reported only on reduced HPA axis reactivity in individuals with positive psychological states or traits such as happiness, internal locus of control, self-esteem, emotional regulation or active coping. No potential associations with burnout or job stress could be identified in this review due to a lack of suitable studies.
Finally, some available studies also investigated HPA axis regulation by using a pharmacological stimulation design. Applying the DST, some studies found higher dexamethasone-induced suppression of endogenous cortisol concentrations in individuals scoring high in work-related exhaustion: Bellingrath et al. (2008) applied a low-dose (0.25mg) DST and found significant associations between dexamethasone-suppressed cortisol levels and measures of burnout, vital exhaustion as well as the subscale rewards for the effort-reward-imbalance model. All measures related to lower cortisol levels. These results are in line with two earlier empirical studies that also applied the DST and that found stronger dexamethasone-induced suppression in subjects reporting high levels of chronic stress (Pruessner et al., 1999) or symptoms of burnout (Sonnenschein et al., 2007). Other studies, by contrast, found no such associations (e.g. Mommersteeg et al., 2006). Applying the combined DEX-CRH test, Wirtz et al. (2010), by contrast, recently reported higher levels of overcommitment to work to be associated with higher total plasma cortisol but not ACTH secretion in response to the test in a large sample of 200 subjects. This finding was interpreted by the authors as demonstrating an increased reactivity of the adrenal cortex but a normal reactivity of the pituitary in individuals scoring high in overcommitment to work. No study so far has applied an ACTH\textsubscript{1-24} stimulation test to explore potential associations between measures of chronic stress or burnout and the reactivity or sensitivity (low-dose test) of the adrenal cortex.

Taken together, considerable divergence in results exists in the available research literature on associations between measures of chronic stress and burnout and the different dimensions of HPA axis functioning.

2.4.2 Theoretical accounts of HPA axis hyper- versus hypoactivity

In a meta-analytical review by Miller et al. (2007), five factors that might shape HPA axis activity following chronic stress exposure are described. The first factor is the time since
onset of a stressor. It is suggested that after an initial period of elevated HPA axis activity, a counter-regulatory decline below normal levels would be observable. This factor corresponds to the time course model of hypocortisolism that has been proposed earlier (e.g. Hellhammer & Wade, 1993; Fries et al., 2005). While an initial activation of the stress axis is needed to cope with an acute stressor, the negative feedback loop of the axis would in the longer run initiate a reduction of circulating levels of cortisol. This would also protect the organism from a too long exposure to high levels of glucocorticoids. As second factor, the exact nature of the stressor is named. It is postulated that prolonged stressors threatening the physical self including also traumatic stressors result in a flattened diurnal cycle with lower morning levels, but also a reduced decline throughout the day resulting in an overall higher daily output. Stressors “only” threatening the social self, i.e. non-traumatic stressors would stimulate the HPA axis activity during the day hours. This idea corresponds to the specificity hypothesis, which proposes that different kinds of stressors elicit different patterns of HPA axis activation. The third factor named by the authors is the emotional reaction elicited by the stressor. For example, chronic feelings of shame seem to yield different cortisol output patterns (higher day profiles) than chronic feelings of loss (flatter day profiles). The fourth factor is the dimension of controllability of the stressor. While acute uncontrollable stress stimulated HPA axis activity, chronic uncontrollable stress leads to a decrease in HPA axis functioning, reflecting tendencies of withdrawal and disengagement. Finally, it is suggested that individual psychiatric consequences might determine the pattern of endocrine activation more than actual features of the stressor itself. Obviously, all these factors are partly intertwined and not always clearly separable. However, this review highlights the importance to develop and test more elaborate biological and psychological hypotheses in this field and demonstrates a number of plausible reasons for HPA axis alteration in the direction of hyperactivity or hypoactivity.
2.4.3 Methodological considerations on HPA axis assessments under chronic stress:
Contributions of the three empirical research studies

As the previous paragraph shows, obviously not all of the reported divergence in study results concerning the direction of cortisol deviance under chronic stress can be attributed to variations in methodological proceedings. Rather, certain stress conditions modify HPA axis activity and change it in the one or other direction. Generally, then, stress has the capacity to increase or decrease HPA axis activity and the mechanisms involved in this process seem to be rather complex (Miller et al., 2007). However, there also are some methodological issues that might help to discern such physiological alterations of the HPA axis from error variance. Aside from compliance monitoring, these issues include for example consideration of relevant, not content-related sources for inter- and intraindividual variation or development of adequate study designs. Here, the intended contributions of the three empirical studies of the present thesis in terms of these aspects will shortly be discussed in the following.

Regarding sources of inter- and intraindividual variation in HPA axis (re)activity, several articles are available that summarize relevant factors (Chida & Steptoe, 2009; Kudielka et al., 2009; Foley & Kirschbaum, 2010). However, our knowledge on influencing factors is still increasing and more and more factors emerge that moderate different dimensions of HPA axis functioning, including reactivity to challenge (Kudielka et al., 2009; Foley & Kirschbaum, 2010) or basal HPA axis activity as indexed for example by the CAR (Chida & Steptoe, 2009). For the CAR, one factor that so far has only been insufficiently investigated is the female menstrual cycle. So far only two cross-sectional studies have investigated potential influences of menstrual cycle phase on the CAR (Kudielka & Kirschbaum, 2003; Bouma et al., 2009). Comparing women in the follicular and in the luteal phase, both studies found no differences in the CAR between these two groups. So far, there is no longitudinal study available to assess the impact of menstrual cycle phase on the CAR
in a within-subject design and also there is no study that has integrated all four phases of the menstrual cycle, especially also the ovulatory period at mid-cycle. Such a research design was thus realized in the first empirical study of this thesis (please see Chapter 3).

Concerning the development of adequate study designs, a recent study by Lovallo et al. (2010) highlighted the importance of including a control day in studies comprising exposure to the TSST. It was demonstrated in this study that a control day helps to appreciate the amount of TSST-induced perturbation of the underlying diurnal cortisol profile as compared to a pre-post-design, which is commonly used in other studies. This issue will be discussed in the second empirical paper of this thesis, where we postulate based on our findings that a control day is especially needed in the assessment of natural stressors (please see Chapter 4). Furthermore, our findings in this study suggest that associations between chronic stress measures and the CAR might sometimes be obscured by acute stress exposure on the day of CAR assessment and that also for this reason inclusion of a resting control day is a favourable complement. Finally, this study also raises the issue of ecological validity and generalizability of findings obtained with laboratory stress designs. Response profiles to the TSST are compared with responses to a natural stressor.

Also in the context of study design, most previous studies on the association between markers of HPA axis activity and chronic stress have investigated only single stress conditions (e.g. only the effort-reward-imbalance model) and have used only one marker or test to assess HPA axis functioning (e.g. only the CAR, only the DEX-CRH test). This approach does not allow to assess differential effects of different stress conditions on different levels or dimensions on HPA axis functioning. In the third study of the present thesis, both the combined DEX-CRH test and ACTH$_{1-24}$ (Synacthen) sensitivity test were applied in one sample of healthy teachers. In this way, it was possible to assess differential
effects of chronic work stress on different functional or anatomical levels of the HPA axis (please see Chapter 5).
2.5 References


Chapter 2: Theoretical background


Chapter 2: Theoretical background


Chapter 2: Theoretical background


Chapter 2: Theoretical background


Chapter 3

The cortisol awakening response (CAR) across the female menstrual cycle


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3.1 Abstract

The cortisol awakening response (CAR) has been established as a useful marker of hypothalamus-pituitary-adrenal (HPA) axis activity and has become a standard tool for stress research in ambulatory settings. Although much knowledge has been accumulated on a variety of factors modulating the CAR, the impact of the female menstrual cycle, especially during ovulation, still remains unclear. To the best of our knowledge, this is the first study that measured the CAR during menses, the follicular phase, ovulation and the luteal phase in a repeated measurement design. For this purpose, a final sample of 29 naturally cycling, healthy, non-smoking, and medication-free women collected saliva samples directly after awakening as well as 30, 45, and 60 minutes later during each of the four different phases. To determine the timing of ovulation, an ambulatory chromatographic ovulation test kit was applied.

A repeated measurements ANOVA resulted in a significant interaction effect sample x cycle phase (p=0.04), with the highest awakening response during ovulation. While awakening cortisol levels were comparable across the four cycle phases (p=n.s.), the net increase was significantly elevated during ovulation (p=0.05). Our data also confirmed earlier cross-sectional results reporting no differences in the CAR between the follicular and luteal phase. Finally, a concurrent assessment of mood applying the POMS (Profile of Mood States) yielded no differences across the four cycle phases (all p=n.s.).

In sum, the present data points to the idea that the CAR is elevated during ovulation, an effect which is presumably mediated by elevated sex steroid levels during the ovulation period.
3.2 Introduction

The cortisol awakening response (CAR) is a sharp and discrete burst of cortisol secretion in the first hour post-awakening that is superimposed on the continuous circadian rise occurring during the second half of the night (Wilhelm et al., 2007; Clow et al., 2010). Since its introduction as an index of adrenocortical activity and reactivity (Pruessner et al., 1997; Schmidt-Reinwald et al., 1999), the CAR has meanwhile become a standard tool for the assessment of hypothalamus-pituitary-adrenal (HPA) axis regulation in ambulatory settings (for reviews see Wüst et al., 2000b; Clow et al., 2004; Kudielka & Wüst, 2008). The CAR has gained high attractiveness for stress research because it was repeatedly shown to be related to a variety of psychosocial and stress-related factors as well as health and disease states (for reviews see Fries et al., 2009; Kudielka & Wüst, 2010). Meanwhile, a fair amount of research has also been dedicated to describe moderating and intervening factors like for example age and gender, health behaviors, genetic factors, personality traits, light exposure and time of awakening (Scheer & Buijs, 1999; Wüst et al., 2000a; Kudielka & Kirschbaum, 2003; Kudielka et al., 2006; Badrick et al., 2007).

So far, only two cross-sectional studies reported on the impact of the female menstrual cycle phase on the CAR. Kudielka and Kirschbaum (2003) found no differences in CAR profiles between healthy women who were either in the follicular or in the luteal phase as determined by subjects’ self-reports. Accordingly, in a much larger study sample of adolescents Bouma et al. (2009) did not observe any differences in the CAR between girls in the follicular versus luteal phase. The hormonal activity of the gonads during the menstrual cycle is regulated by hypothalamic GnRH (gonadotropin releasing hormone) triggering the pituitary polypeptides LH (luteinizing hormone) and FSH (follicle stimulating hormone). LH, FSH, and estradiol levels are low during menstruation and the early follicular phase and sharply increase directly before or during ovulation. After the peak, hormone levels decrease throughout the luteal phase. In contrast, progesterone levels are highest during the luteal
phase. Considering on the one hand evidence from animal and human research showing that gonadal steroids are potent agents that can interact with and modulate HPA axis functioning (see e.g. Carey et al., 1995; Kirschbaum et al., 1996; Kudielka et al., 1999; Viau, 2002) and on the other the pronounced hormonal fluctuations across the menstrual cycle with peak levels during ovulation, it can be speculated whether the CAR might be altered particularly during ovulation.

In sum, to test whether ovulation is characterized by an altered CAR, the present study for the first time compares CAR profiles across the full female menstrual cycle encompassing menses, the follicular phase, ovulation, and the luteal phase following a within-subject design. Additionally, to overcome the weakness of cycle phase determination solely based on self-reports and to ascertain the exact time point for ovulation, an ambulatory chromatographic ovulation test kit was applied.

3.3 Methods

Participants

Thirty-five naturally cycling female students volunteered to participate in the study. Three candidates were not eligible due to habitual smoking. To screen for premenstrual dysphoric disorder (PMDD), the premenstrual symptoms screening tool (PSST) by Steiner et al. (2003) was applied. Three women (9.4%) qualified for PMDD, which is in accordance with incidence rates reported by Steiner et al. (2003), and were therefore excluded from the analyses.

The final sample was comprised of 29 healthy and medication-free subjects as assessed by an anamnestic medical interview. All participants were between 20 to 34 years of age (mean \( \pm \) SD age: 26.3 \( \pm \) 3.9 years) with a normal body mass index (mean BMI: 22.1 \( \pm \) 3.9).
SD BMI: 2.9). Neither of the women had children nor was pregnant. None of the women took oral contraceptives or used any other type of hormonal contraception during the time of the study. Although 20 subjects had at some point in time in the past taken oral contraceptives or other types of hormonal contraception, all had ceased doing so for at least four months before study entry. Cycle duration ranged between 26 and 34 days with a mean of 30.0 ± 2.0 days (SD).

Before entering the study, written informed consent was obtained from all participants. All subjects received a monetary compensation of 25 Euros for their participation in the study. The study protocol was approved by the ethics committee of the German Psychological Society (DGPs).

**Study Protocol and Saliva Collection**

Sixteen cortisol Salivettes (Sarstedt, Nümbrecht, Germany; four Salivettes for each test day), an ambulatory chromatographic ovulation test kit (gabControl by gabmed, Nettetal, Germany), detailed written instructions, and a paper diary were sent to each participant by mail. Any questions regarding the study protocol or the correct administration of the provided materials could be clarified via telephone if necessary. Subjects completed four test days over the period of a complete menstrual cycle. The first test day was completed during menstruation. The second test day was performed during the follicular phase defined as two to six days after offset of menstruation. The third test day took place during ovulation defined as one to two days after the LH (luteinizing hormone) surge as determined by a chromatographic ovulation predictor test kit. According to the instructions given by the manufacturer, subjects self-administered a urine test daily from three days prior to the expected LH surge. The fourth test day was a day during the luteal phase defined as six to nine days after the LH surge.
Participants were instructed to collect four saliva samples during the first hour after awakening on each of the four test days at home: directly after awakening, 30, 45, and 60 minutes after awakening. It was stressed that the first sample needs to be collected directly after awakening while still lying in bed in a supine position in order to avoid a potentially confounding effect of a too long delay between the time of awakening and the first saliva collection on the CAR (Kudielka et al., 2003; Desantis et al., 2010; Okun et al., 2010). Furthermore, participants were requested to choose a constant awakening time between 0600h and 0830h on all four test days and to use an alarm clock to ensure as comparable awakening times across cycle phases as possible. To avoid contamination of saliva with blood, participants were instructed not to brush their teeth before completing the four morning saliva samples. Additionally, eating and drinking beverages containing caffeine or fruit juices were not allowed during the first hour after awakening. Besides these restrictions, participants were free to follow their normal daily routines on the sampling days.

In addition to collecting saliva samples, a paper diary had to be filled out in which participants reported individual awakening times, sampling times, as well as current mood (see psychological assessment) for each of the four sampling days.

Participants completed all four study days within one menstrual cycle. However, if participants for some reason missed a sampling day in one cycle (e.g., anovulation in cycle as indicated by the ovulation test kit, missing of the time of ovulation due to misuse of the ovulation test procedure, minor temporary illness or others), they were allowed to complete their testing within the next cycle. This was the case in five out of the 29 participants.

