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The Role of Saliva Cortisol Measurement in Health and Disease

Editors:

Margareta Kristenson
Linköping University
Sweden

Peter Garvin
Linköping University
Sweden

Ulf Lundberg
Stockholm University
Sweden

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Edited By

Margareta Kristenson

Professor of Social Medicine and Public Health Science
Department of Medical and Health Sciences,
Linköping University
Sweden

Peter Garvin

Post-doc at the Department of Medical and Health Sciences,
Linköping University
Sweden

Ulf Lundberg

Professor Emeritus of Biological Psychology at the Department of Psychology and Centre for Health Equity Studies (CHESS), Stockholm University
Sweden

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FOREWORD

The ability to assess the biomarker cortisol as an index of Hypothalamic Pituitary Adrenal (HPA) axis activity has provided enormous insights about the relations between psychosocial and physical environmental characteristics and the human stress response. Having a reliable and valid index of stress has also proven invaluable in examining the role of stress in mental and physical health. The ability to assess cortisol in saliva samples has opened up vast new areas of scientific exploration, particularly at the borders of social science with public health and medicine. The collection of salivary cortisol is a relatively unobtrusive procedure that can then be analyzed at low cost. It is remarkable that this book on salivary cortisol as a human stress biomarker is authored by a group of scientists from Scandinavia since, along with some prestigious scientists in Germany, much of the pioneering work on neuroendocrine biomarkers of stress has emanated from Sweden and Norway.

This volume arrives at an opportune moment with exponential growth in the use of salivary cortisol as a biomarker of stress coupled with remarkable interdisciplinary research on the borders of the social sciences and health. Thus we can ask two key questions about salivary cortisol and scientific research: What have we learned about the utility of salivary cortisol as a biomarker of stress? How should we use this tool to assess emerging scientific questions? Reading this book provides in depth answers to these questions.

This book provides a balanced, careful, and thorough review of literally hundreds of studies relating salivary cortisol indices to sociodemographic background characteristics of individuals such as socioeconomic status and gender, psychosocial working conditions (*e.g.,* job control), perceived stress, and psychological resources such as social support. Studies of associations between salivary cortisol and biomarkers of cardiovascular and immune function as well as sleep processes are reviewed along with work on the relations between salivary cortisol and major health outcomes (*e.g.* cardiovascular disease, breast cancer) as well as mental health (*e.g.,* depression). The authors identified all potentially relevant articles then applied systematic conceptual and methodological inclusion criteria to filter out irrelevant or sloppily conducted studies. They then systematically analyzed the remaining studies, tabling results in a manner that is easy to read and understand. Each table is organized by methods of saliva collection according to variables of interest (*e.g.,* sociodemographic background, disease outcomes). The results of hundreds of studies are then discussed within each topic area taking into account the patterns of findings and implications for measurement and theory. As the reader will be able to see herein, the quality of data and the clarity of conclusions about salivary cortisol as a stress biomarker vary considerably because of measurement protocols, statistical and methodological controls, and important conceptual issues having to do with static versus dynamic measures and inter versus intra person comparisons.

The authors have done all of us who are interested in the interplay among environment, personal background, stress, and disease, a marvelous favor. They have extensively and accurately reviewed what we know about salivary cortisol as an index of human stress. The authors have provided direction as well for how future research on salivary cortisol as a biomarker of stress should proceed.

Gary W. Evans
Cornell University
USA

PREFACE

This book is based on a combination of fascination and frustration; fascination on the wish to use saliva cortisol measurement because of its many advantages but frustrations over opposing results in the literature. Several discussions at different meetings led to the development of a network of researchers from Sweden, funded by the Swedish National Research Council. This network was soon expanded to also include colleagues from Norway and Denmark. Thus, this was a Scandinavian network working on measurement of Cortisol and the name ScanCort was taken.

The main aim of the group was to try to understand the results from different studies on saliva cortisol measurement and thereby better understand how and when saliva cortisol assessment best could be made. A hypothesis was that, seemingly, divergent findings could be effects of differences in the theoretic assumptions made and methods used.

This led over to a decision to perform a literature review focusing on if the many different ways of evaluating the levels and dynamics of salivary cortisol especially with regard to time points of assessment and analyses of data affect the interpretation of cortisol measurement in various contexts.

The literature review was, of course, more work than expected but it was also a very exciting learning experience! We are grateful for the economic support given by Swedish National Research Council. We thank Gary W. Evans for being insightful, constructive and generous by reviewing all chapters and Lorna O'Brien for skillful language control. As editors we thank all colleagues in the ScanCort group for an unforgettable time together, for stimulating discussions and hard work. My specific thanks goes to my two co-editors professor Ulf Lundberg and PhD Peter Garvin for their work, enthusiasm and friendship.

We do hope that this book will be of use for all those who are involved in the challenging but fascinating field of stress research and want to use saliva cortisol measurement. We do believe that this can be a useful biomarker in many settings, if caution is taken in the choice of methods used.

Margareta Kristenson
Linköping University
Sweden

List of Contributors

Anette Harris, Ph.D. Researcher at Uni Health and Department of Psychology, Bergen University, Bergen, Norway.

Anne Helene Garde, Ph.D. Senior researcher at the National Research Centre for the Working Environment, Copenhagen, Denmark.

Åse Marie Hansen, Ph.D. Senior researcher at the National Research Centre for the Working Environment, Copenhagen, Denmark.

Berndt Karlson, M.D., Ph.D. Doctor in Occupational and Environmental Medicine,at the Department of Public Health and Clinical Medicine, Umeå University, Sweden.

Björn Karlson, Ph.D. Professor at the Department of Psychology, Lund University, Lund, Sweden.

Christin Mellner, Ph.D. Researcher at the Department of Psychology, Stockholm University, Sweden.

Christina Halford, M.D., Ph.D. Researcher at the Department of Public Health and Caring Sciences, Uppsala University, Sweden.

Frida Eek, Ph.D. Associate professor at the Division of Occupational and Environmental Medicine, Lund University, Sweden.

Ingibjörg H. Jonsdottir, Ph.D. Associate professor at the Institute of Stress Medicine, Gothenburg, Sweden.

Lars-Gunnar Gunnarsson, M.D., Ph.D. Medical Doctor in Occupational and Environmental Medicine at Örebro University Hospital, Örebro, Sweden.

Margareta Kristenson, M.D., Ph.D. Professor in Social Medicine and Public Health Science at the Department of Medical and Health Sciences, Linköping University, Sweden.

Nanna Hurwitz Eller, M.D. Medical Doctor in Occupational and Environmental Medicine at Bispebjerg Hospital, Copenhagen, Denmark.

Oskar Lundgren, M.D., Ph.D.-student in Social Medicine and Public Health Science at the Department of Medical and Health Sciences, Linköping University, Sweden.

Peter Garvin, Ph.D, MPH. Post-doc in Social Medicine and Public Health Science at the Department of Medical and Health Sciences, Linköping University, Sweden.

Petra Lindfors, Ph.D. Associate professor in psychology at the Department of Psychology, Stockholm University, Sweden.

Roberto Riva, Ph.D. at the Department of Psychology, Stockholm University, Sweden.

Roger Persson, Ph.D. Senior reseacher at the National Research Centre for the Working Environment, Copenhagen, Denmark.

Torbjörn Åkerstedt, M.D., Ph.D. Professor at the Stress Research Institute, Stockholm University, Stockholm, Sweden.

Töres Theorell, M.D., Ph.D. Professor Emeritus at the Stress Research Institute, Stockholm University, Stockholm, Sweden.

Ulf Lundberg Ph.D. Professor Emeritus of Biological Psychology at the Department of Psychology and Centre for Health Equity Studies (CHESS), Stockholm University, Sweden.

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CHAPTER 1

The Role of Saliva Cortisol Measurement in Health and Disease. Introduction - Why This Book?

Margareta Kristenson[1,*], Peter Garvin[2] and Ulf Lundberg[3]

[1]Professor in Social Medicine and Public Health Science at the Department of Medical and Health Sciences, Linköping University, 58183 Linköping, Sweden; [2]Post-doc at the Department of Medical and Health Sciences, Linköping University, Sweden and [3]Professor in Psychology at the Department of Psychology and Centre for Health Equity Studies (CHESS), Stockholm University, Stockholm University, Sweden.

Abstract: In recent decades, the technique of using ambulatory saliva sampling for measuring cortisol levels has become increasingly popular in field research and clinical studies aimed at investigating bodily responses to psychosocial stress and other psychological and clinical conditions. This interest is paralleled with frustrations on opposing and ambiguous results. To get a deeper understanding of the seemingly contradictory results, the Scandinavian cortisol and stress network (Scancort) was formed, based on 20 researchers from the disciplines of public health, psychology, biology and medicine. This book is based on a critical review of the existing empirical literature on salivary cortisol, aiming to evaluate the usefulness of salivary cortisol as a biomarker in various settings. In particular, this book focuses on how the many different ways of evaluating the levels and dynamics of salivary cortisol (*i.e.*, with regard to time points of assessment and different algorithms used to integrate data from multiple time points) affect the interpretation of cortisol measurements in various contexts. One main question is to find out if it is possible that different results of studies involving cortisol assessments are functions of differences in the theoretic assumptions made and the methods used.

Keywords: Salivary cortisol, stress, cognitive activation theory of stress, adults, ambulatory, single time point measures, deviations measures, area under the curve, laboratory test, dexamethasone.