Biochemical Assays

Participants stored their completed saliva samples in the refrigerator until completion of sampling. Upon completion, saliva samples and diaries were returned by mail and stored at -20°C. When all participants had returned their materials, samples were sent to the
biochemical laboratory at the University of Trier to be assayed. Samples were assayed in duplicate using a time-resolved immunoassay with fluorometric detection (DELFIA). The intra-assay coefficient of variation was between 4.0% and 6.7%, and the corresponding inter-assay coefficients of variation were between 7.1% and 9.0%. The averages of the duplicate values were used in further analyses.

**Psychological Assessment**

Emotional states on each of the four test days were assessed using the Profile of Mood States questionnaire by McNair et al. (1971) in a German short version by Biehl and colleagues (1986). This 35-item version is comprised of four scales with a Cronbach’s α ranging from 0.89 to 0.95, respectively: depression (14 items), vigor (7 items), fatigue (7 items), and anger (7 items). Participants were asked to rate their mood of the present day on a 7-point scale ranging from 1 = not at all to 7 = very much (e.g., “Today I feel exhausted”).

**Compliance Monitoring**

Subjects’ noncompliance to a given sampling procedure can potentially invalidate cortisol data gained in ambulatory settings and different methods have been introduced to control for compliance biases or to improve subjects’ adherence (Kudielka et al., 2003; Kudielka et al., 2007; Desantis et al., 2010; Okun et al., 2010). In a first experimental study, we found that subjects’ compliance can be significantly increased if electronic monitoring devices are applied and, at the same time, subjects are informed about the devices’ nature (Kudielka et al., 2003). With regard to the CAR, two independent recent studies showed that a temporal delay of >15 minutes between wake time and the first cortisol sample collection is the critical time frame that potentially leads to a flattened morning cortisol profile (Desantis et al., 2010; Okun et al., 2010). Therefore, in the present study, participants were informed about the importance of accurate timing of their saliva collection. In addition, subjects were told that their saliva collection timing would be electronically monitored. Actually, however, every participant received a dummy MEMS Track Cap (AARDEX Ltd., Zug, Switzerland).
**Statistical Analyses**

Statistical analyses were carried out using the PASW statistical software package (Chicago, IL, USA; Version 18). Results are expressed as mean ± standard deviation (SD). For all General Linear Models (GLMs), F-values, degrees of freedom, and p-levels were corrected according to Greenhouse-Geisser procedure whenever sphericity was violated. Effect sizes were calculated by partial eta squared ($\eta^2$), expressing the amount of variance explained in the dependent variable cortisol by the respective effect. Cortisol levels were log-transformed before statistical analysis (the figure and table presents untransformed values for illustration reasons).

A first set of ANOVAs for repeated measures showed that neither awakening times nor sleep duration differed across the four cycle phases (for details see table 1). Both, age and BMI did not result in any significant main or interaction effect on cortisol levels as computed by ANOVAs with the two repeated measurement factors samples and cycle phase (all F<1.55, all p>0.21). Therefore, none of these variables were included in the main model.

<table>
<thead>
<tr>
<th></th>
<th>Menses</th>
<th>Follicular phase</th>
<th>Ovulation</th>
<th>Luteal phase</th>
<th>$F$-value</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Awakening time</strong></td>
<td>07:23 ± 00:54h</td>
<td>07:25 ± 01:01h</td>
<td>07:21 ± 01:07h</td>
<td>07:28 ± 00:57h</td>
<td>$F_{2,30, 54.49}=0.54$</td>
<td>p&gt;0.10</td>
</tr>
<tr>
<td><strong>Sleep duration</strong></td>
<td>07:17 ± 01:03h</td>
<td>06:53 ± 01.22h</td>
<td>06:48 ± 00:52h</td>
<td>07:10 ± 00:56h</td>
<td>$F_{3,84}=2.15$</td>
<td>p&gt;0.10</td>
</tr>
<tr>
<td><strong>Cortisol increase</strong></td>
<td>7.98 ± 8.88 nmol/l</td>
<td>7.95 ± 7.97 nmol/l</td>
<td>11.51 ± 9.45 nmol/l</td>
<td>7.71 ± 6.20 nmol/l</td>
<td>$F_{3,28, 63.94}=2.99$</td>
<td>p=0.05</td>
</tr>
</tbody>
</table>
To analyze the effect of cycle phase on the CAR, we then computed a two-way ANOVA for repeated measures comprising the within-subject factors sample (0, +30, +45, +60 minutes post awakening) and cycle phase (menstruation, follicular phase, ovulation, luteal phase). An additional one-way ANOVA for repeated measures with the repeated measurement factor cycle phase was computed with the cortisol increase (defined as difference between the individual maximum cortisol value and the awakening value) as dependent variable. The same analysis was computed with the awakening sample as dependent variable to test whether cortisol concentrations directly after awakening differ across the different cycle phases. Students paired t-tests were applied post hoc to compare cortisol levels between cycle phases.

Finally, potential effects of subjects’ incompliance were analyzed as follows: In line with previous research, we defined incompliance as a temporal delay of $>15$ minutes between the reported time of awakening and the timing of the first saliva sample (Desantis et al., 2010; Okun et al., 2010). In a first step, we compared cortisol increases between compliant and noncompliant subjects per cycle phase, if applicable. In a second step, we inspected the potential impact of the time delay on the cortisol increase in noncompliant subjects (delay expressed in minutes and introduced as continuous covariate in the ANOVA). In a third step, we rerun the main ANOVA analyses testing the effect of cycle phase on the CAR in a subsample excluding all participants with reported incompliance during at least one cycle phase.

### 3.4 Results

The main ANOVA to test the effect of cycle phase on the CAR yielded a significant main effect sample ($F_{1.70, 47.45} = 12.99$, $p<0.001$, $\eta^2 = 0.59$) reflecting the typical course of the CAR. In respect to cycle phase, the main effect cycle phase was non-significant ($F_{3, 84} = 1.15$, $p=0.33$), but a significant interaction effect sample x cycle phase ($F_{4,85}$,
Chapter 3: The CAR across the menstrual cycle

\[ F_{3, 84} = 1.29, p = 0.28 \]

...indicating that the course of the CAR is significantly modulated by cycle phase. As can be seen in Figure 1, the peak at ovulation was later (45 minutes vs. 30 minutes) compared to the other three cycle phases. Furthermore, while the awakening sample did not differ significantly across the four cycle phases (\( F_{3, 84} = 1.29, p = 0.28 \)), the net increase did (\( F_{2.92, 63.94} = 2.99, p = 0.05 \)). Post hoc paired t-tests revealed that the increase was larger during ovulation than during menstruation (\( t_{28} = 2.34, p = 0.03 \)), the follicular phase (\( t_{28} = 2.75, p = 0.01 \)), and luteal phase (\( t_{28} = 2.26, p = 0.03 \)), respectively (see Table 1). However, if a Bonferroni correction of the nominal \( \alpha \)-level is applied to account for multiple testing, the significance level should be set at \( \alpha = 0.017 \) for three comparisons.

**Figure 1:** The Cortisol awakening response (CAR) across the menstrual cycle in N=29 healthy females; for illustrative reasons data presented in nmol/l (raw data), for statistics cortisol levels are logarithmically transformed.
Incompliance with the given saliva sampling schedule, as defined above, was found for two participants during menstruation, four participants during the follicular phase, and two participants during ovulation. No incompliance was found in the luteal phase. Despite these reports of incompliance, paired t-tests revealed that the cortisol net increases did not significantly differ between compliant and noncompliant subjects (all F<1.28, all p>0.21). Additionally, minutes of delay as continuous variable had no significant impact on the course of the CAR in any of the affected phases (interaction effects sample x minutes of delay: all F<1.67, all p>0.20). Thirdly, when we rerun the main ANOVA based on a subsample of only those 23 participants who reported no incompliance across all four cycle phases, a marginally significant interaction effect sample x cycle phase still remained visible (F_{4.52, 99.46}=2.12, p=0.08, \eta^2=0.09).

Finally, emotional states as measured by the four scales of the POMS did not vary across the four cycle phases as analyzed by repeated measures ANOVAs (all F<1.58, all p>0.20).

3.5 Discussion

Considering the marked fluctuations of gonadal steroids across the female menstrual cycle, this study set out to investigate whether the cortisol awakening response (CAR) is significantly modulated by the menstrual cycle using a within-subject design. For the first time, the CAR was not only assessed during the follicular and luteal phase but also during the ovulatory period, the time when estrogen as well as LH (luteinizing hormone) and FSH (follicle stimulating hormone) reach their peak levels. In the present study, the individual timing of ovulation was determined by a chromatographic ovulation test and did not rely on participants’ self-reports. In a sample of 29 healthy, free-cycling women we found a larger cortisol net increase during ovulation compared to the three other phases. Confirming earlier cross-sectional results (Kudielka & Kirschbaum, 2003; Bouma et al., 2009), however, cortisol net increases during the follicular and the luteal phase did not differ significantly.
Furthermore, the present results point to the idea that the CAR during ovulation might be characterized by a delayed peak as well as a delayed return from peak (see Figure 1).

Previous literature on the impact of the female menstrual cycle on HPA axis functioning has yielded somewhat inconsistent results. Reviewing twelve studies on the effects of the menstrual cycle on HPA axis parameters with only two studies reporting significant effects, Leibenluft and colleagues (1994) concluded that the menstrual cycle does not appear to have any systematic influence on the HPA axis under either baseline or challenge conditions. However, a number of more recent studies do report significant effects. Concerning basal circadian fluctuations, two previous studies (Bao et al., 2003; Odber et al. 1998) observed subtle cycle phase induced modulations of salivary cortisol day profiles. However, both studies did not capture the morning cortisol increase. Furthermore, the Odber et al. (1998) study did not include the ovulatory period. Assessing plasma cortisol day profiles, two available studies found either no cycle phase differences (Carandente et al. 1990) or found temporal rather than quantitative measures to differ (Parry et al. 2000). Both studies did not include ovulation. In sum, these studies appear to provide partial support for subtle cycle phase-induced alterations of the basal circadian rhythm. However, they did not explicitly include and assess the ovulatory phase or the response to morning awakening.

There are only two studies available so far that explored the effect of the female menstrual cycle phase on the CAR. Using cross-sectional designs, we (Kudielka & Kirschbaum, 2003) and – with a much larger sample of adolescents – Bouma and colleagues (2009) compared the CAR in women during the follicular phase and the luteal phase. Both studies did not find significant differences in the CAR between these two phases.

Other previous studies assessed day-to-day variations in HPA axis activity by taking (nearly) daily blood draws, but found no support for cyclic modulations (Parker et al., 1981; Abplanalp et al., 1977). An early study by Genazzani et al. (1975), by contrast, observed
lowest cortisol and ACTH levels during the follicular phase, followed by marked increases around the time of ovulation and somewhat elevated levels during the luteal phase. Other studies did not include ovulation and reported no significant differences between the remaining cycle phases (Rubinow et al., 1988; Heitkemper et al., 1991) or reported on increased cortisol levels during the luteal phase compared to the follicular phase (Tersman et al., 1991).

Regarding HPA axis reactivity, we found higher salivary (but not total plasma) cortisol responses to the Trier Social Stress Test (TSST) in the luteal compared to the follicular phase (Kirschbaum et al., 1999) while Bouma and colleagues (2009) did not find such an effect with a similar stress protocol in adolescents. Some earlier studies applying cognitive or speech task protocols (Abplanalp et al. 1977; Collins et al. 1985) or physical exercise (Kanaley et al., 1992; Galliven et al., 1997; Altemus et al., 2001) measured cortisol levels in blood or urine, but mostly found no significant associations. An exception is an earlier study by Marinari et al. (1976), which reported a heightened reactivity during the luteal compared to the mid-cycle phase (ovulation was not assessed). Finally, the adrenal response to exogenous ACTH\textsubscript{1-24} after premedication with high doses of dexamethasone was found to be comparable between the menstrual, follicular and luteal phase (Kruyt & Rolland, 1982) while HPA axis feedback-sensitivity was shown to be reduced in the luteal phase compared to the follicular phase in a low-dose (0.25mg) dexamethasone suppression test (Altemus et al., 1997).

The present study cannot answer but merely speculate on the mechanisms underlying the modulation of the CAR during ovulation as observed here. The ovulatory time window is characterized by peak concentrations of estrogens, LH and FSH as well as by the initiation of the progesterone surge that extends over the luteal phase. Accordingly, different regulatory mechanisms could – possibly in parallel – be involved in mediating the enhanced CAR found during ovulation. However, the naturalistic design used here does not allow us to
draw any specific conclusions about differential, isolated contributions of single gonadal steroids to the modulation of the CAR.

While data on potential LH or FSH effects are scarce (see also below), various underlying mechanisms have been discussed to explain the stimulatory effects of estradiol on HPA axis parameters observed in animal studies (Viau & Meaney, 1991) or in human studies applying administrations of exogenous estradiol (Kirschbaum et al., 1996) or other sex steroids like DHEA (Kudielka et al., 1998). Potential mechanisms include estradiol-induced impairment of negative feedback regulation (Altemus et al., 1997), modulation of mineralocorticoid and glucocorticoid receptor gene expression as well as multiple functional changes in glucocorticoid receptors (Burgess & Handa, 1992, 1993; Altemus et al., 1997; Turner, 1997), altered electrical and chemical features of hypothalamic neurons (Pfaff & McEwen, 1983) and a direct estrogenic enhancement of CRH gene transcription in the hypothalamus (Vamvakopoulos & Chrousos, 1993). Finally, it is also conceivable that estrogen and progesterone may modulate HPA axis function through non-genomic, non-receptor mediated mechanisms (Wong & Moss, 1992; McEwen, 1994). Indirectly, estrogens may additionally alter HPA axis responsivity via increases in levels of circulating vasopressin and oxytocin or by influencing the activity of a number of neurotransmitter systems which contribute to the regulation of the HPA axis including the serotonergic and noradrenergic systems as well as GABA and glutaminergic systems (Calogero et al., 1988; Feldman & Weidenfeld, 1991; Maccari et al., 1992; Bossmar et al., 1995). A further estrogen-associated mechanism directly related to the regulation of the CAR could be a modulation of the suprachiasmatic nucleus (SCN), the body’s endogenous pacemaker which also regulates circadian rhythms of cortisol secretion (Buijs et al., 1997; Buijs et al., 2003). Recently, Abizaid et al. (2004) found an enhancing effect of estradiol on the light-induced expression of transcription factors in the SCN. In a recent review, Clow and colleagues (2010) speculate that the SCN is involved in the fine-tuning of the CAR via an extra-pituitary pathway.
Accepted that the hormonal components of the HPA and HPG (hypothalamus-pituitary-gonadal) axes operate in a tight and bidirectional relationship (Viau, 2002), the possible role of LH in the interplay of the HPA and HPG axes can be considered in a reversed causality line of argumentation. Kerdelhue et al. (2002) suggest that, analogous to findings in the rat model, the activation of the human HPA axis may participate in the initiation of the LH pre-ovulatory surge. Kerdelhue and colleagues (2002) demonstrate a positive coupling between the HPA and HPG axis at the time of the pre-ovulatory LH surge also in humans. It might thus be speculated that a stronger CAR may contribute to the initiation of ovulation.

In the present study, we observed no significant changes in subjective mood across the different cycle phases. This might be somewhat surprising and contrasts the typical finding of higher negative affect during the late luteal phase and menstruation (Collins et al., 1985; Odber et al., 1998; Ossewaarde et al., 2010; Kiesner & Pastore, 2010). However, this could be due to the fact that we excluded participants with premenstrual dysphoric symptoms and that – although not explicitly instructed to do so – participants presumably responded to the mood questionnaire within the saliva sampling times, i.e. in the first hour after awakening when not all daily mood symptoms might already have manifested. It is also conceivable that more elaborate measures would have been necessary to assess subtle mood changes across the cycle, if they really exist in our sample.

Only some of the above cited studies included the ovulatory period, and even less studies used hormonal measures to establish when exactly (if at all) ovulation occurred. Ovulation tests, designed to detect the LH surge, are used in studies by Altemus et al. (1997, 2001) and Bloch et al. (1998). The latter is the only study that used an ovulation test kit to actually determine the ovulatory period in order to compare pituitary-adrenal hormones between women with premenstrual syndrome and healthy controls. The administration of an ovulation test to determine the exact timing of the ovulatory period can thus be considered as
strength of the present study because the determination of the different cycle phases is not based on self-report data, as in many previous studies.

However, there are also some important limitations which should be considered. Although we applied a chromatographic test kit, it would have been an even more ideal approach to verify post-hoc each menstrual phase by respective hormonal assays. However, this was not feasible because participants collected saliva samples in an ambulatory setting; thus, additional blood samples were not available. A second limitation is that if one draws on a Bonferroni correction of the nominal $\alpha$-level due to multiple testing, only the difference in the cortisol net increase between ovulation and the follicular phase remains significant while the other comparisons should only be regarded as statistical trends. A third limitation is the relatively small sample size of 29 participants. However, compared to most other ambulatory studies with even much smaller samples sizes, the present sample size can be regarded as acceptable, especially considering that we used a within-subject design. Furthermore, since a single day CAR measurement is to some extent determined by situational factors (Adam et al., 2006; Hellhammer et al., 2007; Stalder et al., 2009), it would have been advisable to obtain repeated CARs for each cycle phase. A final limitation is that we did not counterbalance the completion sequence of the four cycle phases across participants. Since a fully counterbalanced design would have required a second ovulation test in a subgroup of participants (those starting with the luteal phase), we decided that all participants start during menstruation and complete the testing in the same order. It is conceivable that accuracy of sampling procedures across test days either improves due to heightened familiarity with the protocol or deteriorates due to decreasing test motivation. However, the obtained compliance results do not speak in favour of a systematic sequence bias in either direction.

In sum, the findings of this study appear to strengthen the view that gonadal steroids are potent stimulators of HPA axis regulation in humans and may therefore affect the CAR during ovulation. The present results imply that future research utilizing the CAR should try to
avoid the ovulatory period because the CAR might be generally heightened during the mid-cycle period compared to the other cycle phases. If not controlled for, such an effect might add additional variance to study results that is not related to the original study question, analogous to a variety of earlier reported intervening variables (for reviews see Wüst et al., 2000b; Clow et al., 2004; Chida & Steptoe, 2009; Fries et al., 2009; Kudielka et al., 2009). As the ovulatory phase is limited to a rather short time window (cycle days 12-16 in a regular menstrual cycle), this should be well reconcilable with most study designs. The remaining days of the menstrual cycle appear to equally qualify for CAR data collection.
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3.6 References


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Chapter 4

Cortisol responses to naturalistic and laboratory stress in student teachers: Comparison with a non-stress control day

Stress and Health, in press.

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4.1 Abstract

Ambulatory assessments of hypothalamus-pituitary-adrenal (HPA) axis responses to acute natural stressors yield evidence on stress regulation with high ecological validity. Sampling of salivary cortisol is a standard technique in this field.

In 21 healthy student teachers, we assessed cortisol responses to a demonstration lesson. On a control day, sampling was repeated at analogous times. Additionally, the cortisol awakening response (CAR) was assessed on both days. Participants were also exposed to a laboratory stressor, the Trier Social Stress Test (TSST) and rated their individual levels of chronic work stress.

In pre-post-stress assessment, cortisol levels declined after the lesson. However, post-stress cortisol levels were significantly higher compared to the control day. Also the TSST yielded higher cortisol responses when using the control day as reference baseline. Associations between the CAR and chronic stress measures were observed solely on the control day. There were no significant associations between cortisol responses to the natural and laboratory stressor.

Our results indicate that a control day might be an important complement in laboratory but especially in ambulatory stress research. Furthermore, associations between chronic stress measures and the CAR might be obscured by acute stress exposure. Finally, responses to the laboratory stressor do not seem to mirror natural stress responses.
4.2 Introduction

The response of the hypothalamus-pituitary-adrenal (HPA) axis to an acute stressor is considered an important biomarker indicating individual stress regulation. Exploring HPA axis reactivity and identifying healthy and pathological stress response patterns may help to understand how psychosocial stress relates to health and disease (Chrousos & Gold, 1992; McEwen, 1998). Numerous studies have thus investigated response parameters of the HPA axis using laboratory stress designs (Kudielka et al., 2009; Foley & Kirschbaum, 2010). However, findings on HPA axis reactivity under controlled simulated conditions lack ecological validity and are restricted to the laboratory. Therefore, complementary research including momentary ambulatory monitoring is needed. Ambulatory assessment allows describing processes that occur under real-life conditions and thus yields more generalizable evidence.

Within ambulatory monitoring, the measurement of free salivary cortisol has become a standard procedure in stress research (Hellhammer et al., 2009; Kudielka et al., 2012). This non-invasive sampling technique permits reliable tracing of cortisol concentrations under field conditions while requiring only little sampling time and effort. It is therefore considered a gold standard to monitor stress regulation with high ecological validity.

However, there also are a few issues regarding the application of this method that warrant further investigation. We aim to address some of them in this study. A first issue concerns the study design used to capture hormonal responses to natural stressors. Frequently, event-related designs with pre-stress and post-stress assessments have been employed. Recently, Lovallo et al. (2010) have shown for the laboratory setting that inclusion of a resting control day allows for a better appreciation of how a stressor perturbs the normal
diurnal baseline of cortisol. We hypothesize that a non-stress control day will help to evaluate stress-induced cortisol increases in particular in response to real-life stress.

Another issue regards the time frame covered to capture a stress process taking place in the natural environment. Specifically, we explore whether the assessment of the cortisol awakening response (CAR) on the stress day yields additional information on the stress process. The CAR, a discrete entity superimposed on the circadian cycle representing a response to morning awakening, is an established marker of basal HPA axis activity (Pruessner et al., 1997; Clow et al., 2004; Clow et al., 2010). We assume that an upcoming natural stressor during the day may manifest already upon awakening leading to a heightened CAR (Fries et al., 2009). Such a finding would challenge conventional conceptions of pre-stress ‘baseline’ assessments for real-life stressors.

Furthermore, it was postulated that long-term experience of work stress potentially leads to dysregulations in HPA axis functioning (Hellhammer & Wade, 1993; Fries et al., 2005; Kudielka et al., 2006; Miller et al., 2007). Especially the CAR as marker of chronic psychosocial stress has been empirically related to measures of chronic work stress in numerous studies, although evidence so far remains inconsistent (Chida & Steptoe, 2009; Kudielka et al., 2006). Therefore, we also want to test if such associations are detectable on a day comprising a real-life stressor or a non-stress control day.

Finally, we want to explore how well standardized laboratory stressors such as the widely-used Trier Social Stress Test (TSST) mirror real-life stressors and produce comparable hormonal stress responses. The TSST (Kirschbaum et al., 1993; Dickerson & Kemeny, 2004; Kudielka, 2007b) has become a standard paradigm for the induction of psychosocial stress and for the investigation of stress reactivity.

To address these issues, we assessed salivary cortisol levels before and after a demonstration lesson at school in a sample of healthy student teachers. A graded
demonstration lesson, which is part of the formal teacher education in Germany, is commonly perceived as important, career-relevant and strenuous event and can thus be considered a significant natural stress situation. Furthermore, it widely parallels components of the TSST protocol (e.g. speaking in front of an audience, social-evaluative threat; see Dickerson & Kemeny, 2004). For a non-stress control condition, sampling was repeated at analogous time points on a regular school day, which involved normal daily working routines at school such as preparing and giving regular lessons, correcting and marking exams, supervising students during recess, etc. On both study days, saliva samples were also collected after awakening for the assessment of the CAR. In addition, all participants underwent the Trier Social Stress Test.

In sum, using a sample of healthy student teachers, this study addresses important issues related to sampling of salivary cortisol for the assessment of responses to naturalistic stress. With this, the present study aims to further refine this technique commonly used in ambulatory stress research to monitor stress regulation with high ecological validity.

4.3 Methods

Participants

Twenty-six student teachers volunteered to participate in the study. Five participants failed to complete all samplings and were excluded, rendering a final sample of twenty-one (nine females). All participants were healthy and medication-free as assessed by an anamnestic medical interview. Mean±SD age was 30.7±6.3 years, average body-mass-index (BMI) was 22.9±2.0. To avoid potential effects of menstrual cycle phase (Kirschbaum et al., 1999; Wolfram et al., 2011), females were only eligibly if taking oral contraceptives or using other types of hormonal contraception. Before entering the study, written informed consent was obtained. Participants received a monetary compensation (50 Euros) and feedback
about their individual results. The study protocol was approved by the ethics committee of the German Psychological Society.

**Study design**

We counter-balanced the study design, with thirteen participants first completing the ambulatory test days (day with demonstration lesson and control day) and eight completing the TSST test day first. In all subjects, TSST session and ambulatory test days were separated by a time interval of at least four days. Because of the well-documented diurnal rhythm of cortisol, the sampling times on all three test days were parallelized per individual to allow for comparability of cortisol levels across test days.

*Ambulatory stress assessments on day with natural stress and a control day: cortisol awakening responses and responses to a demonstration lesson*

Cortisol Salivettes (Sarstedt, Nümbrecht, Germany) were used for sample collections. Participants gained saliva samples during the first hour after awakening on both ambulatory test days: directly and 30, 45, and 60 minutes after awakening. The next samples were collected directly before and after as well as 20, 45, 60, and 90 minutes after the demonstration lesson (analogous time points on control day). As subjects’ noncompliance to sampling schedules can invalidate ambulatory cortisol data, participants received dummy versions of electronic monitoring devices (MEMS Track Cap; AARDEX Ltd., Zug, Switzerland) and were informed about the importance of accurate timing of their saliva collection. As shown earlier, electronic monitoring significantly improves subjects’ adherence to saliva sampling protocols (Kudielka et al., 2003). Directly before and after the lesson (analogous time points on control day), participants also indicated their current subjective stress levels on single 5-point Likert items. Finally, to assess perceived stressfulness of the demonstration lesson, nine items were filled out directly before the lesson that covered perceptions of personal importance of the situation (1 item), anticipated mastery (2 items), threat (1 item), strain (3 items), challenge (1 item), and novelty (1 item).
**Laboratory stress assessments: Trier Social Stress Test**

TSST sessions were individually scheduled to match the time of the demonstration lesson. The TSST was conducted according to the standard procedure (Kirschbaum et al., 1993; Kudielka et al., 2007a). Saliva samples were taken directly before, directly after, as well as 10, 20, 30, 45, 60, and 90 minutes after the TSST using cortisol Salivettes. Directly before the TSST, the same nine items on perceived stressfulness as for the demonstration lesson were rated.

**Biochemical assays**

Participants stored their saliva samples in the refrigerator until completion of sampling. Samples were returned personally or by mail and stored at -20°C in the lab. Upon completion of the study, samples were sent to the biochemical laboratory of the University of Trier to be assayed. Samples were assayed in duplicate using a time-resolved immunoassay with fluorometric detection (DELFIA). The intra-assay coefficient of variation was between 4.0% and 6.7%, and the corresponding inter-assay coefficients of variation were between 7.1% and 9.0%. The averages of the duplicate values were used in further analyses.

**Assessment of chronic work stress and burnout**

To assess chronic work stress, we used the well-established effort-reward-imbalance/overcommitment (ERI/OC) questionnaire (Siegrist, 1996; Siegrist et al., 2004). The extrinsic ERI component covers perceived work efforts and experienced or anticipated rewards. A ratio indicating the degree of effort-reward-imbalance is computed according to the formula given by the original authors. It reflects the amount of subjectively experienced work stress by the individual. The intrinsic model component overcommitment to work (OC) captures
difficulties withdrawing from work obligations. For both scales satisfactory internal consistencies (Cronbach’s alpha) have been demonstrated (ERI: 0.61-0.88; OC: 0.64-0.82; Siegrist et al., 2004).

Burnout, referring to feelings of exhaustion resulting from prolonged work-related stress exposure, was measured by a validated German version (Schwarzer & Jerusalem, 2001) of the Maslach Burnout Inventory (MBI; Maslach & Jackson, 1986) rating the frequency of burnout-related experiences. The questionnaire comprises the subscales emotional exhaustion, depersonalization and lack of accomplishment. Satisfactory internal consistencies (Cronbach’s alpha) have been documented (emotional exhaustion: 0.86-0.88; depersonalization: 0.61-0.73; lack of accomplishment: 0.82-0.83; Schwarzer & Jerusalem, 2001).

**Statistical analyses**

Statistical analyses were carried out using the SPSS statistical software package (IBM; Version 20). The significance level was set at \( p < 0.05 \). Results are expressed as mean±standard deviation (SD). For the assessment of the CAR and responses to stressors, individual cortisol delta increases as well as area under the curve (AUC with respect to ground, see Pruessner et al., 2003) were calculated. Cortisol values were log-transformed before statistical analysis (figures and tables present untransformed values for illustration reasons).

For General Linear Models (GLMs), F-values, degrees of freedom, and \( p \)-levels were corrected according to Greenhouse-Geisser whenever sphericity was violated. Effect sizes were calculated by partial eta squared (\( \eta_p^2 \)). If applicable, post-hoc tests were calculated via t-tests. To identify relevant covariates, we tested for potential main and interaction effects of age, sex and BMI on cortisol concentrations in a first set of ANOVAs for repeated measures. Only for the CAR, the variable sex showed significant effects on cortisol levels and was therefore entered as a covariate in subsequent analyses. To analyze effects of chronic stress
on cortisol profiles, the different work stress variables were entered as continuous questionnaire scores one by one into separate GLMs.