INTRODUCTION

Cortisol is a stress hormone that can be measured in blood, urine and saliva. In recent decades, the technique of using ambulatory saliva sampling for measuring cortisol levels has become increasingly popular in field research and clinical studies aimed at investigating bodily responses to psychosocial stress and other psychological and clinical conditions. This non-invasive method is easy to administer and therefore can be implemented in large-scale study designs. Additional advantages are that, compared with blood sampling, saliva measurements do not induce discomfort or pain and do not interfere with the participants' normal activities and environment. Furthermore, saliva samples provide practical advantages; the rate of deterioration of cortisol is low in samples stored at room temperature. It has been shown that cortisol concentrations are relatively stable even after storing for a week at room temperature [1, 2]. Moreover, it has been shown that saliva can be stored in a refrigerator for at least 3 months without loss of cortisol, and cortisol concentrations are not affected by freezing of samples to any major extent [2, 3].

This interest in the use of saliva cortisol measurements is paralleled with frustrations on opposing and ambiguous results. Several psychological and physiologic conditions have been associated with increased and decreased cortisol levels.

Context

To get a deeper understanding of the seemingly contradictory results, the Scandinavian cortisol and stress

***Address correspondence to Margareta Kristenson:** Professor in Social Medicine and Public Health Science at the Department of Medical and Health Sciences, Linköping University, 58183 Linköping, Sweden; Tel: +46 10 103 5075; Fax: +46 10 103 1865; E-mail: margareta.kristenson@liu.se

Margareta Kristenson, Peter Garvin and Ulf Lundberg (Eds)

network (Scancort) was formed. The network consists of about 20 researchers from the disciplines of public health, psychology, biology and medicine. It has been financed by the Swedish Research Council to gather competence and experience in Scandinavia regarding cortisol measurements.

In particular, the aim of the network has been to evaluate (and further develop):

a. The theoretic basis in the existing literature regarding saliva cortisol and its assumed association with psychological and biological parameters.

b. Saliva cortisol in relation to perceived stress and the possible role of cortisol in stress mediated pathogenesis.

c. When and how measurements on saliva cortisol are and should be made.

d. Different statistical approaches on saliva cortisol measurements and their interpretations, in particular on psychobiological associations.

The aim of the network has not been to investigate or compare performance characteristics and feasibility of different laboratory methods for quantification. For further reading on this topic, we recommend earlier work published elsewhere, for instance by Hansen and colleagues [4, 5].

This book has been written for other researchers who are interested in cortisol research. The sections on cortisol and stress theories are kept to introductory overviews. The main part of the work has involved scanning the existing literature and compiling the results on cortisol and various variables.

AIM

This book is based on a critical review of the existing empirical literature on salivary cortisol, aiming to evaluate the usefulness of salivary cortisol as a biomarker in various settings and what the results from measurements of cortisol mean in different study designs. In particular, the book focuses on how the many different ways of evaluating the levels and dynamics of salivary cortisol (*i.e.*, with regard to time points of assessment and different algorithms used to integrate data from multiple time points) affect the interpretation of cortisol measurements in various contexts. One main question is to find out if it is possible that different results of studies involving cortisol assessments are functions of differences in the theoretic assumptions made and the methods used.

CORTISOL

Cortisol is one of the main stress hormones, and essentially prepares peripheral organs for action. The release of cortisol is mediated by the Hypothalamus-Pituitary-Adrenal (HPA) axis. The principal stimulus of the HPA axis is Corticotrophin-Releasing Hormone (CRH), which is locally produced in hypothalamus. When facing an acute stressor, CRH increases markedly and induces increased secretion of Adrenocorticotropic Hormone (ACTH) from the pituitary gland. ACTH stimulates the cortex of the adrenal glands to secrete cortisol into the circulation.

The role of cortisol has been discussed in 2 main areas: metabolism and inflammation. An increase in cortisol levels increases the supply of energy and oxygen, which translates to a temporary increase in blood pressure, blood glucose levels and free fatty acids. Therefore, it has been suggested that long-term dysregulation of cortisol leads to metabolic abnormalities [6].

The anti-inflammatory properties of cortisol are well known and have led to the widespread clinical use of exogenous cortisol (hydrocortisone). However, the role of endogenous cortisol is much more complex than regulating metabolism and inflammation. It has been suggested that cortisol modulates expression in approximately 10% of the body's genes [7].

Cortisol acts on the intracellular nuclear receptors found in most cell types throughout the body, regulating the transcription of target genes [8]. Thus, in contrast to stress hormones such as adrenaline and noradrenaline, which rapidly enter the bloodstream after exposure to a stressful situation, the secretion of cortisol is generally slower and peaks typically after 20-30 min [7].

In normal physiology, a typical pattern for most individuals shows a distinct diurnal variation in cortisol levels, peaking approximately 30-45 min after awakening and declining throughout the day, with lowest levels at night, around 04:00 h [9, 10] (Fig. **1**). It is not uncommon for the peak values in the morning to be 10-fold higher or more compared with the levels at night.

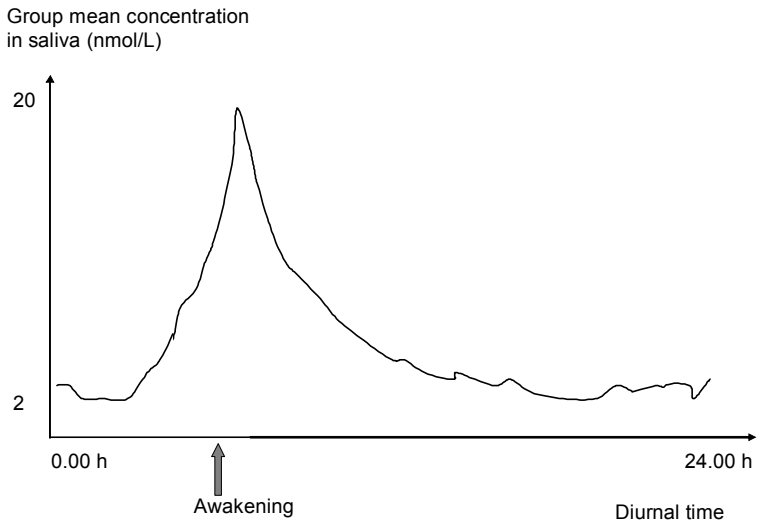

Figure 1: Diurnal variation of cortisol secretion. Figure modified from Ranjit *et al.* [7] and Kudielka *et al.* [8].

STRESS

The word stress has several connotations and is sometimes used in different ways. Our work is based on the definition developed by Levine and Ursin in Brown *et al.* [11]. They divide the term stress into 4 different entities, as seen in Table **1**. These 4 meanings of stress can and should, according to Levine and Ursin, be measured separately to further understand the concept of stress and the role of psychoneuroendocrinology in health and disease [11, 12].

Table 1: Four formal definitions of stress according to Levine and Ursin [11]

Entities of stress
Exposure (stressor, stimulus)
Experience and feelings of the situation (based on self-reports)
Psychoneuroendocrinological activation
Experience and feelings of the somatic response

This introduction begins by discussing the psychobiological stress response and how this is, or could be, linked to measures of stressors and self-reported feelings. Hans Selye, often claimed to be the father of the stress concept, conceptualized stress as the General Adaptation Syndrome (GAS). Based on experimental animal studies, Selye postulated that exposure to stressors is followed by a generalized response consisting of 3 stages as shown in Fig. **2** [13].

Stage 1, the alarm stage or acute stage, is triggered by exposure to any kind of potentially harmful stimulus. This stage has been described by Cannon as the well-known catchphrase “fight or flight,” when the body quickly mobilizes energy to handle a potential threat [15]. An acute response is characterized by a

hormonal shift towards a catabolic state. The stress hormones cortisol, adrenaline, and noradrenaline increase, whereas anabolic hormones promoting repair and growth such as insulin and sex steroids decrease [16]. After exposure and this rapid mobilization, the physiologic response declines [14]. The second stage is referred to as the stage of resistance, or stage of adaptation, when the physiologic response remains high in order to meet the demands of a prolonged stressor. If the duration of exposure is further prolonged, the body eventually reaches the third stage, the stage of exhaustion. The exposure to stressors has now triggered a dysfunctional state and hormonal imbalance, in which the physiologic response is weak, despite being exposed to a stressor normally triggering a strong response.

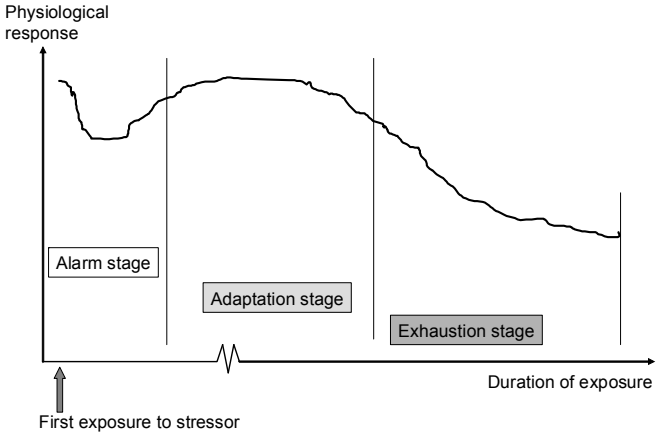

Figure 2: The three stages of General Adaptation Syndrome. Modified from Selye [13, 14].

When transferred to laboratory stress tests of humans or ambulatory sampling in normal populations in everyday life, individuals who are in the exhaustion stage based on GAS should, according to this model, be less responsive when exposed to new acute stressors.

This is supported by empirical data, comparing groups with clinical or subclinical signs of fatigue and exhaustion with groups with less or no signs of exhaustion, the latter group showing a higher responsiveness [17-19]. However, opposite findings with higher cortisol responsiveness in patients characterized by stress-related fatigue have also been reported [20]. Thus, an important notion in stress theory is that the response to an acute stressor is determined more by individual characteristics and the history of previous and current stressors, than by the actual tested acute stressor itself [12]. These observations have led to the incorporation of cognitive function in stress theory, a contribution not originally included in Selye's early work.

THE COGNITIVE ACTIVATION THEORY OF STRESS

There are several stress theories, somewhat overlapping, which incorporate cognitive function in frameworks to understand stress and how a stimulus is translated into a physiologic response. One of the more widespread is the Cognitive Activation Theory of Stress (CATS), formalized by Ursin and Eriksen [12]. The main component in CATS is the feedback to the brain from the outcome of the response, which alters both the exposure to the stimulus and the perception of the stimulus in similar situations henceforth (Fig. **3**). Whether a stimulus is considered exciting or threatening depends on previous experiences and expectations of the outcome [12]. The process is dynamic; the stressor and outcome are evaluated and re-evaluated in similar future situations.

Consonant with this, the feeling of being stressed can be linked with both positive and negative outcome expectancy. Thus, according to CATS, there is no point in trying to measure stress by objectively focusing on the external load of exposure. Attempts to measure stress should be focused on the subjective experience and feelings elicited by the stressors and the stress response [12]. CATS has implications on GAS as it

leads to the conclusion that it is not possible to make a general dose-response association between load of exposure and stage of exhaustion.

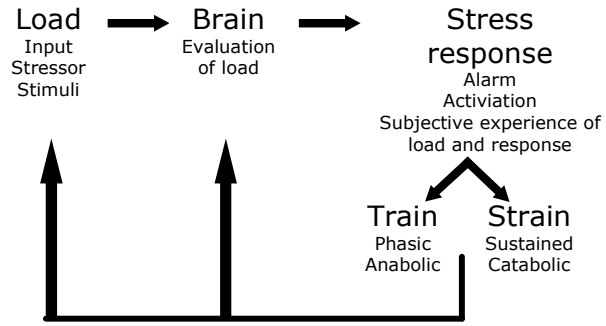

Figure 3: The cognitive activation theory of stress. From Ursin and Eriksen [12].

Folkman *et al.* [21] have summed up the importance of cognitive function in filtering stress load with the statement that "it is not stress *per se* (referring to exposure to a given stimulus), but how people cope with it, that affects health and well-being".

According to CATS [12], the stress response depends on acquired expectancies of the outcomes of stimuli and available responses. Exciting or threatening depends on the individual appraisal of the situation, which is based on previous experience and expectations of the outcome. Thus, the stimulus expectancies may be distorted by psychological defense mechanisms, at least in humans. The response outcome expectancies to the available responses are defined as positive, negative or none. This offers formal definitions of concepts such as coping, hopelessness, and helplessness. For example, helplessness means that individuals cannot see any relation between their actions and the outcome of a threatening situation; hopelessness means that they believe that any action would lead to failure or even a catastrophe. The theory suggests that, if the man/animal is coping successfully, the threat or demands has a short phasic training effect on the body. If the man/animal is in a state of hopelessness or helplessness, it may lead to sustained activation and a catabolic strain effect on the body or lack of adequate response.

THE CONCEPT OF ALLOSTASIS

In 1988, Sterling and Eyer [16] introduced the concept of allostasis in stress theory. The term allostasis literally means "to stand in variability," denoting stability through change. It was introduced as an antonym to the well-known term homeostasis meaning "to stand equally," denoting stability through constancy. Allostasis is based on the observation that most physiologic variables have a diurnal variation, determined by specific behavioral states and environmental events. Sterling and Eyer [16] argued that the term homeostasis may be misleading as it wrongly implies that different systems are kept constant at a "normal level." They claimed that more important for maintaining health is the ability to respond, thereby causing an appropriate arousal when facing an environmental challenge. A more adequate terminology would address the variation rather than the chronic state that homeostasis implies. In their allostatic model, health is defined as a state of responsiveness, including also the ability to restitute. An insufficient restitution leads to a sustained arousal, which in turn inevitably leads to the inability to respond appropriately.

This concept has been further elaborated by McEwen and Wingfield [22] who also pointed out that although homeostasis applies to a limited number of systems essential for life such as maintenance of an adequate body temperature, blood pH and glucose level, and oxygen tension, allostasis is a necessary process to support homeostasis in these systems.

Both Sterling and Eyer [16] and McEwen and Wingfield [22] acknowledge that regulation of allostasis is multileveled, involving feedback at several levels from several hormones. However, cortisol is still described as a key player in allostatic regulation.

CORTISOL MEASUREMENTS

Ambulatory Saliva Sampling

Cortisol in saliva has been shown to reflect concentrations in serum with good precision [23, 24]. In particular, it is suggested that cortisol in saliva reflects the concentration of free cortisol (unbound to carrier proteins), which is believed to be the biologically active form. Convenient methods for ambulatory saliva sampling have been developed. A common, simple, and well-known device consists of a plastic tube and a cotton/polyester swab by which people can collect saliva themselves, for example during a normal day at home or at work, and then send the samples to the laboratory for analysis.

In addition to the marked diurnal variation, the day-to-day variation in cortisol levels within the same individual is also considerable. The correlation between cortisol levels on consecutive days has been reported to be around r=0.5 in several studies [18, 25, 26]. This variation can be explained to a large extent by temporary states in individuals, which may vary from day to day [18, 26, 27]. In order to obtain more reliable values, sampling over several days is often used. The mean levels over 2 or 3 days give more reliable results when testing whether people have a general capacity to respond in a certain way to a new challenge, for example, to working conditions compared with non-work at home.

In addition to the variation within subjects on different days, the variation between subjects is also high [9]. Therefore, in order to compensate for inter- and intra-individual variations in statistical analysis, it is commonly recommended to use relatively large samples of participants for meaningful intergroup comparisons, for example, between patients and healthy controls.

Standardized Laboratory Stress Testing

Cortisol secretion in response to a defined stressor exposure such as external stimuli (*e.g.*, light) or cognitive and emotional activation is sometimes of interest as an indicator of the regulation of the HPA axis. Several models have been developed, such as the Trier Social Stress Test (TSST) and other standardized stress tests, *e.g.*, the Stroop Color Word Test, Anger Recall, Mental Arithmetic, and the Cold Pressor Test.

The Physiologic effects vary widely across tasks [28], making it hard to compare studies with each other if only one stressor is used and therefore a combination on two ore more stressors are recommended. Test-retest stability of laboratory stress responses increases if the laboratory stress includes more than one stressor [17].

Standardized laboratory testing and ambulatory saliva sampling have pros and cons. Using standardized testing in laboratory settings allows full control over the setting, including type of exposure and time for sampling. However, in addition to higher cost, problems are that according to cognitive stress theory, people with different experiences and expectations have different physiologic responses to the same stressor [11, 12]. Also, the stressors chosen may not be valid and easily transferred into everyday life conditions, and generalization to other contexts may be reduced.

Ambulatory saliva sampling under natural conditions, on the other hand, may be representative of the individuals' responses to everyday life, but may lack precision in defining the stressors as well as the time point when the samples were taken [29].

Dexamethasone Suppression Test

The dexamethasone suppression test is a specific test of the regulation of the HPA axis. Dexamethasone is a synthetic steroid, more potent than cortisol, which exerts a negative feedback to the pituitary to suppress the secretion of ACTH and, consequently, of cortisol. It is used clinically by monitoring cortisol after administration of the drug. In short, in a functional HPA axis, cortisol levels should be suppressed, whereas non-suppression of cortisol after administration indicates dysregulation of the HPA axis.

Standardization and Control of Confounders

All saliva sampling for cortisol assessment requires careful control in terms of sampling procedure and information on factors potentially influencing cortisol levels. The circadian variation of cortisol puts strong demands on these measurements to be clearly defined in terms of the actual time for sampling and the individual's circadian rhythm, such as time of awakening in the morning. Even small variations in time of sampling, especially in the morning, could have significant effects on the results. Clear instructions and compliance by the participants are needed to control for food intake, beverages, tobacco, alcohol, physical activity, medicine, sleep the night before, and other confounders.

Different Cortisol Measurements

This book focuses on how the many different ways of measuring and evaluating the level and dynamics of salivary cortisol may influence the results. In the literature on saliva cortisol assessments the main types of measurements can be divided into the following groups:

a. Single time points, including means (or sums) of several single measurements.

b. Deviations/slopes between 2 or more measurements.

c. Area under the curve (AUC) calculated from 2 or more measurements, and

d. Effects of the dexamethasone test.

Single Time Points

Measurements at single time points were more common in earlier studies, and are still common for serum cortisol. For example, a blood sample for cortisol analysis can be taken at a morning visit to the research laboratory.

With regard to saliva cortisol, repeated ambulatory sampling is common, where people collect saliva samples at home and/or at work at different times of the day. Measurements from these single time points are presented in several studies. It is also common that means or sums of 2 or several measurements over one day or several days are used to determine the level of cortisol. For example, Cohen *et al.* [30] measured salivary cortisol for several days in order to investigate the relation between cortisol and socioeconomic status (SES) defined by education and income. The term static can be used here to describe the use of single time points (or means) to determine a certain concentration. These measurements do not provide any information on the dynamics of the system, *i.e.*, whether the individual can respond adequately to a challenge or unwind and relax after stress exposure. Sometimes, one of these single time point measurements is called the basal or rest level, when it represents a rest period or the level observed before exposure to a standardized stress test.

However, to evaluate if the level is high or low relative to other samples or other individuals, it is crucial to know when a sample is taken, because cortisol levels typically follow a diurnal variation.

Deviations/Slopes Between 2 or More Measurements

When more than one saliva sample is used to assess changes in cortisol levels with time, these measurements can be used to quantify variations in activation of the HPA axis. These dynamic measurements aim to determine a change in secretion and indicate the individual's capacity to respond to or recover after stressful stimulation.

The main types of dynamic measurements seen are differences or slopes between 2 or more measurements. In ambulatory research, several deviations can be identified.