Interrelationships between cortisol parameters (AUCs, increases, pre-stress levels) were tested using Pearson correlations. Subjective stress ratings were compared using Student’s t-tests.

4.4 Results

**Cortisol profiles on day with natural stressor and control day**

Using the pre-post-stress assessment technique, we found that only 6 of our 21 participants (28.6%) showed a significant increase in cortisol concentrations as direct response to the demonstration lesson (defined as individual cortisol increase >2.5nmol/l; Schommer et al., 2003). Accordingly, we observed an average decrease of mean cortisol levels from pre-stress to post-stress (12.88±10.05 versus 10.06±7.45nmol/l). Using the control day as reference baseline, an analysis of variance for repeated measures with factors test day (demonstration lesson versus control day) and samples (6 samples across pre/post assessment period) yielded a significant test day by samples interaction effect ($F_{2.82,56.29}=3.13$, $p=0.035$, $\eta_p^2=0.14$) indicating distinct cortisol profiles on the two days. On the control day, cortisol values show the well-documented gradual diurnal decline over time; on the stress day, cortisol values start at a higher level and follow a steeper decline thereafter (see Fig 2). Post-hoc paired t-tests confirm that mean cortisol concentrations directly after the demonstration lesson were significantly higher than mean concentrations at the corresponding time point on the control day (10.06±7.45 versus 4.81±3.39nmol/l; $t_{20}=2.37$, $p=0.028$) while cortisol levels collected directly before the demonstration lesson compared to the respective control sample tended to be higher (12.88±10.05 versus 6.08±4.10nmol/l; $t_{20}=1.86$, $p=0.077$). In line with cortisol data, subjective stress ratings were significantly higher...
directly before \( (t_{20}=8.64, \ p<0.001) \) as well as after \( (t_{20}=7.28, \ p<0.001) \) the lesson compared to analogous time points on the control day.

Furthermore, a significant negative correlation between pre-stress cortisol levels and cortisol increases in response to the demonstration lesson emerged \( (r=-0.779, \ p<0.001) \), indicating that the higher the pre-stress levels, the lower the hormonal response to the natural stressor.

**Figure 2:** Salivary cortisol levels (mean+SEM) after awakening as well as before/after the demonstration lesson (shaded area) and on a regular working day.
Chapter 4: Cortisol responses in student teachers

Cortisol awakening responses on day with natural stressor and control day

Concerning the CAR, we found no differences between the lesson and control day. An ANCOVA for repeated measures with factors sampling day and samples (4 samples) yielded neither a main effect of sampling day nor an interaction effect (both \( p=n.s. \)). Paired t-tests confirmed that neither increase nor AUC of CARs differed significantly (both \( p=n.s. \)).

Cortisol profiles on day with laboratory stressor and control day

An ANOVA for repeated measures yielded a significant main effect of samples on the day of the laboratory stressor (8 samples, \( F_{3,20,63.95}=23.45, p<0.001, \eta_p^2=0.54 \)), reflecting the significant TSST-related rise in cortisol levels. Again, pre-stress cortisol levels negatively correlated with increases (\( r=-0.519, p=0.016 \)), suggesting that also for the TSST higher pre-stress levels were associated with lower stress-related increases.

Using the control day as reference baseline, an analysis of variance for repeated measures with factors test day (lesson versus control day) and samples (6 samples across pre/post assessment period) yielded a significant test day by samples interaction effect (\( F_{3.07,61.44}=4.46, p=0.006, \eta_p^2=0.18 \)), thus again indicating distinct cortisol profiles on the two days. While cortisol concentrations gradually declined throughout the observed period of the control day, they rose in response to the acute stressor on the day of the TSST (see Fig 3). While TSST pre-stress values (5.60±2.57nmol/l) did not differ from corresponding levels on the regular working day (6.08±4.10nmol/l; \( t_{20}=0.09, p=n.s. \)), post-stress cortisol levels measured directly after the TSST (6.21±2.76nmol/l) were significantly higher than levels in the respective control sample (4.81±3.39nmol/l; \( t_{20}=2.13, p=0.046 \)).
Associations of cortisol profiles with chronic work stress and burnout

There were no associations between the CAR on the day of the demonstration lesson and any of our measures of chronic work stress (all $p$=n.s.). Interestingly, however, we did find significant associations between self-reported chronic work stress and the CAR on the non-stress control day. A higher effort-reward-imbalance ratio was linked to a lower CAR on the regular working day ($p=0.023$). Similarly, higher scores in the MBI scale ($p=0.003$) as well as in all MBI subscales (all $p<0.038$) were significantly associated with lower cortisol responses to awakening on the control day. For all F- and $p$-values of main effects and interaction effects (questionnaire scores by 4 CAR samples) please see Table 2.
Chapter 4: Cortisol responses in student teachers

There were no significant associations between measures of chronic work stress or burnout and cortisol responses to the demonstration lesson or the TSST (all \( p = \text{n.s.} \)).

Table 2: Main effects (questionnaire scores on 4 CAR samples) and interaction effects (questionnaire scores by 4 CAR samples) on day of demonstration lesson and on regular working day.

<table>
<thead>
<tr>
<th>Questionnaire</th>
<th>Day of demonstration lesson</th>
<th>Regular working day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Main effects</td>
<td>Interaction effects</td>
</tr>
<tr>
<td><strong>ERI</strong></td>
<td>( F_{1,18}=1.87 ) ( p=0.188 )</td>
<td>( F_{2.15, 36.75}=2.88 ) ( p=0.065 )</td>
</tr>
<tr>
<td><strong>OC</strong></td>
<td>( F_{1,18}=0.01 ) ( p=0.939 )</td>
<td>( F_{1.85,33.33}=1.32 ) ( p=0.278 )</td>
</tr>
<tr>
<td><strong>MBI</strong></td>
<td>( F_{1,18}=1.95 ) ( p=0.180 )</td>
<td>( F_{1.96,35.24}=0.63 ) ( p=0.536 )</td>
</tr>
<tr>
<td><strong>MBI-EE</strong></td>
<td>( F_{1,18}=0.88 ) ( p=0.360 )</td>
<td>( F_{1.99,35.76}=0.89 ) ( p=0.419 )</td>
</tr>
<tr>
<td><strong>MBI-LA</strong></td>
<td>( F_{1,18}=0.40 ) ( p=0.534 )</td>
<td>( F_{1.91,34.32}=0.61 ) ( p=0.542 )</td>
</tr>
<tr>
<td><strong>MBI-DP</strong></td>
<td>( F_{1,18}=3.44 ) ( p=0.080 )</td>
<td>( F_{1.95,35.17}=0.91 ) ( p=0.412 )</td>
</tr>
</tbody>
</table>

Note: ERI = Effort-reward-imbalance; OC = Overcommitment; MBI = Maslach Burnout Inventory; EE = Emotional exhaustion; LA = Lack of accomplishment; DP = Depersonalization.

Cortisol profiles and perceived stressfulness of natural and laboratory stressor

Comparing cortisol responses to the natural versus laboratory stressor, we found no significant correlations in terms of either pre-stress levels, response magnitude (AUC) or
response height (increase) (all $p=n.s.$). Concerning perceived stressfulness of the two stressors, the TSST was rated as more ‘novel’ ($t_{20}=4.95$, $p<0.001$) while the demonstration lesson was perceived as more ‘important to master’ ($t_{20}=5.84$, $p<0.001$). All other items (anticipated mastery, threat, strain, challenge) did not significantly discriminate between the two stressors (all $p=n.s.$).

### 4.5 Discussion

The present study addressed important issues related to sampling of salivary cortisol in the assessment of responses to a natural stressor. In a sample of healthy student teachers, we found that average cortisol levels declined from pre-assessment to post-assessment after a graded demonstration lesson. At first glance, this would have lead to the surprising conclusion that the lesson, though commonly perceived as significant stressor, did not trigger significant cortisol stress responses in the majority of our participants. However, a different picture emerges when the control day is used as reference baseline. This method revealed that cortisol levels after the lesson are significantly elevated when compared to a regular working day. Lovallo et al. (2010) have recently shown for laboratory stressors that a control day is a more valid reference baseline than pre-stress samples. Because cortisol values decline throughout a normal non-stress day, the comparison with a control day yields a more realistic estimate of how a stressor perturbs the underlying diurnal rhythm of cortisol. Our data potentially extents these findings by showing that using a non-stress control day as reference baseline is an essential supplement also in the assessment of naturalistic stress events. In our study, the difference identified by this method is even more pronounced for the natural stressor than for the laboratory stressor (TSST), indicating that a control day as baseline period might be even more important for real-life stressors.

These results might be interpreted in terms of anticipation occurring way before the onset of a natural stress event, thus leading to an altered timing of the stress process (i.e. an
earlier onset of the stress response). Anticipation, especially of a highly relevant natural stressor may alter the diurnal profile at much earlier stages of the day and result in significantly elevated pre-stress cortisol levels. Supporting this idea, we found higher subjective stress ratings before and after the natural stressor, in this case a career-relevant and examination-like event, than on the control day. Presumably anticipated stress is also higher before a natural than before a laboratory stressor as indicated by the subjects’ appraisal of higher ‘importance to master’ the demonstration lesson than the laboratory stressor. This would be in line with our finding that pre-stress cortisol levels on the day of exposure to the TSST were not elevated compared to a corresponding time point on the non-stress control day.

However, it also seems that in general higher pre-stress levels (for example due to an earlier onset of the stress response because of anticipation) are associated with difficulties to mount a further cortisol increase upon stressor onset. This is reflected in the negative correlations between pre-stress levels and cortisol increases for both the natural and the laboratory stressor. This general principle would lead to the recommendation for future research to avoid high pre-stress cortisol levels, e.g. by testing participants during afternoons, when cortisol baseline levels are lower compared to morning levels, or by ensuring a sufficiently long pre-stress resting period before stressor onset.

Regarding our second research issue, the CAR did not significantly differ between the day of the demonstration lesson and the regular working day. This stands in contrast to our expectations and to previous research findings, where a higher CAR has been observed on stressful days (Kunz-Ebrecht et al., 2004; Liberzon et al., 2008). Considering our relatively small study sample, we are inclined to assume that this was, at least in part, due to insufficient statistical power. Interestingly, associations between different scales on self-reported chronic work stress and the CAR were found on the non-stress control day but no associations were observed on the stress day. Both a perceived imbalance between efforts
and rewards indicating high levels of job stress as well as burnout, conceptualized as strain resulting from prolonged stress exposure, were associated with a lower response to awakening on the regular working day. This pattern is in line with the hypocortisolism model of exhaustion introduced earlier (Hellhammer & Wade, 1993; Fries et al., 2005; Kudielka et al., 2006; Miller et al., 2007). The fact that such associations were not observed on the stress day, however, raises the idea that associations between chronic psychosocial stress and the CAR might be obscured by acute stress-related processes taking place in anticipation of an upcoming stress situation later on the same day. Such masking effects might have contributed to the existing divergence in available literature on associations between measures of chronic stress and the CAR, where numerous studies report significant associations, but others fail to do so (Kudielka et al., 2006; Chida & Steptoe, 2009).

Concerning our final issue on the equivalence of stress responses obtained under naturalistic versus laboratory conditions, the real-life stressor and the laboratory stressor TSST turned out not to be comparable in terms of cortisol responses. There were no significant correlations between pre-stress levels, response magnitude or response height. Thus, in this study the TSST did not mirror stress processes taking place in the natural environment. This seems surprising, because both stressors require the subject to stand and speak in front of an audience and both stress situations perceptibly convey social-evaluative threat. As mentioned above, different levels of anticipated stress before the demonstration lesson and the TSST might contribute to this effect. Perceived stressfulness ratings show that the TSST situation was appraised as more novel while the lesson was more important for participants, thus supporting the idea that natural and laboratory stressors might evoke different stress responses. Future studies are warranted to investigate this issue in more detail. Nevertheless, the present finding of non-equivalent response profiles to the natural and the laboratory stressor again highlights the need for ambulatory stress studies to complement findings from the laboratory setting. Such research allows drawing conclusions about individual stress regulation with high ecological validity.
An obvious limitation of the present study is the relatively small sample size. Furthermore, although sampling times were kept as analogous as possible, the laboratory and the natural stressors had somewhat different time durations, which might have contributed to different response profiles. It also remains unclear if the present results are generalizable to other kinds of naturalistic stressors, other laboratory stress protocols and beyond the teacher profession. Finally, naturally cycling females were excluded from the present study rendering findings restricted to a sample of males and of females using oral contraceptives. With this, we avoided potential confounding effects of menstrual cycle phase in a study involving several test days, which can also be considered as strength of the present study. According to Kirschbaum et al. (1999), women using oral contraceptives show a reduced salivary cortisol response to acute stress. This would be in line with the overall low responses to the stressors in the present study.

In sum, the most important finding of the present study is that a control day is an essential complement in laboratory but especially in ambulatory stress research using salivary cortisol sampling. A control day can serve as a more realistic baseline reference to assess cortisol responses to acute stress. Furthermore, associations between chronic work stress measures and the CAR could be obscured by acute stress exposure, potentially explaining the so-far inconsistent existing literature. A control day might help to uncover such associations. Finally, the study also indicates that it is questionable if responses to laboratory stressors mirror natural stress response.
4.6 References


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Chapter 5

Emotional exhaustion and overcommitment to work are differentially associated with hypothalamus-pituitary-adrenal (HPA) axis responses to a low-dose ACTH1-24 (Synacthen) and dexamethasone-CRH test in healthy school teachers

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5.1 Abstract

Evidence for a detrimental impact of chronic work stress on health has accumulated in epidemiological research. Recent studies suggest altered hypothalamus-pituitary-adrenal (HPA) axis regulation as possible biological pathway underlying the link between stress and disease. However, the direction of dysregulation remains unclear with reported HPA hyper- or hyporeactivity.

To disentangle potential effects on different functional HPA levels, we examined responses using two pharmacological stimulation tests in 53 healthy teachers (31 females, 22 males; mean age: 49.3 years, age range: 30-64 years): A low-dose ACTH$_{1-24}$ (Synacthen) test assessing adrenal cortex sensitivity and the combined dexamethasone-CRH (DEX-CRH) test examining pituitary and adrenal cortex reactivity. Blood and saliva samples were collected at -1, +15, +30, +45, +60, +90, +120 minutes. Emotional exhaustion (EE), the core dimension of burnout, was measured with the Maslach Burnout Inventory. Overcommitment (OC) was assessed according to Siegrist’s effort-reward-imbalance-model.

We found a significant association between EE and higher plasma cortisol profiles after Synacthen ($p = 0.045$). By contrast, OC was significantly associated with attenuated ACTH ($p = 0.045$), plasma cortisol ($p = 0.005$) and salivary cortisol ($p = 0.023$) concentrations following DEX-CRH.