One of the most commonly used dynamic measurements is the difference between awakening and adjacent time points, typically 15 min and 30 min after awakening [31]. This deviation/difference is often called the

Cortisol Awakening Response (CAR) or Awakening Cortisol Response (ACR) and can be defined as absolute and relative. For example, Riva *et al.* compared absolute CAR between female patients with fibromyalgia and healthy controls [32] and Olsson *et al.* [20] investigated relative CAR in patients suffering from stress-related fatigue.

Another measurement derived from ambulatory saliva sampling is the difference between morning and evening levels of cortisol, the cortisol decline over a day, which is often called diurnal variation. Although simplified, it is believed to capture patterns of secretion over the day by measuring cortisol at different points in time throughout the day or days. In the literature, this has been done by either deliberately including the peak value in the morning and subtracting evening values (see for instance [33]), or deliberately excluding the peak value in the morning and subtracting evening values (see for instance [34]). The rationale for the former would be to study the general capacity of the dynamics of cortisol secretion. The rationale for the latter would be that the peak value has a large effect on the deviation [34].

In addition, deviations have been presented between midday/afternoon, or late evening. For example, Nater *et al.* [35] compared morning versus evening salivary cortisol between people with Chronic Fatigue Syndrome (CFS) and a non-fatigued group.

Using standardized laboratory stress testing, dynamic measurements can focus on either reactivity during the stress test or recovery after the stressor. Reactivity is commonly measured as the difference between peak/stress level and baseline/pretest level, answering the question "how high was the increase in cortisol?" Recovery is commonly measured as either the difference peak/stress level and poststress level (after a fixed time point) or how long it takes in minutes to return to baseline values after finishing the test. For example, Kristenson *et al.* [17] investigated cortisol reactivity to a standardized stress test (TSST) in Lithuanian (Vilnius) men exposed to long-term psychosocial stress compared with healthy Swedish (Linköping) men (Fig. **4**).

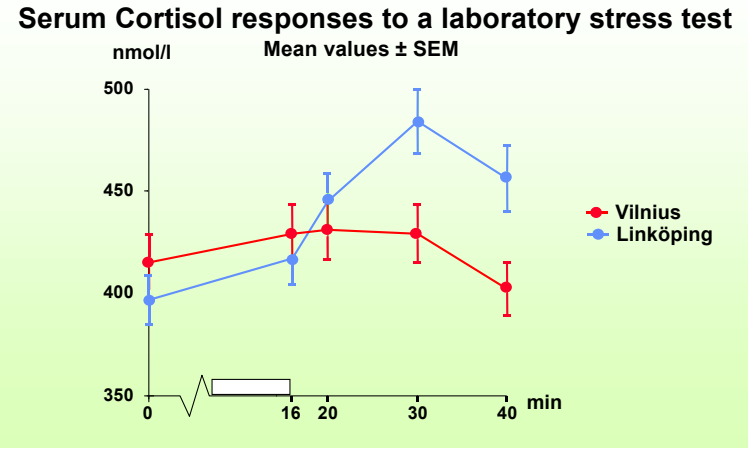

Figure 4: Results of a standardized laboratory stress test comparing two populations (reproduced from Kristenson *et al.* [36]).

Area Under the Curve Calculated from 2 or More Measurements

The AUC captures an integrated value of cortisol excretion over a period of time. It is believed to combine information from several consecutive time points into one variable to facilitate statistical analysis. This calculation is done in 2 (principally different) ways [37]. AUC with respect to increase calculates the area under the curve using the first value as reference. In other words, it captures a change in secretion (*e.g.*, after a certain stressor) at a certain time. The AUC with respect to ground also includes the area created by the basal level (pre-test level). In other words, it captures the overall concentration at a certain time. It has also been shown empirically that there is a reasonably good correlation between AUC with respect to ground and levels of free cortisol in 24-h urine samples (r=0.4) [38].

As with the deviations mentioned earlier, the values of AUC are highly dependent on the time frame used in calculation. AUCs can be categorized as morning, mid-day, diurnal, and laboratory stress test. Both AUC with respect to ground and AUC with respect to increase may be computed for any time frame.

Effects of the Dexamethasone Suppression Test

This test typically measures the overnight effect of oral administration of 1 mg dexamethasone. Most healthy participants seem to respond well and their HPA axis is totally suppressed the day after, although in other cases the cortisol levels are not affected. This is commonly described as non-suppression. For example, Lange *et al.* [39] performed the Dexamethasone Suppression Test (DST) in 21 female borderline patients and 23 healthy controls [39].

Relation of Saliva Cortisol Levels to Covariates - Stressors, Buffers and Outcomes

The main aim of several studies using saliva cortisol measurements was to examine the importance of health determinants in relation to cortisol levels. In addition to the sampling procedure and standardization of the test setting, validity of results is also related to proper use of statistical analyses and control of confounders.

The distribution of saliva cortisol in a population is usually negatively skewed, which can be solved by logarithmic transformations of the data before use of parametric methods. This means that the influence of a few very high values is reduced. As logarithmic measurements are difficult to interpret, measurements are usually transformed back to actual values (*e.g.*, nmol/L) before presentation in tables and figures.

Lack of control for age and sex, time when the cortisol sample is taken, the individual's normal awakening time, various forms of medication, *e.g.*, antidepressant treatment, and many other factors may confound the result and modify the effects on the cortisol responses.

CORTISOL AND HEALTH

Cortisol is related to several health determinants. To a large extent, these health determinants can be logically organized in a hierarchy, as described in the model from Marmot and Wilkinson [40] (Fig. **5**).

This structure is also relevant according to CATS theory, *i.e.*, in terms of these factors being stressors, buffers, physiologic correlates, or measures of morbidity.

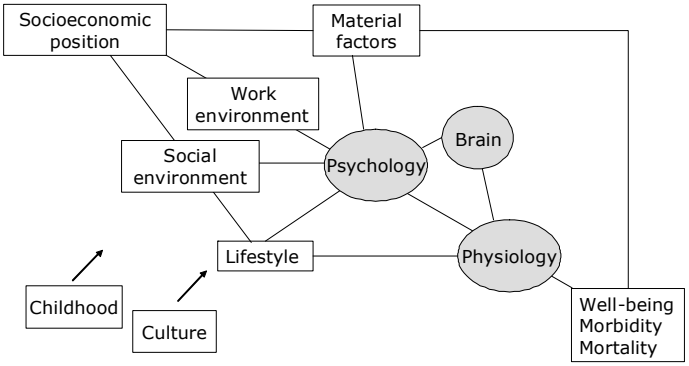

Figure 5: Model suggesting a link between external factors and health. Modified from Marmot and Wilkinson [40].

Stressors include life conditions in terms of individual SES and ethnicity and psychosocial work environment. Low SES, immigrant status, a poor work environment in terms of high job strain and poor social support, and poor balance between effort and rewards, are factors known to be associated with a higher risk of premature death and ill-health, most pronounced for mental ill-health, Cardiovascular Disease (CVD), and musculoskeletal disorders.

With regard to buffering resources, according to the CATS model, the stressor, *per se*, might not be informative enough regarding an individual's response in terms of straining or training to exposure. Whether the exposure is

stimulating and exciting or threatening depends on the individual's appraisal of the situation, which in its turn is based on previous experiences and expectations of the outcome. The stimulus expectancies may, however, be distorted by psychological defence mechanisms, *e.g.*, denial or wishful thinking. This response outcome expectancy can be measured using psychometric scales of psychological resources, such as mastery, coping, self-esteem, sense of coherence [41], and psychological well-being [42].

Sleep is of particular importance in this context as it is the most important part of the restitution. During sleep, deep slow-wave (according to electroencephalography) sleep in particular, catabolic processes are replaced by anabolic processes and immune functions are enhanced. Sleep is also of importance for adequate metabolic functioning and it has been indicated that memory consolidation is strengthened during sleep.

With regard to physiologic correlates, long-term exposure to stressful conditions, such as work-related stress, is known to lead to increased risk for several disease groups, of which CVD is one. Therefore, the relationship between cortisol levels and established biomarkers for CVD risk are relevant. These include Body Mass Index (BMI), waist hip ratio, lipid levels, blood pressure, and insulin resistance (plasma glucose and insulin levels).

The concept of allostatic load combines these factors with catecholamines (as a measure of the autonomic nervous system activity) and increased coagulation (*e.g.*, fibrinogen level). Another physiologic pathway to observed outcomes of high stress (or stressor load) is immunologic factors; *e.g.*, Inter Leukins (IL), especially IL-6, and factors related to vulnerability (matrix metalloproteinase). Additional physiologic measurements include other hormones, *e.g.*, growth hormones.

With regard to morbidity measures, several mental and somatic health problems have been associated with cortisol secretion. Examples of mental health problems are perceived stress (as defined by Cohen [43]), depression, vital exhaustion, and burnout. Examples of somatic health problems are CVD, cancer, musculoskeletal disorders, and pain [44-47].

Table 2: Example of main table as used in the chapters

References	Year	Exp.	Des.	n. cort.	M/W	Single time points (or sum/mean of two or more time points)					Deviation (difference/ slope between two or more time points)					AUC								Dexamethasone Suppression test
						a1	a2	a3	a4	a5	b1	b2	b3	b4		c1		c2		c3		c4		d
Exposure/outcome 1																								
Last name [1]	1996		Exp	87	87/0									0								0		
Last name [2]	1996		C-S	180	88/92						↓		0							0				
Last name 3 [3]	1999		C-C	66	24/42						0													↑
Last name 4 [4]	2000		Pros	59	59/0		0			0							↑							
Last name 4 [5]	2003		C-S	36	10/26						0		0				↑				0			

Information in table:

Reference Last name of first author.

Year Year of publication.

Exp Exposure (or outcome depending on content in chapter).

Des. Study design where C-S stands for cross-sectional, C-C for case-control, Pros. for prospective and Exp. for experimental design.

n cort Number of participants with cortisol measurements.