Results support the notion of altered HPA axis regulation in chronically work-stressed teachers showing differential patterns of hyper- and hyporeactivity depending on individual stress condition and tested functional level of the HPA axis.
5.2 Introduction

During the last decade, an increasing number of studies focused on the impact of adverse psychosocial working conditions on health outcomes (e.g. Grzywacz and Dooley 2003; Butterworth et al. 2011). To date, evidence from epidemiological and prospective studies has been accumulated showing that chronic stress at work is a relevant risk factor for the development and progression of manifest disease (e.g. Bosma et al. 1998; Stansfeld et al. 1999; Kivimäki et al. 2002; Kumari et al. 2004; Siegrist 2005). In line with this evidence, recent psychoneuroendocrine work suggests that chronic work stress according to the effort-reward-imbalance/overcommitment (ERI/OC) model and work-related exhaustion manifest in measures of physiological wear and tear, called allostatic load (Bellingrath et al. 2009). In particular, ERI/OC and emotional exhaustion might lead to alterations in the regulation of the hypothalamus-pituitary-adrenal (HPA) axis, the main endocrine stress responsive system (Chrousos and Gold 1992); however, so far results on chronic stress are inconsistent regarding the direction of dysregulation. Generally, HPA axis dysfunction can manifest in hyper- or hypo-(re)activity and considerable divergence exists regarding HPA axis regulation in chronically distressed individuals or patients suffering from stress-related diseases (Yehuda et al. 1993; Heim et al. 2000; Miller et al. 2007). Since HPA axis dysregulation represents one potential biological pathway linking ERI/OC or work-related exhaustion to manifest disease (Tsigenos and Chrousos 1994; McEwen 1998a), it is important to understand the psychobiological pathways and, in particular, the physiological sites of such alterations in more detail. Different functional levels of HPA axis regulation might be differentially affected by distinct work stress conditions.

Overcommitment to work (OC), the intrinsic component of the ERI/OC work stress model, captures a permanent motivational or cognitive disposition to react with exaggerated efforts on work obligations (Siegrist 1996; Siegrist et al. 2004). Overcommitted individuals are prone to overwork, exhaust own resources and show high ambition, high need for control
and approval as well as low detachment from work. In the present study, we assessed OC according to the well-established effort-reward-imbalance/overcommitment model (Siegrist 1996). With this model, we also assessed the extrinsic component of work stress (effort-reward-imbalance) capturing the dimensions perceived efforts spent at work, perceived rewards received in return and the effort-reward-imbalance (ERI) ratio reflecting the amount of work stress experienced by the individual. Emotional exhaustion is considered the core dimension or central quality of burnout, a non-psychiatric condition which refers to feelings of being overextended and having overly exhausted one’s emotional and physical resources (Maslach et al. 2001). To measure work-related emotional exhaustion (EE), we applied the widely used Maslach Burnout Inventory (MBI; Maslach and Jackson 1986). The given constructs capture related although different aspects of the stress process. Overcommitment, which is conceptualized as enduring cognitive-motivational disposition or personality trait, is thought to be a stable psychological risk factor for chronic exposure to stressful experiences due to exaggerated intrinsic work motivation. By contrast, EE as core component of burnout, and ERI, the extrinsic component of the ERI/OC model, reflect more state-dependent characteristics, as they capture current feelings or symptoms of distress resulting from the perception of work stress. In the present study, distinct constructs were assessed to allow for a broad characterization of individual stress conditions and a differentiated analysis of distinct stress facets on HPA axis regulation.

Available research methods from clinical endocrinology allow addressing different functional and structural levels. However, there is still a paucity of studies that investigate effects of different facets of work-related stress on the various levels of HPA axis functioning. In a recent experimental study on HPA axis stress reactivity (Bellingrath and Kudielka 2008), we applied the Trier Social Stress Test (TSST) to induce moderate psychosocial stress in a highly-controlled laboratory setting. In this study, we observed hyporeactive HPA axis responses in individuals overly committed to work. Other studies so far investigated HPA
axis feedback sensitivity in relation to burnout using the dexamethasone suppression test (DST). A dexamethasone-induced suppression of endogenous corticosteroids is thought to reflect sensitivity of glucocorticoid receptors in mediating feedback inhibition of the HPA axis. To date, the DST has become a standard tool in the assessment of HPA axis alterations and so far some studies have used it to assess HPA axis functioning in relation to exhaustion due to chronic work stress. While some studies report stronger cortisol suppression in the DST in individuals scoring high in work-related exhaustion (Pruessner et al. 1999; Sonnenschein et al. 2007; Bellingrath et al. 2008), others failed to find this pattern (Mommersteeg et al. 2006). However, the DST has been criticized for its low sensitivity. Compared to the DST, a superior sensitivity in the detection of HPA axis alterations in psychiatric conditions has been demonstrated for the combined dexamethasone-CRH test (DEX-CRH test; Holsboer et al. 1987; Heuser et al. 1994). In this protocol, a CRH dose is administered to stimulate ACTH and cortisol release after dexamethasone pre-treatment and to challenge the stress system after prior suppression. Thus, the paradigm is designed to test joined pituitary and adrenal cortex reactivity. Recently, Wirtz et al. (2010) used the combined DEX-CRH test for the first time to assess potential alterations in individuals with high levels of overcommitment to work (OC). They reported an enhanced total plasma cortisol but not ACTH response to CRH after DEX premedication in individuals with high versus low levels of OC. This pattern of results was interpreted as heightened reactivity of the adrenal cortex but a normal reactivity of the pituitary in overcommitted individuals. However, to the best of our knowledge no study has yet explored potential alterations of adrenal cortex sensitivity in overcommitted or chronically work-stressed individuals by applying the low-dose ACTH$_{1-24}$ (Synacthen) test. In contrast to the DEX-CRH test, a Synacthen test is applied to trigger directly cortisol responses from the adrenal cortex. Therefore, with application of a low dose of synthetic ACTH, the response sensitivity of the adrenal cortex can be tested pharmacologically.
Thus, in order to simultaneously test whether potential alterations at different functional levels of the HPA axis are differentially related to chronic work stress in terms of ERI/OC and EE in otherwise healthy individuals, we applied both pharmacological tests in the same study population.

A high risk for chronic work stress and exhaustion is often described for employees in so-called people-oriented occupations requiring high degrees of social interaction. Especially the teaching profession has gained much attention due to increased rates of early retirements and stress-related disorders (Kyriacou and Sutcliffe 1978; Kyriacou and Pratt 1985; Weber et al. 2001; Weber et al. 2004). To capture a wide range of subjective stress levels due to workplace characteristics, we thus selected healthy school teachers for the current study.

Generally, we expect that our chronic stress measures will be associated with altered HPA axis response patterns to Synacthen or DEX-CRH challenge. More specifically, in line with our previous findings on hyporeactive HPA axis responses to acute psychosocial stress in relation to overcommitment (Bellingrath and Kudielka 2008), but at the same time in contrast to findings from the reported Wirtz et al. (2010) study, we hypothesize that OC will be associated with an attenuated HPA axis reactivity if probed by pharmacological stimulation. In respect to the more momentary stress measures, we do not state a directed hypothesis regarding the direction of HPA axis dysregulation because of the obvious paucity of previous studies and inconsistencies in results of available studies.

Taken together, to the best of our knowledge this is the first study exploring whether chronic work stress in terms of ERI/OC and EE are differentially associated with HPA axis responses to a low-dose ACTH$_{1-24}$ (Synacthen) challenge and combined DEX-CRH test in a sample of 53 healthy school teachers.
5.3 Subjects and Methods

Subjects

A sample of 53 currently employed healthy school teachers from the region of Bremen, Germany, was recruited via local newspaper announcements and personal visits to local schools. Before entering the study, all procedures were explained and described thoroughly and written informed consent was obtained from all participants. An anamnestic medical interview and a brief bodily examination were conducted to exclude subjects taking medication or suffering from chronic health problems or diseases potentially interfering with our tests. In particular, volunteers with psychiatric disorders, serious endocrine or heart diseases or intake of corticosteroid or psychotropic medication and persons in psychotherapeutic treatment were not eligible. Pregnant women and habitual smokers were excluded from study participation.

Subjects were between 30 and 64 years of age (mean ± SD: 49.3 ± 9.4 years) with a normal body mass index (mean ± SD BMI: 24.0 ± 2.6). Twenty-two participants were male. Of the thirty-one female participants, eleven used oral contraceptives at the time of the study, ten were in the postmenopausal phase, and ten were tested during the luteal phase of their menstrual cycle.

The study protocol was approved by the ethics committee of the German Psychological Society (DGPs) as well as the ethics committee of the Bremen State Medical Association. The principles of the Helsinki Declaration were followed. Subjects received a monetary compensation of twelve Euros per hour for their participation in the study and an individualized feedback about the study results.
Psychological Assessment

We applied the 17-item effort-reward-imbalance (ERI) questionnaire (Siegrist 1996; Siegrist et al. 2004). The extrinsic component covers the two dimensions perceived efforts (six items on quantitative and qualitative work load as well as increase in work load over time, e.g. “I am often pressured to work overtime”) and experienced or anticipated rewards (eleven items on aspects of job security and promotion as well as esteem rewards, e.g. “I receive the respect I deserve from my superiors”). Responders are asked to indicate on a five-point rating scale whether the given assertions apply to their present job situation and, if so, the degree of experienced distress caused by it. The total sum scores derived from each of these two subscales were then used to compute a ratio according to the procedure described by Siegrist et al. (2004). Overcommitment to work (OC), the intrinsic model component, was measured with the one-dimensional six-item scale of the ERI model (Siegrist et al. 2004). Responders indicate their agreement versus disagreement on four-point Likert-scaled statements capturing individual efforts spent on work and inability to withdraw from obligations (e.g. “Work rarely lets me go, it is still on my mind when I go to bed”). The scale results in a single sum score ranging from 6 - 24 with higher scores indicating higher levels of overcommitment. In the present sample, the internal consistency (Cronbach’s alpha) was satisfactory for all scales (effort: 0.70, reward: 0.78, OC: 0.74).

Burnout was assessed by a validated German 22-item version (Schwarzer and Jerusalem 2001) of the widely-applied Maslach Burnout Inventory (MBI; Maslach and Jackson 1986). The frequency of burnout-related experiences or feelings is rated on a seven-point Likert scale ranging from zero = “never” to six = “daily”. Within three subscales, the domains of emotional exhaustion (nine items), depersonalization (five items), and lack of accomplishment (eight items) are covered. The subscale emotional exhaustion (e.g. “I feel emotionally drained from my work”) is considered the core dimension of burnout (Maslach et al. 2001). This subscale results into a sum score ranging from 0 - 54. The following internal
consistencies (Cronbach’s alpha) were observed in the present sample: 0.89 (emotional exhaustion), 0.78 (depersonalization), and 0.89 (lack of accomplishment).

Depressive symptoms were measured using the validated German version (Herrmann et al. 1995; Cronbach’s alpha: 0.81) of the Hospital Anxiety and Depression Scale – Depression subscale (HADS-D; Zigmond and Snaith 1983), in which participants self-rate frequency and intensity of depressive symptoms by choosing one from four given response alternatives (seven items, e.g. “I still enjoy the things I used to enjoy”).

**Experimental Protocol**

Participants reported twice to our lab on afternoons (appointments between 1400h and 1600h). On the first test day, the ACTH$_{1-24}$ (Synacthen) sensitivity test was administered, followed by the DEX-CRH test on the second test day. On average, time elapsed between the two tests was 12.2 days (+ 15.4 SD) with a range of 1 to 70 days due to individual scheduling of subjects. Because of extremely fast elimination of Synacthen from blood (terminal phase reached after 3 hours), no spill-over effects are expected for consecutive test days (Note: in the present data time elapsed between test days did not affect any results). On both test days, participants arrived at the lab having abstained from alcohol, caffeinated beverages, physical exercise and a heavy lunch throughout the present day. The first test day involved a short health check by a medical doctor (cardiovascular and anthropometric measures; a chromatographic pregnancy test in premenopausal women). Then, an intravenous catheter was inserted into either the antecubital or the medial cubital vein and a first blood sample (2.7ml) was gained for the analysis of corticosteroid binding globulin (CBG). Sixty minutes later, at time point -1 minute, a pre-test blood (9ml) and saliva sample were collected, directly followed by an intravenous injection of a low dose (1µg) of ACTH$_{1-24}$ (Synacthen®, Novartis Pharma, Nürnberg, Germany). In time intervals of +15, +30, +45, +60, +90, +120, +180, +240, +360, and +480 minutes post-injection, blood samples (2.7 ml each) were obtained.
+90, and +120 minutes after Synacthen administration, consecutive blood (9ml) and saliva samples were collected.

The night before the second test day, participants self-administered an oral dose of 1.5mg dexamethasone (Fortecortin®, Merck, Darmstadt, Germany) at 2300h. After insertion of the intravenous catheter, participants rested for 45 minutes. At time point -1 minute, a pre-test blood and saliva sample were collected. Immediately afterwards, the HPA axis was challenged with an injection of 100µg human CRH (Ferring, Kiel, Germany). Further blood (9ml) and saliva samples were collected +15, +30, +45, +60, +90, and +120 minutes after hCRH administration.

Biochemical Analyses

Venous blood was collected using Monovettes (Sarstedt, Nümbrecht, Germany) for determination of ACTH (EDTA Monovettes) and total plasma cortisol (EDTA Monovettes) as well as CBG (Serum Monovettes). Saliva samples were collected using Cortisol Salivettes (Sarstedt, Nümbrecht, Germany). Blood samples were immediately stored on ice and centrifuged at 2000g and 4°C for 15 minutes in an adjacent room and plasma or serum was pipetted into aliquots. Aliquots were then frozen at -20°C until the end of the test day and in the evening at -80°C until further analysis. Saliva samples were stored at -20°C. Upon completion of the study, all samples were sent either to the Psychobiological Research Laboratory of the University of Trier, Germany (ACTH, total plasma and free salivary cortisol) or to IBL International Hamburg, Germany (CBG) on dry ice for being assayed.

Free salivary cortisol was measured in duplicate using a time-resolved immunoassay with fluorometric detection (DELFIA, intra-assay variation: 4.0% - 6.7%, inter-assay variation: 7.1% - 9.0%). The detection limit was 0.1nmol/l. For the determination of total plasma cortisol and ACTH concentrations, blood samples were assayed in duplicate using a commercially
available enzyme-linked immunosorbent assay (ELISA) kit (total plasma cortisol: intra-assay variation: 3.2% - 8.1%, inter-assay variation: 6.5% - 7.7%, detection limit: 0.1ng/ml; ACTH: intra-assay variation: 3.1% - 4.2%, inter-assay variation: 5.8% - 6.2%). For the determination of CBG concentrations, blood samples from Serum Monovettes were assayed using a commercially available radioimmunoassay (RIA) kit (intra-assay variation: 2.9% - 3.9%, inter-assay variation: 2.4% - 5.5%).