M/W Indicates number of me and women in the study group.

Arrow up indicates a positive significant association, arrow down a negative significant association and 0 a nonsignificant finding.

OUTLINE OF THIS BOOK

In the chapters of this book, a literature review of recent empirical studies is presented, relevant for the relations described earlier for health determinants/outcomes in relation to cortisol. This was done by sorting the evidence according to the methods used for measuring cortisol: single time points, deviation/slope, and AUC

measurements or dexamethasone test according to the following schemes (Table **2**). All chapters present results in tables following this template. Each chapter includes 2 types of tables: 1 with full information on the study design, type of sample, and the methods used for sampling saliva, measurements of covariates and results; according to the three schemes above. The second table summarizes the findings from the first table, using arrows (up or down) and zeros to indicate positive, negative, and nonsignificant findings.

5 different categories of single time points are used for presenting results, 4 for deviations and 4 for AUC. These are defined as follows:

Single Time Points

a1. At awakening (immediately on awakening; normal awakening time).

a2. In the morning (including the morning peak).

a3. At midday (from 12:00 to 18:00 h).

a4. In the evening (normally late evening, in a restful state, before going to bed).

a5. Mean or sum of several measurements over the day.

Deviation

Using ambulatory saliva sampling:

b1. Morning (difference between awakening and adjacent time point, absolute or relative).

b2. Midday.

b3. Morning-evening: pattern of secretion over the day.

Using standardized laboratory stress testing:

b4. Standardized laboratory test; reactivity (difference between peak and baseline/pretest level) and/or recovery (difference between peak and poststress level, *i.e.*, time when return to baseline was expected); when relevant; baseline before exposure to stressor.

AUC

c1. Morning (increase/ground).

c2. Midday (increase/ground).

c3. Morning-evening (increase/ground).

c4. Laboratory test (increase/ground).

Dexamethasone Suppression Test

No stratification on different doses in dexamethasone test has been made.

Book Content

The book contains 7 chapters on salivary cortisol in relation to a broad spectrum of factors. (Table **3**).

Table 3: Evaluated factors in relation to salivary cortisol

Chapter number	Content	Factors included (search terms)
2	Socioeconomic status and demographic variables	Education attainment Occupational status Income Ethnicity Age Sex
3	Psychosocial work stressors	Effort-reward-imbalance Demand-control-support-models
4	Perceived stress and psychosocial resources	Perceived Stress Scale Locus of Control Mastery Self-Esteem Sense Of Coherence
5	Biological markers	Body Mass Index Waist circumference Waist/hip ratio Blood pressure Cholesterol (Total; LDL; HDL) Triglycerides Blood glucose Heart rate Heart rate variability C-reactive protein Interleukin-1 Interleukin-6 Tissue Necrosis Factor alpha
6	Sleep	Sleep duration Overall sleep quality Difficulty falling asleep Disturbed/restless sleep Sleep deprivation
7	Mental health problems	Depression Vital exhaustion Burnout
8	Somatic disease	Cardiovascular Disorders Cancer Rheumatoid Arthritis Pain

In each chapter, and in the summary in Chapter **9**, the findings are discussed in relation to the question: Is it possible that divergent results of studies are related to different theoretic assumptions and methods used?

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CHAPTER 2

Socioeconomic Status, Demographic Variables and Salivary Cortisol

Peter Garvin[1,*], Nanna Hurwitz Eller[2] and Anette Harris[3]

[1]Post-doc at the Department of Medical and Health Sciences, Linköping University, 581 83 Linköping, Sweden; [2]Medical Doctor in Occupational and Environmental Medicine at Bispebjerg Hospital, Copenhagen, Denmark and [3]Researcher at Uni Health and Department of Psychology, Bergen University, Bergen, Norway

Abstract: This chapter evaluates the association between salivary cortisol and socioeconomic variables (level of education, occupational status, income and related composite measures), ethnicity, age and sex. There were many non-significant findings for all variables, indicating that the associations with cortisol levels are relatively small. Regarding the significant results, there were some consistent trends. It is implied that high SES, regardless of how it is measured, is associated with a higher cortisol deviation throughout the day, and a higher capacity to react with increase in cortisol following a laboratory stress test. Regarding ethnicity, results consistently hint at a higher deviation throughout the day amongst Caucasians in comparison to Hispanics and Afro-Americans. Analyses on sex were not fully consistent, possibly due to influences of the menstrual cycle on cortisol levels. In addition, it has been reported that men and women respond differently to different stressors used in laboratory stress tests. For age, the significant findings found may hint at a small but general increase in cortisol levels throughout the day with increasing age.

Keywords: Salivary cortisol, socioeconomic status, educational level, occupational status, income, ethnicity, age, sex, single time point measures, deviations measures, area under the curve.

INTRODUCTION

Socioeconomic Status

Low Socioeconomic Status (SES), whether measured as educational level, income, occupational status, or other indicators are consistently associated with increased morbidity and mortality regardless of context [1-3]. The mechanisms have been discussed for decades, but are as yet not fully elucidated. Suggested mechanisms are typically based on a lower material standard and lower financial resources, a higher exposure for both environmental risk factors and behavioral risk factors, and/or lower psychosocial resources such as coping and higher psychosocial risk factors such as depressive mood and hopelessness [4-9]. As it has been hypothesized that behavioral factors and psychosocial factors may have an impact on stress hormones (see Chapters **4** and **7** for overviews), it has been suggested that at least part of the detrimental effects of low SES are mediated by stress hormones, in particular with reference to dysregulation of the Hypothalamo-Pituitary-Adrenal (HPA) axis [10].

A recent review of the literature on salivary cortisol and SES has been performed by Dowd *et al.* [11]. The review was based on 14 studies, and suggests that lower SES is related to a blunted pattern of cortisol secretion, although there are many inconsistencies in the results [11]. In the summary of the 14 studies, 4 showed an association between low SES and higher cortisol levels, 2 showed an association between low SES and lower cortisol levels (regardless of when cortisol was measured), 4 showed mixed results and 4 were non-significant [11]. The authors suggest that part of the explanation for the inconsistency was the variation in approaches regarding cortisol measurements and state that a better theory and study design should help clarify the expected and observed relationships between SES and cortisol levels. In this chapter, we go through the papers covered in the review by Dowd *et al.* [11] and add some more papers not included in the review. A brief review of ethnicity is also included in this chapter, as is sex and age.

***Address correspondence to Peter Garvin:** Post-doc at the Department of Medical and Health Sciences, Linköping University, 581 83 Linköping, Sweden; Tel: +46 10 103 8875; Fax: +46 10 103 1865; E-mail: peter.garvin@liu.se

Margareta Kristenson, Peter Garvin and Ulf Lundberg (Eds)

AIM

To examine to what extent associations between different measures of SES and cortisol measurements and can be found, and which of the cortisol measurements seem to be of highest relevance. A second aim was to examine to what extent different cortisol measurements were related to ethnicity, age and sex.

METHOD

Search Strategies

In a first step, an online search of the NCBI PubMed database was conducted (National Library of Medicine, National Institutes of Health, Bethesda, MD, USA. http://www.ncbi.nlm.nih.gov/PubMed). The search covered the period up to October 2009 (allowing e-publications if a full paper was published electronically prior to journal publication). Search terms were selected with reference to relevant PubMed terms and key words (see detailed description for each of the biological markers below), in combination with salivary cortisol in its truncated form ("saliva*"). The limitations were set only to include studies matching "human" and "English".

In a second step, studies on patient populations were excluded (*e.g.,* cancer, diabetes, and major depressive disorder). Studies on genome variations, pregnant women, and pharmacological interventions were also excluded.

In a third step, all articles retrieved from each search were briefly read. If no direct statistical analysis between salivary cortisol and the evaluated factors were presented in tables, figures, or text, the paper was excluded. Intervention studies were included if associations with the factors of interest were presented as baseline characteristics. However, the effects on salivary cortisol in response to the intervention are not included in this review. Articles were also excluded if another (prior) publication from the same study material was already included in the evaluation.

SES

The terms "socio", "socioeconomic" and "SES" were used in combination with truncated salivary and cortisol as three searches. These yielded in 62 hits. In combination with truncated salivary and cortisol, the term "educational" yielded 39 hits, "income" yielded 40 hits, and "occupational" yielded 83 hits.

Ethnicity

The terms "ethnicity" and "race" were used in combination with truncated salivary and cortisol as two searches. These yielded 46 hits.

Sex

In addition, papers were included if sex-specific analyses were reported in other papers found in searches on socioeconomy. In total, there were 84 hits.

Age

The term "age difference" in combination with truncated salivary and cortisol was used. In addition, papers were included if analyses on age were reported in other papers found in searches on socioeconomy. In total, there were 61 hits.

RESULTS

Several studies control for the effects of SES and/or ethnicity, without presenting associations between cortisol and SES or ethnicity per se. Similarly, most studies control for the effects of sex and age, but do not explicitly present associations with those factors. Therefore, the number of studies is reduced in comparison to the numbers found in the searches. After meeting all exclusion criteria, 21 papers remained, describing at least one

measure of salivary cortisol in relation to any of the measures of SES. There were 6 papers on ethnicity, 18 on sex and 12 on age. In total, 210 associations between salivary cortisol and any of the variables evaluated have been studied, comprising 108 associations on SES, 17 on ethnicity, 50 on sex, and 35 on age. The proportions between salivary cortisol and all markers tested were: for single time points, 108 (51%); for deviations, 73 (35%); for Area Under Curve (AUC), 26 (12%); and for dexamethasone tests, 4 (2%).

Educational Level

Quantitative Analysis on the Studies Evaluated

In 13 studies [12-24], there were 44 analyses on the relationship with salivary cortisol Table **1a**. Of these 27 were on single time points, 12 on deviations, 5 on AUC, and 1 on dexamethasone suppression test. In total, 12 of the analyses (27%) showed significant associations with salivary cortisol, whereas the other 32 (73%) were non-significant. Of these, 8 of the significant findings were found for single time points (30%), 3 for deviations (25%), and 1 for AUC (20%). The significant findings were mainly clustered in two categories: higher level of education was associated with low evening samples in 3 out of 6 studies (50%); higher level of education was associated with a higher capability to react on laboratory stress tests in 2 out of 3 studies (67%).

Table 1a: Summary of main findings of associations between measures salivary cortisol and high educational status sorted by year of publication

References	Year	Design	No. cortisol	Sex m/w	Single time points (or sum/mean of two or more time points)					Deviation (difference/slope between two or more time points)					AUC								Dexamethasone suppression test
					a1	a2	a3	a4	a5	b1	b2	b3	b4		c1		c2		c3		c4		d
Brandstädter [12]	1991	C-S	767	387/380		↑	0	0															
Kristenson [13]	2001	C-S	183	183/0		0							↑										
Bennett [14]	2004	C-S	56	22/34		↑				0													
Wright [15]	2005	C-S	81	40/41	0					0	0												
Steptoe [16]	2005	C-S	158	67/91			0						0										
Vreeburg [17]	2006	C-S	491	199/292	0	0		0				0			0	0							0
Daniel [18]	2006	C-S	129	0/129							0												
Cohen [19]	2006	C-S	193	95/98		0	0	0												↓			
Cohen [20]	2006	C-S	781	328/453	0	0	↓	↓		0		↑								0			
Neupert [21]	2006	C-S	74	58/26		0	0						↑										
Dockray [22]	2008	C-S	83	55/28											0								
Garcia [23]	2008	C-S	86	35/51	0	↓	↓	↓		0													
Hong [24]	2009	C-S	26	0/26	0	0	0	↓															

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory stress test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory stress test increase/ground; C-S, cross-sectional.

Consistency of the Material

Most of the associations evaluated were non-significant findings. None of the studies report any association between the level of education and cortisol levels at awakening [15, 17, 20, 23, 24], or between cortisol levels and Cortisol Awakening Response (CAR) [14, 15, 20, 22, 23]. These reports included small-scale studies and large-scale studies in different contexts. Arrows in opposite directions were found only for single time points of cortisol measurements in the morning; two studies report associations between a high cortisol level and high educational level [12, 14], whereas one study suggest an association between a low cortisol level and a high education level [23].

Methodological or Contextual Explanation for Divergent Findings

The studies reporting inconsistent results for morning values are based on study designs that differ greatly. Based on the sampling protocol in each study, the highest control of when samples were actually taken is found in Bennett *et al*. [14], where all participants received a telephone call at normal awakening time, with a second call 30 min after as a reminder to take the second sample (constituting the morning sample in the study). Brandstädter *et al.* present a large-scale study, in which participants were instructed to leave a morning sample between 07:00 h and 09:00 h [12]. In the study by Garcia *et al.* participants were instructed to leave samples at 07:00 h as the second sample in the study [23].

Occupational Status

Quantitative Analysis on the Studies Evaluated

In 5 studies [12, 13, 25-27], there were 17 analyses on the relationship with salivary cortisol. Of these Table **1b**, 11 were on single time points, 5 on deviations, and 1 on a dexamethasone suppression test. In total, 6 of the analyses (35%) showed significant associations with salivary cortisol, whereas the other 11 (65%) were non-significant. Of the significant findings, 2 were found for single time points (18%), 3 for deviations (60%), and 1 for dexamethasone suppression test (100%).

Table 1b: Summary of main findings of associations between measures salivary cortisol and high occupational status sorted by year of publication

References	Year	Design	No. cortisol	Sex m/w	Single time points (or sum/mean of two or more time points)					Deviation (difference/slope between two or more time points)				AUC								Dexamethasone suppression test
					a1	a2	a3	a4	a5	b1	b2	b3	b4	c1		c2		c3		c4		d
Brandstädter [12]	1991	C-S	767	387/380		↑	0	0														
Rosmond [25]	2000	C-S	284	284/0					0			↑										↓
Kristenson [13]	2001	C-S	183	183/0			0/↓						↑									
Steptoe [26]	2003	C-S	163	87/76		0	0	0	0	0		0										
Kunz-Ebrecht [27]	2004	C-S	128	69/59	0					↓												

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory stress test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory stress test increase/ground; C-S, cross-sectional.

Consistency of the Material

The number of studies is too low to fully evaluate the association of occupational status and salivary cortisol. However, the reported finding of deviations hint at a higher diurnal variation [25] as well as a higher capability to respond to a laboratory stress test [13] amongst subjects with higher occupational status.

Methodological or Contextual Explanation on Divergent Findings

Steptoe *et al.* [26] report non-significant associations between salivary cortisol and occupational status when evaluating the entire study population. They report that there is a difference between men and women; women with higher grades of employment tend to have somewhat higher levels of cortisol throughout the day compared with women with lower grades, whereas men with high grades of employment tend to have somewhat lower cortisol levels throughout the day compared with men with lower grades [26]. The possible sex difference may have a contextual explanation in manual/nonmanual work; the former may be associated with higher levels of stress hormones as a preparation for physical tasks.

Income

Quantitative Analysis on the Evaluated Studies

In 7 studies [12, 13, 19, 20, 22, 23, 28], there were 25 analyses on the relationship with salivary cortisol Table **1c**. Of these were 15 on single time points, 5 on deviations, and 5 on AUC. In total, 13 of the analyses (50%) showed significant associations with salivary cortisol, whereas the other 13 (50%) were non-significant. Of the significant findings, 8 were found for single time points (50%), 2 for deviations (40%), and 3 for AUC (60%).

Table 1c: Summary of main findings of associations between measures salivary cortisol and high income sorted by year of publication

References	Year	Design	No. cortisol	Sex m/w	Single time points (or sum/mean of two or more time points)					Deviation (difference/slope between two or more time points)					AUC								Dexamethasone suppression test
					a1	a2	a3	a4	a5	b1	b2	b3	b4		c1		c2		c3		c4		d
Brandstädter [12]	1991	C-S	767	387/380		↑	↑	0															
Kristenson [13]	2001	C-S	183	183/0		0							↑										
Cohen [19]	2006	C-S	193	95/98		0	0	0												0			
Cohen [20]	2006	C-S	781	328/453	0	0	↓	↓		0		↑								↓			
Dockray [22]	2008	C-S	83	55/28											↓								
Garcia [23]	2008	C-S	86	35/51	0	↓	↓	↓		0										0			
Kraft [28]	2009	C-S	94	37/57									0								↑		

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory stress test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory stress test increase/ground; C-S, cross-sectional.

Consistency of the Material

As with educational status, there is a possible contradiction in the findings presented by Brandstädter *et al.* [12] compared with Garcia *et al.* [23] regarding morning values of salivary cortisol (see above). Regarding other significant associations, there seems to be a consistency in that the findings suggesting a higher diurnal variation amongst subjects with higher income [20] are in line with findings of lower evening values [20, 23] and a lower AUC with respect to ground throughout the day [20]. Also, the reported higher capability by Kristenson *et al.* to respond to a laboratory stress test [13] amongst subjects with higher income are in agreement with Kraft *et al.* [28], who suggest that high family income is associated with higher levels of cortisol throughout a laboratory stress test (speech task).

Methodological or Contextual Explanation for Divergent Findings

There are no apparent systematic differences between the studies showing associations and the studies reporting non-significant findings. Thus, no clear contextual explanations can be found.

Other SES Measures

Using the search terms described earlier, there were a number of studies that used measures of SES that deviate from the classic three: education, occupational status, and income. Those are combined in this section.

Quantitative Analysis on the Evaluated Studies

In 6 studies [15, 19, 29-32], there were 22 analyses on the relationship with salivary cortisol Table **1d**. Of these were 10 on single time points, 10 on deviations, and 2 on AUC. Four of the analyses (18%) showed significant associations with salivary cortisol, whereas the other 18 (82%) were non-significant. Of the significant findings, 3 were found for deviations (30%), and 1 for AUC and (50%).

Table 1d: Summary of main findings of associations between measures salivary cortisol and other measurements of SES

References	Year	Design	No. cortisol	m/w	Single time points (or sum/mean of two or more time points)					Deviation (difference/slope between two or more time points)					AUC								Dexamethasone suppression test
					a1	a2	a3	a4	a5	B1	b2	b3	b4		c1		c2		c3		c4		d
High Composite SES (education × income)																							
Cohen [19]	2006	C-S	193	98/95		0	0	0												↓			
High Composite SES (education × income × material wealth)																							
Decker [29]	2000	C-S	31	31					0														
High Composite SES (education × income × self perceived SES)																							
Wrosch [30]	2007	C-S	215	103/112	0					0		0								0			
High subjective socioeconomic position																							
Wright [15]	2005	C-S	81	40/41	0					↓													
Low material hardship																							
Ranjit [31]	2005	C-S	188	W	0					↑		0											
Low financial strain																							
Wright [15]	2005	C-S	81	40/41	0					0	0												
Changes to lower financial strain																							
Steptoe [32]	2005	Pros.	114	63/51	0	0				↓/0		0											

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory stress test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory stress test increase/ground; d, DST, dexamethasone suppression test; C-S, cross-sectional; Pros, Prospective.

Consistency of the Material

Most of the associations evaluated were non-significant findings. None of the cortisol measures based on single time points showed any significant association with SES measures. Cohen *et al.* [19] suggest that, although there are non-significant findings regarding single time points in both studies, a high SES (as composite measure) is associated with a lower AUC with respect to ground throughout the day.