Statistical Analyses

Statistical analyses were carried out using the SPSS statistical software package (IBM SPSS Statistics, Version 19; Chicago, IL, USA). Results are expressed as mean ± standard deviation (SD). For all General Linear Models (GLMs), F-values, degrees of freedom, and p-levels were corrected according to Greenhouse-Geisser procedure whenever sphericity was violated. The significance level was set at $p < 0.05$. Effect sizes were calculated by partial eta squared ($\eta_p^2$), expressing the amount of variance explained in the dependent variable by the respective effect. Averages of duplicate values (see biochemical analysis) were used in statistical analyses. Rates below detection limit were set at the lowest detectable value. Cortisol and ACTH concentrations were log-transformed before statistical analysis (the figures present untransformed values for illustration reasons).

Test of Potential Covariates

At first, potentially occurring main or interaction effects of age, sex, BMI, and CBG concentrations on ACTH, total plasma cortisol, or free salivary cortisol concentrations were assessed in a set of ANOVAs for repeated measures for each stimulation procedure. In this way, we checked for any effects of potential covariates on hormonal responses in our sample including men, women taking hormonal contraception, postmenopausal women, and women in the luteal phase. As age did not yield any significant main or interaction effects for neither
ACTH nor cortisol (all \( p = \text{n.s.} \)) and to avoid overfitting of our GLMs (Babyak 2004), this variable was not included as covariate in the statistical models (see below). For sex, BMI, and CBG concentrations significant effects on HPA axis parameters were found and they were thus included as covariates in all subsequent analyses. However, none of these covariates showed significant associations with questionnaire scores (all \( p = \text{n.s.} \)).

Main Models

To analyze hormonal responses to stimulations, ANCOVAs with the repeated measure factor samples (-1, +15, +30, +45, +60, +90, +120 minutes after Synacthen or CRH injection) were run. In a next step, in separate GLMs for repeated measures effects of the different work stress variables on HPA axis responses were analysed entering the continuous score of the respective questionnaire. Questionnaire scores were entered into ANCOVAs as continuous stress variables to avoid any reduction of available information by artificial grouping (see Aiken and West 1991; Royston et al. 2006) as applied earlier (Schlotz et al. 2004; Kudielka et al. 2006; Bellingrath et al. 2008).

5.4 Results

Sample Characteristics in Respect to Psychological Variables

With a mean ± SD ERI ratio of 0.81 ± 0.27 and a range from 0.39 to 1.47 our sample displayed high albeit rather heterogeneous levels of work-related stress. A small ratio close to zero is interpreted as favourable whereas values above 0.72 (Lehr et al. 2009) have empirically been identified to indicate adverse working conditions. The mean ± SD OC sum score resulted in 16.57 ± 3.49; with a minimum of 8 and a maximum of 23 again a broad range of scores was found. The mean ± SD emotional exhaustion score of 18.47 ± 10.01 in our sample can be regarded as average according to results from a normative community sample (Schaufeli and Van Dierendonck 1995). With a range from 2 (minimum) to 53
(maximum), however, 21 of our 53 participants scored above 20 which corresponds to subjects in the highest tertile of this comparison sample. Concerning HADS-D measuring depressive symptoms, the mean ± SD score in our sample was 6.42 ± 2.36. In our sample, 35 of our 53 participants (71.1%) had a score below 8, which is in approximate accordance with values from a normative German sample (Hinz and Brähler 2011). Three individuals with a score of 11 and one subject with a score of 12 just reached the cut-off of the HADS-D scale defined by values of 11 to 21. For further sample characteristics please see Table 3.
Table 3: Sample description

<table>
<thead>
<tr>
<th></th>
<th>N=53</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>22</td>
<td></td>
<td></td>
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<tr>
<td>Women in the luteal phase</td>
<td>10</td>
<td></td>
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<tr>
<td>Women using oral contraception</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postmenopausal women</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>49.3 ± 9.4</td>
<td>30 – 64</td>
<td></td>
</tr>
<tr>
<td><strong>Body Mass Index (BMI)</strong></td>
<td>24.0 ± 2.6</td>
<td>20 – 29</td>
<td></td>
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<tr>
<td><strong>Corticosteroid Binding Globulin (CBG) concentrations (µg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Men</td>
<td>59.77 ± 12.19</td>
<td></td>
<td></td>
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<tr>
<td>Women in the luteal phase</td>
<td>70.81 ± 10.66</td>
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<tr>
<td>Women using oral contraception</td>
<td>101.59 ± 45.32*</td>
<td></td>
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<tr>
<td>Postmenopausal women</td>
<td>72.24 ± 9.91</td>
<td></td>
<td></td>
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<tr>
<td><strong>Years of employment</strong></td>
<td>19.3 ± 11.5</td>
<td>3 – 42</td>
<td></td>
</tr>
<tr>
<td><strong>German school types</strong></td>
<td></td>
<td></td>
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<tr>
<td>Elementary/primary school (Grundschule)</td>
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<td></td>
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<tr>
<td>Basic level secondary school (Hauptschule)</td>
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<td></td>
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<tr>
<td>Secondary school (Realschule)</td>
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<td></td>
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<tr>
<td>Grammar school (Gymnasium)</td>
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<td></td>
<td></td>
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<tr>
<td>Comprehensive school (Gesamtschule)</td>
<td>8</td>
<td></td>
<td></td>
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<tr>
<td>Vocational school (Berufsbildende Schule)</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not further specified</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Effort-Reward-Imbalance (ERI) scores</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effort</td>
<td>18.66 ± 4.14</td>
<td>11 – 27</td>
<td></td>
</tr>
<tr>
<td>Reward</td>
<td>44.31 ± 6.63</td>
<td>30 – 55</td>
<td></td>
</tr>
<tr>
<td>Effort-reward-imbalance ratio</td>
<td>0.81 ± 0.27</td>
<td>0.39 – 1.47</td>
<td></td>
</tr>
<tr>
<td><strong>Overcommitment (OC) scores</strong></td>
<td>16.57 ± 3.49</td>
<td>8 – 23</td>
<td></td>
</tr>
<tr>
<td><strong>Maslach Burnout Inventory (MBI) scores</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emotional exhaustion</td>
<td>18.47 ± 10.01</td>
<td>2 – 53</td>
<td></td>
</tr>
<tr>
<td>Depersonalization</td>
<td>5.45 ± 5.41</td>
<td>0 – 30</td>
<td></td>
</tr>
<tr>
<td>Lack of accomplishment</td>
<td>12.75 ± 8.21</td>
<td>0 – 36</td>
<td></td>
</tr>
<tr>
<td><strong>Hospital Anxiety and Depression Scale, Subscale Depression (HADS-D) scores</strong></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>6.42 ± 2.36</td>
<td>3 – 12</td>
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</table>

* One-way ANOVA shows that CBG levels are significantly higher in women using oral contraception (F_{3,49} = 8.55; p < 0.001).
Chapter 5: Work stress & HPA axis dysregulation

**ACTH$_{1-24}$ (Synacthen) Test**

Initial ANCOVAs for repeated measures with the main factor samples confirmed significant responses in both total plasma and free salivary cortisol to ACTH$_{1-24}$ injection (total plasma cortisol: \( F_{2.07, 107.63} = 173.92; p < 0.001; \eta^2_p = 0.77 \); free salivary cortisol: \( F_{2.41, 125.55} = 269.93; p < 0.001; \eta^2_p = 0.84 \)).

Next, to ascertain whether measures of work-related stress were associated with altered cortisol responses to the test, questionnaire scores were entered one by one as additional continuous independent variables. In respect to burnout, the total MBI score was not associated with total plasma cortisol concentrations following Synacthen injection (main effect total MBI score: \( F_{1.48} = 3.20; p = 0.080; \eta^2_p = 0.06 \); interaction samples*total MBI score: \( F_{1.98,94.97} = 1.81; p = 0.170 \), but the core construct emotional exhaustion was significantly associated with a higher plasma cortisol profile (main effect EE: \( F_{1.48} = 4.25; p = 0.045; \eta^2_p = 0.08 \); interaction samples*EE: \( F_{2.00,95.75} = 1.77; p = 0.175 \)). The other two subscales (depersonalization and lack of accomplishment) showed no significant associations (main or interaction effects) with total plasma cortisol output (all \( F < 2.02 \); all \( p > 0.14 \)). Overcommitment to work (OC) was not related to total plasma cortisol concentrations (main effect OC: \( F_{1.48} = 0.10; p = 0.753 \); interaction samples*OC: \( F_{2.06,98.71} = 1.04; p = 0.358 \)).

Neither the ERI subscale effort (main effect effort: \( F_{1.48} = 3.58; p = 0.065; \eta^2_p = 0.07 \); samples*effort interaction: \( F_{2.02,96.94} = 0.44; p = 0.650 \)), nor reward (main effect reward: \( F_{1.48} = 1.44; p = 0.237 \); samples*reward interaction: \( F_{2.03,97.56} = 0.05; p = 0.956 \)), nor the ratio between efforts and rewards (main effect ERI: \( F_{1.48} = 3.27; p = 0.077; \eta^2_p = 0.06 \); samples*ERI interaction: \( F_{2.03,97.53} = 0.23; p = 0.800 \)) were associated with total plasma cortisol concentrations following Synacthen injection. Concerning free salivary cortisol, no significant associations (main or interaction effects) for any of the questionnaire scores were found (all \( F < 1.05 \); all \( p > 0.258 \)).
Figure 4: Mean total plasma cortisol response (± SEM) to injection of 1µg ACTH$_{1-24}$ in subjects with high vs. low emotional exhaustion (EE).

Note: For illustration reasons the sample was artificially divided by median split into groups with high (N=27) versus low (N=26) EE; statistics are based on continuous EE questionnaire scores; the figure presents untransformed raw values.

ANCOVA analysis for repeated measures controlling for sex, body mass index and corticosteroid binding globulin concentrations revealed that higher EE (entered as continuous variable) was associated with higher concentrations of total plasma cortisol in response to the ACTH$_{1-24}$ injection ($F_{1.48} = 4.25; p = 0.045; \eta^2_p = 0.08$).
Chapter 5: Work stress & HPA axis dysregulation

**DEX-CRH Test**

Initial ANCOVAs for repeated measures with the main factor samples again revealed significant responses in all HPA axis parameters to CRH injection (ACTH: $F_{1.91, 99.47} = 112.80; p < 0.001; \eta^2_p = 0.68$; total plasma cortisol: $F_{1.82, 94.00} = 66.69; p < 0.001; \eta^2_p = 0.56$; free salivary cortisol: $F_{1.49, 77.24} = 30.29; p < 0.001; \eta^2_p = 0.37$).

In subsequent analyses, we then added the different questionnaire scores one by one to the GLMs to test for their impact on hormonal responses to the CRH injection. Overcommitment to work (OC) yielded significant main effects on all endocrine parameters: For ACTH and total plasma cortisol, a significant OC main effect emerged (ACTH main effect OC: $F_{1, 48} = 4.24; \eta^2_p = 0.08$; samples*OC interaction: $F_{1.82, 87.42} = 2.90; \eta^2_p = 0.06$; total plasma cortisol main effect: $F_{1, 48} = 8.46; \eta^2_p = 0.15$; samples*OC interaction: $F_{1.81, 86.64} = 2.22; \eta^2_p = 0.12$). For free salivary cortisol, significant results were found for both the main effect ($F_{1, 48} = 5.49; \eta^2_p = 0.10$) as well as the samples*OC interaction ($F_{1.55, 74.50} = 6.45; \eta^2_p = 0.12$). For all HPA axis parameters, higher OC was associated with lower responses to DEX-CRH. Neither effort, reward, the ERI ratio, the total score of the MBI nor any MBI subscale was significantly related to endocrine responses (all $F < 1.40$; all $p > 0.243$).
Figure 5: Mean ACTH (A), total plasma cortisol (B) and free salivary cortisol (C) response (± SEM) to injection of 100µg CRH in subjects with high vs. low overcommitment (OC) to work.
Chapter 5: Work stress & HPA axis dysregulation

Note: For illustration reasons the sample was artificially divided by median split into groups with high (N=26) versus low (N=27) OC; statistics are based on continuous OC questionnaire scores; the figures present untransformed raw values.

ANCOVA analyses for repeated measures controlling for sex, body mass index and corticosteroid binding globulin concentrations revealed that higher OC (entered as continuous variable) was associated with lower concentrations of ACTH (F\(_{1, 48} = 4.24; p = 0.045; \eta^2_p = 0.08\)), total plasma cortisol (F\(_{1, 48} = 8.46; p = 0.005; \eta^2_p = 0.15\)) and free salivary cortisol (F\(_{1, 48} = 5.49; p = 0.023; \eta^2_p = 0.10\)) in response to the CRH injection.

Complementary Analyses

In the main analyses, EE yielded a significant effect in the Synacthen test while OC was significantly associated with HPA axis responses in the DEX-CRH test. As discussed earlier, emotional exhaustion and overcommitment to work show some symptom overlap with depressive symptomatology (Ahola et al. 2005; Bellingrath and Kudielka 2008; Bellingrath et al. 2008; Wirtz et al. 2010). In accordance with these earlier data, emotional exhaustion, overcommitment to work and depressive symptomatology showed significant intercorrelations of medium height (see Table 4). Thus, in complementary analyses, we tested whether the above reported significant effects are partialled out or remain stable if simultaneously controlling for the other two variables, respectively. Results show that the main effect of EE on total plasma cortisol responses to Synacthen became even stronger when additionally controlling for OC and HADS-D (F\(_{1,46} = 7.20; p = 0.010; \eta^2_p = 0.14\)).

The above reported OC main effects in the DEX-CRH test also became more robust (ACTH: F\(_{1,46} = 6.17; p = 0.017; \eta^2_p = 0.12\); total plasma cortisol: F\(_{1,46} = 15.27; p < 0.001; \eta^2_p = 0.25\); free salivary cortisol: F\(_{1,46} = 8.02; p = 0.007; \eta^2_p = 0.15\)). Additionally, the respective samples*OC interaction effects gained significance (ACTH: F\(_{1.84, 84.64} = 3.20; p = 0.050; \eta^2_p = 0.07\); total plasma cortisol: F\(_{1.85, 84.91} = 4.41; p = 0.039; \eta^2_p = 0.07\)) or, in the case of free salivary cortisol, became more pronounced (F\(_{1.57, 72.40} = 8.02; p = 0.002; \eta^2_p = 0.15\)).
Table 4: Pearson correlation matrix for MBI-EE, OC and HADS-D

<table>
<thead>
<tr>
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<th>MBI-EE</th>
<th>OC</th>
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<tr>
<td>OC</td>
<td>0.585**</td>
<td></td>
</tr>
<tr>
<td>HADS-D</td>
<td>0.503**</td>
<td>0.407**</td>
</tr>
</tbody>
</table>

MBI: Maslach burnout inventory; EE: emotional exhaustion; OC: overcommitment to work; HADS-D: Hospital anxiety and depression scale, subscale depression. **p < 0.01

5.5 Discussion

In a sample of 53 healthy school teachers, we found emotional exhaustion, the core component of burnout, and overcommitment to work to be differentially related to HPA axis functioning as indexed by responses to a low-dose ACTH$_{1-24}$ challenge and a combined DEX-CRH test. To the best of our knowledge, this is the first study on chronic work stress according to the effort-reward-imbalance/overcommitment (ERI/OC) model and emotional exhaustion (EE) that tests for differential effects in stress signal processing at the various anatomical, respectively functional levels of the HPA axis by administering two different endocrine stimulation procedures in the same study population.