Ranjit *et al.* [31] suggest that low material hardship is associated with a steeper slope following the morning peak (based on a higher peak). This is in line with the results by Steptoe *et al.* [32] where an increased financial strain is associated with a lower awakening response. Although different constructs are used to measure SES, this may be somewhat in contrast to Wright *et al.* [15] who suggest that participants with lower (self-rated) SES have a higher awakening response.

Methodological or Contextual Explanation on Divergent Findings

There are no apparent systematic differences between the studies showing associations and the studies reporting non-significant findings. Thus, no clear contextual explanations can be found.

SES (All Measurements)

As there is a considerable overlap in construct between common measures used to capture SES, the results for education status, occupational status, income level, and other measures of SES are aggregated and presented together as one entity.

Quantitative Analysis on the Evaluated Studies

In 21 studies [12-32], there were 108 analyses on the relationship with salivary cortisol. Of these were 63 on single time points, 31 on deviations, 12 on AUC, and 2 on dexamethasone suppression test. Of these, 34 analyses (31%) showed significant associations with salivary cortisol, whereas the other 64 (69%) were

non-significant. Of the significant findings, 17 were found for single time points (27%), 11 for deviations (35%), 5 for AUC and (41%) and 1 for dexamethasone suppression test (50%)

Consistency of the Material

There are more non-significant findings than significant findings. However, there are few studies contradicting a general pattern where subjects with higher SES tend to have a somewhat higher diurnal deviation throughout the day, a lower AUC with respect to ground throughout the day, and a higher capacity to react with an increase in cortisol following a laboratory stress test.

Methodological or Contextual Explanation on Divergent Findings

The studies using a measure of SES that deviates from the classic three (education, occupational status, and income), *i.e.,* using a composite measure, subjective SES, or other measure, do not seem to show significant findings to the same extent as the other three (with the exception of AUC; both studies that included this measure reported a significant association).

Ethnicity

Quantitative Analysis on the Evaluated Studies

In 7 studies [14, 20, 22, 33-36], there were 17 analyses on the relationship with salivary cortisol Table **1e**. Of these were 6 on single time points, 9 on deviations and 2 on AUC. In total, 10 of the analyses (58%) showed significant associations with salivary cortisol, whereas the other 7 (42%) were non-significant. Of the significant findings, 3 were found for single time points (60%) and 6 for deviations. The significant findings on deviations were clustered in category b3 (deviation throughout the day) where 6 out of 6 findings were significant.

Table 1e: Summary of main findings of associations between measures salivary cortisol and ethnicity sorted by year of publication.

References	Year	Design	No. cortisol	Sex m/w	Single time points (or sum/mean of two or more time points)					Deviation (difference/slope between two or more time points)					AUC								Dexamethasone suppression test
					a1	a2	a3	a4	a5	b1	b2	b3	b4		c1				C3		c4		d
Caucasians vs Afro-Americans																							
Bennett [14]	2004	C-S	56	22/34		↑				0													
Wilcox [33]	2005	Exp	28	0/28		0							↓										
Cohen [20]	2006	C-S	781	328/453	↑	0	0	↓		0		↑								0			
McCallum [34]	2006	C-S	127	0/127								↑											
DeSantis [35]	2007	C-S	257	67/190								↑											
Caucasians vs Hispanics																							
Gallagher-T [36]	2006	C-S	48	0/48								↑											
DeSantis [35]	2007	C-S	257	67/190								↑											
Caucasians vs South-east Asians																							
Dockray [22]	2008	C-S	83	55/28											0								
Hispanics vs Afro-Americans																							
DeSantis [35]	2007	C-S	257	67/190								↑											

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory stress test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory stress test increase/ground; d, DST, dexamethasone suppression test; C-S, cross-sectional; Exp, experimental.

Consistency of the Material

There is a high degree of consistency in the presented results. All three studies that compared Caucasians and Afro-Americans and evaluated a deviation throughout the day reported a higher deviation among Caucasians [20, 34, 35]. It is also suggested that Caucasians have higher deviation throughout the day than Hispanics [35, 36], and Hispanics have higher deviation than Afro-Americans [35]. The results on deviations is also supported by difference at single time points by two studies, where it is reported that Caucasians have a higher level in the morning and a lower level in the evening in comparison to African-Americans [14, 20].

Methodological or Contextual Explanation for Divergent Findings

There are no apparent clear contradictions in the results. Wilcox *et al.* [33] examined stress reactivity in caregiving postmenopausal women and found that more African Americans (58%) than Caucasians (14%) showed >50% increase in cortisol during a test interview about negative aspects of being a caregiver. This laboratory task might reflect a more uncontrollable and stressful situation as a caregiver amongst African Americans than amongst Caucasians.

Sex

Quantitative Analysis on the Evaluated Studies

In 18 studies [12, 15-17, 22, 27, 30, 37-47], there were 50 analyses on the relationship with salivary cortisol Table **1f**. Of these were 21 on single time points, 22 on deviations, 6 on AUC, and 1 on dexamethasone test. Of these, 20 of the analyses (40%) showed significant associations with salivary cortisol, whereas the other 30 (60%) were non-significant. Of the significant findings, 5 were found for single time points (23%), 11 for deviations (50%), 3 for AUC (50%) and 1 for dexamethasone test (100%).

Table 1f: Summary of main findings of associations between measures salivary cortisol and sex sorted by year of publication

References	Year	Design	No. cortisol	Sex m/w	Single time points (or sum/mean of two or more time points)					Deviation (difference/slope two or more time points)					AUC								Dexamethasone suppression test
					a1	a2	a3	a4	a5	b1	b2	b3	b4		c1		c2		c3		c4		d
Sex, women vs men																							
Brandstädter [12]	1991	C-S	767	387/380		↑	↑	0															
Kirschbaum [37]	1992	C-S	153	75/78			0						↓										
Kirschbaum [38]	1999	C-S	81	20/61									↓[a]										
Stroud [39]	2002	C-S	50	24/26									↑↓[b]										
Kudielka [40]	2003	C-S	105	53/52	0					0					0								
Hansen [41]	2003	C-S	120	37/83	0	0/↑	0			0													
Kunz-Ebrecht [27]	2004	C-S	128	69/59	0					↑/0[c]													
Wright [15]	2005	C-S	81	40/41	0					↑	↑												
Steptoe [16]	2005	C-S	158	67/91									0										
Dockray [22]	2008	C-S	83	55/28											0								
Wrosch [30]	2007	C-S	215	103/112	0					0		↑							↑				
Therrien [42]	2007	C-S	82	51/31						0													
Steptoe [43]	2007	C-S	2873	2126/747					↑														
van Stegeren	2008	C-S	80	21/59			0						0										

[44]																					
O'Donell [45]	2008	C-S	479	309/170						0		0					↓				
Vreeburg [17]	2009	C-S	491	199/292	0	0		0		↑		↓			↑	0					↓
Bouma [46]	2009	C-S	644	352/292						0			↓								
Filaire [47]	2009	C-S	52	26/26		0/↑	0	0					0								

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory stress test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory stress test increase/ground; d, DST, dexamethasone suppression test; C-S, cross-sectional.

[a] Differences vary with menstrual cycle.

[b] Different results for different stressors.

[c] Difference for workdays but not for weekends.

Consistency of the Material

Overall, there were small differences reported between women and men. The highest consistency was found in laboratory stress tests, where men had a higher response than women in 4 studies (50%) [37-39, 46]. The associations in the early hours of the day hint at a possible difference, where women tend to have somewhat higher values in the morning, as assessed by CAR or single values in the morning (41%) [12, 15, 17, 27, 41, 47].

Methodological or Contextual Explanation for Divergent Findings

It has been reported by Stroud *et al.* [39] that different stress tests have different patterns for women and men. Women appear more physiologically reactive to social rejection challenges (such as being systematically excluded by associates during a conversation), but men react more to achievement challenges (where study participants were told that the investigator studied the relation between intelligence and performance) [39]. This might have implications on choice of stressors depending on whether men or women are to be studied.

Several studies indicate that cortisol levels are influenced by the menstrual cycle, which complicates analyses on sex differences. It is suggested that estradiol induces changes in corticosteroid-binding protein levels [38]. Kirschbaum *et al.* [38] suggest that men in general have a stronger hypothalamic drive in response to stressful stimulation than women. However, although the difference is consistent between men and women in the follicular phase or women using contraceptives, there were no difference between women in the luteal phase [38].

Age

Quantitative Analysis on the Evaluated Studies

In 12 studies [12, 16, 17, 21, 22, 29, 30, 40, 41, 45, 48, 49], , there were 34 analyses on the relationship with salivary cortisol Table **1g**. Of these, 17 were on single time points, 11 on deviations, 5 on AUC, and 1 on dexamethasone test. Eleven of the analyses (32%) showed significant associations with salivary cortisol, whereas the other 23 (68%) were non-significant. Of the significant findings, 5 were found for single time points (29%), 4 for deviations (36%), 1 for AUC (20%) and 1 for dexamethasone test (100%).

Consistency of the Material

Most of the associations evaluated were non-significant findings. The reported significant associations may hint at a general increase in salivary cortisol levels with increasing age. Four independent studies show that increased age was associated with increased cortisol levels, at different time points throughout the day. Moreover, two out of two studies conclude that increasing age is associated with a higher reactivity in laboratory stress tests.

Methodological or Contextual Explanation for Divergent Findings

It has been suggested that age differences are more apparent in older ages [41]. There were however no apparent differences in mean age between the studies reporting significant differences and the studies reporting non-significant findings.