First of all, our results show that work-related emotional exhaustion is associated with a higher total plasma cortisol output to a low dose of exogenous ACTH. This finding reflects a heightened sensitivity of the adrenal cortex in emotionally exhausted but otherwise healthy teachers. Second, in line with our hypotheses, overcommitment to work is associated with a significantly blunted HPA axis response to CRH challenge after dexamethasone premedication. Strikingly, these effects are even stronger when additionally controlling for
depression and EE or OC, respectively. That means, by partialling out mutually overlapping facets of the different constructs the observed effects become even more evident.

To date, the combined DEX-CRH test is considered the most sensitive pharmacological test for the detection of alterations in HPA axis functioning (Heuser et al. 1994). This stimulation procedure tests for joined pituitary and adrenal cortex reactivity to an exogenous CRH stimulus under dexamethasone feedback inhibition. It could be argued that the DEX-CRH test primarily acts at a pituitary level because the synthetic glucocorticoid dexamethasone only poorly penetrates the blood brain barrier (De Kloet 1997). However, central regulation downstream the hippocampus is also clearly involved in the response to DEX-CRH provocation due to feedback signalling of suppressed endogenous glucocorticoid concentrations to the brain. In the current study, we observed hyporeactive pituitary as well as adrenal cortex responses as reflected in reduced ACTH, total plasma and free salivary cortisol concentrations in overly work committed teachers. We therefore assume that such an attenuated HPA axis response is not solely of pituitary and adrenal but also likely of central origin. This interpretation would also be perfectly in line with findings from two previous studies. In an independent sample of male and female teachers overly committed to work we recently observed of a reduced ACTH, total plasma and free salivary cortisol response to acute psychosocial stress induced by the Trier Social Stress Test (TSST) (Bellingrath and Kudielka 2008). Accordingly, Wirtz et al. (2008) reported higher overcommitment to be significantly associated with lower free salivary cortisol and noradrenaline responses after the TSST in working men. Evidence from these studies clearly suggests a hypo-(re)active stress system in overcommitted individuals. Strikingly, these results are in contrast with another recent finding by the same authors (Wirtz et al. 2010) who reported an increased total plasma cortisol response to the combined DEX-CRH test in overcommitted subjects. Since ACTH reactivity was not related to overcommitment in this study, the authors speculated that an increased response to exogenous CRH may be due to enhanced adrenal cortex sensitivity that might have developed under chronic OC-conditions as an adaptive counter-regulatory reaction to compensate for a blunted stress-induced CRH release. Our
present empirical results do not support the latter interpretation by Wirtz et al. (2010) as we do not find any indication for increased adrenal cortex sensitivity in subjects with high overcommitment to work as tested in the Synacthen test. We can only speculate on possible reasons for the discrepant findings in the DEX-CRH test in that study and our own study, e.g. an on average younger and less well educated sample including participants from various occupational fields with an unemployment rate of 20 percent as well as a lower mean OC score in the Wirtz et al. study (16.57 versus 13.25). Interestingly, Wirtz et al. generally report small to medium sized effects, while we find medium to large effects in our study.

Strikingly, however, our results in the DEX-CRH test appear to be in agreement with findings by Rydmark et al. (2006) who found markedly attenuated HPA axis responses to DEX-CRH in patients on long-term sick leave with job stress-induced depression compared to a matched healthy control group. The authors of this study speculate that the observed pattern is indicative of a hypoactive central stress-axis circuitry and in this way comparable to conditions found in atypical depression. In a follow-up study by Wahlberg et al. (2009), the same sample was retested with the DEX-CRH test and the hyporeactive response pattern compared to the control group was found to be stable even after clinical improvement of depressive symptoms and full remission in the majority of patients. Wahlberg and colleagues (2009) interpret this finding as initial evidence for hyporeactivity as manifested in the DEX-CRH test being a trait rather than a state marker of disease in this population. An attenuated response to DEX-CRH would thus reflect a stable marker of a pre-existing vulnerability. Based on these findings, it is hypothesized that in affected individuals, the inability to mount an adequate stress response may impede successful coping with stressful job conditions and ultimately lead to the development of depressive symptoms. Alternatively, it might be speculated whether a sustained hyporeactivity could also reflect a “scar”-marker rather than a vulnerability marker. Individuals could have acquired the hyporeactive response pattern during a period of extended stress or a state of psychopathology and maintained it thereafter. In our present study, we found overcommitment to work to be associated with dampened
responses to DEX-CRH. OC in turn has been identified as risk factor for the development of
depression in earlier studies (Dragano et al. 2008). Integrating these various findings, it might
either be speculated that both OC and a hyporeactive central stress circuitry are
(interrelated) risk factors for the development of depression or that a coping style
characterized by high overcommitment to work results in a hyporeactive stress circuitry,
which eventually leads to manifestations of stress-induced depressive symptomatology. As
we included only healthy participants at one measurement occasion so far, our present study
cannot clarify this question.

How could the differential effects of individual stress condition on HPA axis regulation
as observed in the present study be explained? This seems not to be an easy endeavor,
especially since OC and EE are significantly intercorrelated. We would like to offer two
potential explanations.

First, unlike emotional exhaustion as core component of burnout and also unlike ERI,
OC is conceptualized as underlying and enduring motivational pattern to cope with job
demands and is even considered as stable personality trait. Work-related stress due to
overcommitment can thus be assumed to be relatively stable over time and relatively
invariant to changes in the work place or the environmental context. By contrast, both EE
and ERI might reflect more momentary or even state-dependent characteristics that result as
a consequence of present adverse working conditions. It is thus conceivable that a
dispositional permanently overcommitted coping style affects the HPA axis differently than
subjective distress or feelings of work-related exhaustion. However, previous research
repeatedly showed that EE is also related to dispositional variables like for example
neuroticism/low emotional stability or low agreeableness (Goddard et al. 2004; Cano-García
et al. 2005; Bakker et al. 2006). This suggests that some individuals are more prone to
actually experience states of burnout or emotional exhaustion than others. Exhaustion can
thus neither be regarded as independent of personality aspects nor as purely "trait". Rather,
EE seems to be the result of a dynamic interaction of individual disposition and situational factors. Thus, taken together, empirical evidence challenges the idea that OC and EE can be reliably separated by trait versus state aspects.

However, a second and alternative mechanistic pathway is conceivable. This pathway implicates a time course model of HPA axis hypo- and hyper(re)activity. As outlined above, an overcommitted coping style constitutes a psychological risk factor, e.g. for depression (Dragano et al. 2008). Overcommitted individuals are at an increased risk to experience chronic work stress because they expose themselves more frequently and constantly to high demands at work and exaggerate their work-related efforts. This coping style might initially and under sound working conditions be successful. However, it might over time trigger an overload of stressful experiences and eventually promote feelings of exhaustion or depressive symptoms. Due to their high intrinsic needs for control and approval overcommitted individuals are probably prone to experience feelings of exhaustion when they meet frustrating working conditions. This would explain the high intercorrelations between OC, EE and HADS-D. From a psychobiological perspective, a hypo-(re)active stress system in overcommitted subjects might be a mechanism to prevent hypercortisolism in an organism frequently exposed to stressful experiences. So far, this pattern would be in line with the time course model of hypocortisolism that has been proposed earlier (Hellhammer and Wade 1993; Fries et al. 2005; Kudielka et al. 2006; Miller et al. 2007). It proposes an initial period of enhanced stress responsiveness to be followed by a phase of blunted responsiveness under prolonged stress. An overcommitted coping style, which imposes chronic stress on the body, would over time lead to attenuated HPA axis responses. If, however, as a consequence of frustrated efforts feelings of emotional exhaustion and subjective distress arise, a counter-regulatory increase in adrenal sensitivity might take place. Conceivably, this could then be interpreted as a bodily attempt to reinstall homeostasis and overall functioning. Our findings would therefore extend the established
time course model by a phase, in which the organism strives to re-enhance HPA axis responsiveness after a period of blunted functioning.

These scenarios obviously must remain speculations. In this respect, an apparent limitation of the present study is its cross-sectional design. Only longitudinal approaches would be able to assess the assumed gradual alterations of HPA axis regulation over time. Besides important limitations (e.g., cross-sectional design, medium-sized and heterogeneous study sample including men, naturally cycling women in the luteal phase, women using oral contraception, and women in the postmenopausal phase, no use of a standardized clinical interview in recruitment of participants, and no constant time interval between the two test days) there are also some strengths of the current study, like the broad characterization of the HPA axis (including ACTH, total plasma cortisol and free salivary cortisol) as well as the application of both an ACTH$_{1-24}$ sensitivity and combined DEX-CRH test in one study sample. The simultaneous test administration allowed for testing of potential alterations at different levels of HPA axis functioning under different conditions of chronic stress. Furthermore, our sample of healthy school teachers provided a model for studying the impact of chronic work-stress on physiological functioning. Reported average levels of work-related stress were medium to high with a sufficiently broad range of stress levels covering highly stressed and barely stressed individuals.

Finally, the observation that the effect of EE on cortisol responses to Synacthen reached the level of significance in total plasma cortisol but not salivary cortisol warrants some commentaries. First of all, the EE effect on salivary cortisol approached the level of significance if controlling for HADS-D and OC ($p = 0.07$) and additional analyses on net increases or area under the curve responses showed that total plasma cortisol and salivary cortisol after Synacthen stimulation are significantly intercorrelated. Considering the generally lower effect sizes in the Synacthen test compared to the DEX-CRH test raises the idea that the effect on salivary cortisol might have failed to reach significance due to insufficient statistical power. Further, the fact that we applied a low-dose Synacthen
sensitivity test might have contributed to the discrepancy. However, the present findings also support to the well-known observation that plasma cortisol and salivary cortisol responses are often highly intercorrelated, but can show significant dissociations (for review see Hellhammer et al. 2009). Especially under response conditions, there is not necessarily always a linear relationship between the two HPA axis indicators, pointing to the necessity of distinguishing between the two HPA axis parameters (Kirschbaum et al. 1999; Kudielka and Kirschbaum 2005).

In sum, our results support the notion of HPA axis dysregulation under conditions of chronic work-related stress. While emotional exhaustion is associated with increased adrenal cortex sensitivity, overcommitment to work is related to hyporeactive pituitary and adrenal cortex responses. Thus, our data show a differential pattern of hyper- and hypo-(re)activity in relation to work-related emotional exhaustion and overcommitment to work depending on the tested level of HPA axis functioning and individual stress condition. According to McEwen’s concept of allostatic load (McEwen 1998b) both chronic over- as well as underactivity of functional parts of the stress system should be regarded as wear-and-tear of body and brain, such that both patterns of dysregulation contribute to translating adverse psychosocial conditions into bodily changes and ultimately bringing about manifest disease.
5.6 References


Chapter 5: Work stress & HPA axis dysregulation


Chapter 6

Summary and general discussion
This chapter seeks to summarize the three empirical studies presented throughout the last three chapters and discuss and integrate their findings. Some general strengths and limitations of the current work as well as ideas for future research building upon the evidence findings will be described.

6.1 Summary

The present dissertation aimed to contribute to our knowledge of how chronic stress exposure modifies HPA axis (re)activity and to deepen our understanding of how cortisol can be used as biological stress marker in this context. The presented empirical studies covered the three different dimensions of HPA axis functioning described in Chapter 2: Basal activity, i.e. the cortisol awakening response (Study 1), HPA axis reactivity to acute (natural and laboratory) stress (Study 2), as well as HPA axis regulation as assessed by two different pharmacological stimulation procedures (Study 3). Taken together, all three studies focussed on the assessment of cortisol as marker of HPA axis activity in the context of psychoneuroendocrinological stress research.

Several methodological conclusions and recommendations for future research can be derived from the presented work. First of all, Study 1 indicates that future research should try to avoid the menstrual mid-cycle period in assessments of the CAR in free-cycling female study participants. During this short time window of ovulation, when the gonadal steroids estrogen and luteinizing hormone reach their peak levels, the CAR is significantly increased. A thereby heightened CAR might confound data collected during this time with additional variance that is unrelated to the original research question at hand. Confirming earlier cross-sectional research (Kudielka & Kirschbaum, 2003; Bouma et al., 2009), by contrast, the CAR does not seem to be significantly altered in any of the other cycle phases including menstruation, the follicular phase as well as the luteal phase suggesting that these phases
equally well qualify for CAR data collection. This is a positive result in terms of practical implications for future research, because it is easy to determine and avoid the ovulatory period as only critical time period during the menstrual cycle and only a small number of days within a given cycle are at all affected by this limitation.

Some further methodological conclusions for future research stem from findings gained in Study 2. A main result of this study was that it is of major importance to include a non-stress control day into research studies collecting cortisol data in response to acute stress or challenge, and especially to natural stress. In line with an earlier report (Lovallo et al., 2010), it could be shown in this study that such a control day is pivotal to understand how an acute stressor actually perturbs the normal underlying diurnal cortisol rhythm. This was the case for a laboratory stressor, namely the TSST, but even more so for a natural stressor, namely a graded demonstration lesson in student teachers. In a pre-post-stress comparison, the estimated impact of the stressor at hand was significantly smaller or, as in the case of the natural stressor, even absent in the majority of participants. Only the comparison with a non-stress control day allowed realistically estimating how the underlying diurnal rhythm was perturbed by stress and, presumably, stress anticipation. This finding gains specific relevance in light of the fact that many studies use a pre-post-assessment design, which would, according to our data, underestimate the true impact of a given stressor on cortisol profiles.

Furthermore, Study 2 also showed that the non-stress control day allowed detecting associations between measures of chronic stress or burnout and a blunted CAR. Interestingly, these associations were clouded on a day of acute stress exposure. This finding implies that stress processes such as anticipation that take place on a day of a natural stress event might obscure existing associations with measures of chronic stress.
Hence, both reported findings strongly suggest that additional cortisol samples should be collected on a non-stress control day.

Besides this, Study 2 indicates that the Trier Social Stress Test, which is widely used to induce moderate psychosocial stress in laboratory research settings, might not mirror natural stress processes occurring under real-life conditions. No significant correlations in terms of either response height or response magnitude could be found between the two types of stressors: the TSST on the one hand and a graded demonstration lesson in student teachers on the other hand. This finding is somewhat disconcerting because the TSST – as other laboratory stress induction procedures – is commonly used in the tacit assumption that the stress response profile triggered by this protocol in some way mirrors response profiles that would be observable under naturalistic conditions. However, this assumption so far has not been empirically tested. The presented results from Study 2 might call into question whether a “natural” stress response can be obtained by applying laboratory stress protocols such as the TSST.

In this context, it is briefly described in the Discussion of Study 2 that especially anticipatory processes that can occur before a real-life stressor might alter the stress profile under natural conditions in comparison to laboratory conditions. While, in general, anticipation may also occur before a laboratory stressor, the psychological processes taking place in the two different stress scenarios could be different in numerous ways and this is also reflected in the psychological ratings about the two different stressors in Study 2. Perceivably, a real-life stressor, if sufficiently relevant, will be considered as more important for an individual. This corresponds to the observation that in our study, participants rated the natural stressor as significantly more “important to master” than the laboratory stressor. Similarly, a bad performance in the stress situation should be evaluated as more detrimental by the individual as negative consequences of some kind may follow. Furthermore, a person
likely has more knowledge about the nature of an upcoming stress event under real-life conditions. By contrast, while a laboratory stressor might be unpleasant and stress provoking as well, the exact contents and procedures will in most cases be a surprise for the participant and anticipation occurring beforehand, if at all, will be more vague. This is in line with the ratings of our participants in Study 2 who perceived the TSST as significantly more “novel” than the real-life stressor. However, the here described finding regarding the limited comparability of natural and laboratory stressors remains restricted to the two specific stressors that were used, namely the TSST and a demonstration lesson in student teachers. It obviously also remains unclear whether significant correlations between response profiles could for example have been found if a more spontaneous or unplanned natural stressor would have been used instead of the demonstration lesson. If differences in stress anticipation indeed were responsible for the lack of correlation, then responses to such a more unplanned stress event could perceivably have resembled the TSST response profile to a greater extent. In addition, different time durations might have further limited comparability of the two stressors as mentioned in the respective Discussion in Chapter 4. In any case, the here discussed result from Study 2 highlights the need for ambulatory stress research with high ecological validity to complement findings derived from the laboratory setting.

A final methodological recommendation can be derived from Study 3. Here, it was demonstrated that the simultaneous administration of two different pharmacological stimulation procedures within one study sample revealed interesting and differential associations between distinct stress conditions and alterations at different hierarchical levels of HPA axis functioning. Because in previous research only single test procedures were used in a given study sample, such differential effects could not be found and reported so far. A general recommendation for future research therefore would be to similarly aim at a broad characterization of HPA axis functioning and include multiple simultaneous stimulation
procedures or assessment techniques and, if possible, also multiple HPA axis output parameters (i.e. ACTH, total plasma cortisol and free salivary cortisol) in a given study sample. Such a broader methodological procedure could help to overcome the difficulties in integrating the many different single, punctual and sometimes apparently contradictory findings that cumulate with ongoing research.

Regarding the other research goal of the present dissertation, namely the exploration of associations between chronic stress exposure and HPA axis (re)activity, several conclusions can be drawn from the presented work. As shown in Study 3, distinct stress conditions seem to be differentially related to alterations at various levels of HPA axis functioning. While overcommitment to work was associated with hyporeactive responses at the level of the pituitary and the adrenal cortex, the sensitivity of the adrenal cortex was increased under conditions of emotional exhaustion as central component of the burnout syndrome. The simultaneous administration of both stimulation procedures, namely the combined DEX-CRH test and the ACTH$_{1-24}$ (Synacthen) sensitivity test in one study sample allowed assessing such alterations at different functional levels of the HPA axis associated with distinct stress states. This procedure allowed us to contribute a new aspect to the earlier described ongoing debate about hyper- versus hypoactivity of the HPA axis under chronic stress exposure, namely the possibility that both kinds of dysregulation exist in parallel. This would mean that certain earlier findings could potentially be complementary rather than contradictory, because different functional HPA axis levels were targeted by the respective assessment technique or because distinct stress conditions were evaluated.

Furthermore, as shown in Study 2, a blunted CAR was found in subjects scoring high in measures of burnout and job stress as assessed by effort-reward-imbalance and overcommitment to work. Interestingly, as discussed already above, this pattern was only found on a control day, while the association apparently was obscured on a day of acute
stress exposure. This finding could imply that some previous research might have failed to discover significant associations because these were clouded by acute stress processes.

In sum, all three studies contribute to our understanding of how stress can be “measured” by using cortisol as biological, pre-clinical stress marker. Various methodological issues were addressed concerning the assessment of different dimensions of HPA axis functioning and recommendations for future research in the area of psychoneuroendocrinological stress research were given. Furthermore, some important conclusions regarding altered HPA axis activity under conditions of chronic stress exposure could be drawn as well.
6.2 General discussion

The findings from Studies 2 and 3 are in line with previous research reporting on stress-induced alterations of HPA axis functioning (Chrousos & Gold, 1992; Kudielka et al., 2006). These alterations are assumed to be the biological intermediaries that link psychological stress with manifest disease. All findings were obtained from healthy participants as ensured by the strict inclusion criteria described in the individual studies. Thus, the present research results confirm that cortisol seems to be a promising candidate for a pre-clinical biomarker indicating subtle changes already in apparently healthy adults. Such a pre-clinical marker would substantially help in the early identification of individuals at risk and would also be useful in the monitoring of stress interventions.

However, as described in the Introduction as well as in the section on theoretical background information, existing discrepancies in the available literature complicate such an application of cortisol in practical settings. The present work intended to shed some more light on this current state of research and the unsolved debate about hyper- versus hypoactivity of the stress axis. At first glance, however, the presented studies seem to make the issue even more complicated: First of all, evidence was provided that still another variable needs to be controlled for in the assessment of the CAR, because ovulation results in an increased CAR. Furthermore, it was demonstrated that the widely-used TSST does not seem to mirror natural stress processes, possibly indicating that the tracing of real-life stress events with high ecological validity would yield more valid information on individual stress regulation. Moreover, the realization of a resting control day in study designs was found to be an important additional complement in studies on stress reactivity, thereby recommending a further lengthening of in many cases already laborious and time-consuming data acquisition phases. Finally, it was argued on the basis of empirical results from the third study that the simultaneous administration of different pharmacological stimulation tests within one study sample allows for a differential assessment of distinct functional levels of the HPA axis. It
was concluded that different stress conditions seem to be differentially associated with alterations at different levels of HPA axis functioning. In sum, various conclusions and recommendations are given that at first sight even would seem to further complicate the assessment of cortisol as psychobiological marker for chronic stress and burnout.

However, as described in the paragraph on theoretical accounts for HPA axis hyper- and hypoactivity in Chapter 2, the mechanisms involved in these processes indeed seem to be rather complex and chronic stress exposure might effectively result in either increased or decreased HPA axis activity depending on a variety of factors in the respective stress process. Because of this physiological complexity in HPA axis regulation over time, methodological rigour is needed to obtain valid results in psychoneuroendocrinological stress research. Only when confounding factors are excluded and when study designs are used that are adequate to capture the dynamics and complexities of the physiological stress process, it is possible to obtain clear and unconfounded data. Ultimately, such a stringent and rigorous methodological procedure might lead to cortisol data that is easier to interpret and to make use of in practical settings.

The present work also has some important limitations. Besides the specific limitations that are discussed in the respective articles, a general limitation that applies to all of the three presented studies is the rather small size of investigated study samples. However, this limitation has to be considered in the light to a number of factors related to study design and study recruitment, which will be briefly described in the following: Because all studies required the application of some laboratory or ambulatory test procedures (the application of an ovulation test in Study 1, exposure to the TSST in Study 2, administration of both DEX-CRH test and Synacthen test in Study 3) and various biochemical analyses had to be conducted (salivary cortisol samples in all three studies, CBG, ACTH as well as plasma cortisol samples in Study 3), the potential number of participating subjects was limited by considerations regarding financial and labour costs as well as personnel requirements.
beforehand. Furthermore, recruitment of study participants was complicated by strict inclusion criteria regarding gender (in Study 1 obviously only female subjects were included), health status and health behaviours such as smoking (all studies), occupation (in Studies 2 and 3 only student teachers or teachers were included, respectively) or use of hormonal contraceptives (in Study 1 only naturally cycling women were included; in Study 2 only women using hormonal contraception were included). Moreover, all study designs imposed a rather high working load on participants, who had to monitor a whole menstrual cycle and autonomously administer an ovulation test kit over several consecutive days (Study 1), repeatedly collect saliva samples and fill out accompanying paper diaries on mood and situational characteristics after awakening (Studies 1 and 2) and over two complete working days (Study 2). Further challenges for our study participants were the high time burden (especially in Study 3, which comprised two test days with three to four hours of assessment each), the stressful experience of being exposed to the TSST (Study 2), and especially also the invasive test procedures of Study 3 (medical health check including pregnancy test in female participants, insertion of an intravenous catheter on two test days, intake and administration of three different synthetic pharmacological agents: Synacthen, dexamethasone, and CRH, repeated collection of blood and saliva samples). In sum, there were a number of reasons that made it difficult to obtain large sample sizes. Consequently, statistical power might partly have been insufficient and might thus account for some non-significant results.

Besides this overarching limitation, there are also some strengths that apply to the current work. In addition to the specific strengths discussed in the individual studies, the use of salivary cortisol as a by now well-established marker of HPA axis activity can be regarded as one strong point of the present dissertation. Throughout the recent decades, salivary cortisol has emerged as an easy-to-measure and valid marker of HPA axis activity and reactivity. In contrast to total plasma cortisol, free salivary cortisol encompasses the biologically free and active fraction of total cortisol that is actually available to the organism.
Chapter 6: Summary and general discussion

(Kudielka et al., 2012). A further strength is the consideration of compliance issues in the two ambulatory studies (Studies 1 and 2). This is important because insufficient adherence to sampling times can potentially invalidate data collected under field conditions. This is especially critical for the CAR, where a too long delay between wake time and the first sample was shown to lead to a flattened response profile to awakening (Desantis et al., 2009; Okun et al., 2009). While in Study 1 real track caps as electronic monitoring devices were used and data were analyzed in consideration of sampling times as captured by the respective records of the caps, participants used dummy track caps in Study 2. It was shown earlier that already the knowledge about the nature of the monitoring devices and the careful instruction of participants concerning compliance issues significantly enhances adherence to sampling protocols (Kudielka et al., 2003).

Building upon the results summarized and discussed in the previous paragraphs, I now would like to propose some ideas for future research agendas that could follow up from the present dissertation. Specifically regarding the results from Study 1, future research should explore the potential influence of ovulation on other dimensions of HPA axis activity, including reactivity to acute stress or responses to pharmacological stimulation or suppression. For acute stress reactivity, exposure to the TSST could be used to induce acute psychosocial stress. Here, the problem of habituation after repeated exposure to the same stress protocol in various phases of the menstrual cycle could be solved by a counterbalanced design. As described in the Discussion of Chapter 3, some previous studies addressed the question of how menstrual cycle phases can alter the response to acute stress. Some of these studies reported heightened cortisol responses to stress during the luteal phase of the menstrual cycle (Marinari et al., 1976; Kirschbaum et al., 1999), but others failed to find corresponding effects (Collins et al., 1985; Kanaley et al., 1992; Galliven et al., 1997; Bouma et al., 2009). However, none of the cited studies included the ovulatory period, which, according to the data from this dissertation, would be the most critical phase. Also regarding HPA axis (feedback) regulation as captured by pharmacological suppression
or stimulation, only the menstrual phase, follicular phase and luteal phase have been investigated, but not ovulation (Kruyt & Rolland, 1982; Altemus et al., 1997). These outlined research ideas are part of a more general future research agenda, which will need to further explore and identify relevant factors that modify and determine the physiological stress response and impact on individual stress regulation.

Concerning the findings on HPA axis alterations under conditions of chronic stress from Study 2 and especially Study 3, a key aspect for future research endeavours will be the use of longitudinal approaches to monitor the assumed gradual alterations over time. The cross-sectional approaches that have been used in the present work do not allow drawing definite conclusions about the temporal dynamics of the underlying physiological processes. First longitudinal evidence has already revealed interesting features of stress regulation. For example, Rydmark et al. (2006) used the DEX-CRH test to assess HPA axis regulation in a sample of women who were on long-term sick leave due to work-stress induced depression. Contrary to the authors’ expectations and to what has commonly been described for major depression, the authors found an attenuated response to the test procedure in this sample compared to a healthy matched control sample. After twelve months, the same group of researchers retested the sample of participants using the same test procedures (Wahlberg et al., 2009). At this follow-up assessment, the majority of participants had made marked clinical improvements up to a full recovery. However, interestingly, the hyporeactive response profile to DEX-CRH persisted in this group of patients, independent of clinical improvement. The stability of this pattern is interpreted by the authors as indicative of a trait rather than a state marker. A hyporeactive HPA axis would thus reflect a pre-existing vulnerability. Alternatively, it could also be considered as a “scar marker” that has been acquired during the depressive episode and that stays thereafter. In any case, this example demonstrates that longitudinal research can yield important insights into physiological stress regulation that cannot be achieved by cross-sectional studies. Regarding the findings from the present dissertation, it would be interesting to see whether the results by Wahlberg et al.
(2009), which are derived from a clinical sample, could be replicated with a non-clinical sample such as the teacher sample used in Study 3. Here, we found a similarly hyporeactive response pattern to DEX-CRH in apparently healthy individuals, who score high in overcommitment to work (OC). It would be interesting to find out whether this profile persists over time, whether it is altered for example after changes in the work environment such as during a sabbatical year, after retirement, after a job change, or after a stress intervention program such as psychotherapy.

The present dissertation confirmed and extended our knowledge on the possibly detrimental effects of chronic work stress on HPA axis regulation. Subtle forms of dysregulation were present already in our two independent samples of apparently healthy and currently working school teachers or student teachers. This underlines the importance to develop and implement effective and early stress prevention, stress reduction and stress intervention strategies for individuals affected or individuals at risk. The teaching profession has been identified as highly stressful occupation (Weber et al., 2001, 2004) and thus teachers should be especially supported in their efforts to cope with the high demands imposed on them by their work obligations at school. These obligations increasingly not only include the communication of contents and general school education, but more and more also social casework and compensation for deficits in parenting. Stress reduction strategies for teachers could encompass interventions as diverse as opportunities for supervision and collegial cooperation, possibly also including facilitation of cooperation with experts outside of school such as social workers, youth welfare services, psychologists and others, strengthening of a sound work-life-balance, time and classroom management techniques, cognitive interventions, relaxation techniques or social assertiveness training. Also more flexible work time models could help to match individual needs and resources. Finally, a more understanding and appreciative societal attitude towards teachers, who are still often considered as privileged part-time workers, will be needed.
However, work stress is a phenomenon that widely spreads beyond the teaching occupation. In general, employers and employees as well as health experts and health policy makers will need to pay more attention to stress-related aspects of work including fairness at work, work-life-balance, early identification of stress-related symptoms, offering of stress management interventions etc. in order to protect health and well-being of the workforce in an increasingly stressful working life.
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6.3 References