Table 1g: Summary of main findings of associations between measures salivary cortisol and age sorted by year of publication

References	Year	Design	No. cortisol	Sex m/w	Single time points (or sum/mean of two or more time points)					Deviation (difference/slope between two or more time points)					AUC							Dexamethasone suppression test
					a1	a2	a3	a4	a5	b1	b2	b3	b4		c1		c2		c3		c4		d
Age, increasing																						
Brandstädter [12]	1991	C-S	767	387/380		0/↓	0	0														
Decker [29]	2000	C-S	31	31					0													
Seeman [48]	2001	C-S	40	16/24			↑															
Kudielka [40]	2003	C-S	105	53/52	↑					↓					↓							
Hansen [41]	2003	C-S	120	37/83	0	0	0			0												
Steptoe [16]	2005	C-S	158	67/91			↑						↑									
Vreeburg [17]	2006	C-S	491	199/292	0	0		↑		0		0			0	0						↑
Neupert [21]	2006	C-S	74	58/26		0	0						↑									
Wrosch [30]	2007	C-S	215	103/112	0					0		↑							0			
Ahn [49]	2007	C-S	359	167/192								0										
Dockray [22]	2008	C-S	83	55/28											0							
O'Donell [45]	2008	C-S	540	350/192						0		0										

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory stress test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory stress test increase/ground; d, DST, dexamethasone suppression test; C-S, cross-sectional.

Most studies, with significant and non-significant findings, had a fair sample size. Thus, lack of statistical power is an unlikely explanation to the large number of non-significant findings.

DISCUSSION

General Remarks

Before interpreting the results further, there are 3 aspects that should be considered.

First, we studied salivary cortisol levels alone, focusing on the feasibility of using this approach with regard to socioeconomic and demographic variables. A number of other studies using concentrations in sera and/or urine that we omitted may be of relevance when determining associations between cortisol and the factors studied.

Second, a large proportion of the articles are based on relatively small study populations. This leads to the possibility of a high number of beta errors in the presented non-significant findings. On the other hand, the results may suffer from publication bias, where non-significant findings are not reported explicitly in some papers, even though analyses were done on cortisol and socioeconomic or demographic variables. It is somewhat of a scientific oddity that there are numerous studies that adjust for age, sex, SES, and ethnicity without reporting if there are associations between cortisol and these factors to begin with. Indeed, several studies report low R^2 values for regressions on cortisol levels, also when including SES, ethnicity, age, and sex in the models. For example, O'Donell *et al.* [45] reports an R^2 value of about 0.13 when studying various cortisol measures adjusting for income, sex, age, body mass index, depression, smoking, self-rated health, and awakening time.

Third, the search strategies used may be somewhat incomplete. It is likely that associations between any of the variables covered in this investigation and cortisol in saliva have been studied and presented in papers that could not be identified in our search. In particular, this might be the case for age and sex, where analyses are presented but hidden from key words and titles, as those analyses are not a primary aim of the paper.

A description of all papers covered in this section can be found in Table **2**.

Table 2: Included studies on socioeconomic status and demographic variables sorted by appearance in text in this chapter

References	Outcome	Study design/group characteristics	Sampling	Laboratory method of standardization in sampling	Statistical approach for cortisol measure	Statistical analysis, in relation to outcome	Results	Discussion
Brandstädter 1991 [12]	Educational level Occupational status Income Age Sex	Design: C-S No: 767 M/W: 387/380 Age: 30–60 years Group: Population based recruitment of married couples from urban area, Germany Excl: not known P rate: 77%	Days: 1 Samples per day: 3 Times for sampling: Sample 1 between 07:00 and 09:00 h Sample 2 between 15:00 and 17:00 h Sample 3 between 20:00 and 22:00 h Setting: ambulatory	RIA kit. Salivettes were mailed with instructions and a short questionnaire on health status and use of medication	Cortisol data: log transformed Measurement(s): single time points at a2, a3 and a4	Correlations on age and sex. Spearman rank correlations for SES measures	a1 significantly higher for all three SES variables and for men. a2 positively associated with income and higher for men a3: No associations reported. Age was negatively associated with a1 amongst women, but not amongst men. No associations for age to a2 or a3	Cortisol levels assessed later in the day have clearly less differential predictive value, which is most probably due to decreasing variance of cortisol levels throughout the day
Kristenson 2001 [13]	Educational level Occupational status Income	Design: C-S No.: 310 M/W: men Age: 50 years Group: Population based samples in one Lithuanian city and one Swedish city Excl: advanced cancer P rate: 79%	Days: 1 Samples per day: 3 Times for sampling: afternoon Setting: laboratory setting. Baseline level and response to a standardized stress test	Not stated	Cortisol data: continuous Measurement(s): single time point at a3 Laboratory stress test (b4)	Spearman rank correlations for SES measures. Linear regressions with all SES variables in the same model	In linear regression models, after controlling for the city, blue-collar occupations and low education related to low saliva reponse to stress (p=0.045 and 0.006, respectively)	Our results indicate that men in low social class had more psychosocial stress and an attenuated cortisol response to a laboratory stress test. This same pattern is found in Vilnius and Linköping men.
Bennett 2004 [14]	Educational level Ethnicity	Design: C-S No.: 56 M/W: 22/34 Age: 36 years (11) Group: White/African American recruited by public announcement Excl: Not being fully employed P rate: not stated	Days: 1 Samples per day: 2 Times for sampling: a1. Awakening a2. 30 min later Setting: Work day; refrain from eating, drinking, smoking, or tooth brushing until after second sample	*RIA. Samples frozen at –20 °C. Salivette™ non-coated.* All participants received an automated phone call at their normal time of awakening and 30 min after	Cortisol data: log transformed Measurement(s): a2. Mean awakening and 30 min after b1. Difference between awakening and 30 min after awakening	ANOVAs using one within-group (time) and two between-group (ethnicity, education) design. Adjustment for sex, age , sampling time, BMI and managerial status	Higher levels of a2 but not b1 among individuals with higher education Higher levels of a2 but not b1 among whites vs blacks. A significant ethnicity by education interaction: Whites with high education had significantly higher b1 than the others	Compared with other findings, the low cortisol levels found among lower educated African Americans were particularly surprising
Wright 2005 [15]	Education level Subjective socioeconomic position.	Design: C-S No.: 81 M/W: 40/41 Age: 65-80	Days: 1 Samples per day: 5 Times for sampling: at waking + 10, 20, 30 and 60 mins after	Immunoassay with fluorescence detection Take the first sample in bed. Instructed not to eat,	Cortisol data: Continuous Measurement(s): Single time point at	Comparing dichotomies on socioeconomic status and sex.	A higher subjective socioeconomic position was associated with a lower deviation in b1. No relations with education	The study was based on the hypothesis that higher SES individuals would display smaller CARs than less priveleged groups, and this

	Financial strain Sex	Group: Recruited from general practices for study on ageing and health Excl: Coronary heart disease, tachycardia, dementia, psychosis, cancer P rate: n.a	awakening Setting: Ambulatory at home	drink, smoke or brush teeth before fourth sample, otherwise follow daily routine.	a1. Deviations at b1 (30-minutes sample minus waking sample) Deviations at b2, repeated measures of variance on all five cortisol measurements	Controlling for body mass index, smoking and time of waking	level and financial strain.	might indicate the operation of more health-protective psychobiological processes in this group.
Steptoe 2005 [16]	Educational level Sex Age	Design:C-S No.: 158 M/W: 67/91 Age: 27-42+ 65-80 years Group: Excl: heart disease, dementia, psychosis, cancer P rate:	Days:1 Samples per day: 2 Times for sampling: Differs among participants. Morning and afternoon Setting: Laboratory environment. Before (at res) and after stress task.	Immunoassay with flourometric detection. Standardised cognitive tasks.	Cortisol data: continuous a3: resting value before stress test b4: selecting highest value after stress test in comparison to level before stress test	Mean comparisons between groups adjusted for BMI, chronic illness, medication and time of day of testing.	a3: No differences between groups b4: Older groups showed larger cortisol changes than younger participants. SES did not influence the increased cortisol responsivity of older participants.	Educational background had no impact on cortisol responses, the hypothesis that higher SES might be protective could not be confirmed. The study involves cognitive tasks that were not very activating in terms of neuroendocrine responses.
Vreeburg 2006 [17]	Education level	Design: No.: 491 M/W: 199/292 Age: 43 years (SD 15) Group: Excl: No lifetime history of anxiety or depressive disorder. No glucocorticoid treatment P rate: not stated	Days:2 Samples per day: 6 day 1, 1 day2 Times for sampling: Awakening, + 30 min, +45 min, +60 min, 22:00 h, 23:00 h and awakening next day (DEXA) Setting: Ambulatory, instructed to be assessed on a typical (working) day. 0.5 mg DEXA ingested after sample 23:00 h	EIA. No eating, drinking or smoking 15 min prior to sampling. No dental work 24 h prior to sampling	Cortisol data: continuous for b1, b3 and c1. Log transformed for a1, a4 and d Measurement(s): a1. at awakening, a2: +30, +45 and +60 min a4. mean of 22.00 and 23.00 h b2 Deviation first four samples b3 Deviation between awakening and 23:00 h divided by numbers of hours in between c1. AUC ground and increase d: DEXA	All values higher than 2 SD over mean excluded ($n = 40$). Linear regressions Confounders: age, sex, number of pain days, somatic disease, time of awakening, daylight, sleep and mentioned outcome	No association between educational level and a1, a2, a4, b2, b3, c1 or d	Sex, smoking, physical activity and months with daylight most consistent determinants. An explanation why educational status was not associated with cortisol was that the gradients were large in other studies
Daniel 2006 [18]	Educational level	Design: C-S No.: 129 m/w: 0/129 Age: 21-66 Group: workers at industrial sites in North Carolina Excl: P rate: 11%	Days:1 Samples per day: 2 Times for sampling: At awakening and midday (before lunch) Setting: Ambulatory, typical working day.	High sensitivity immunoassay. Instructions verbally and in writing: to avoid alcohol, eating and brushing teeth prior to sampling.	Cortisol data: Continuous Measurement(s): b2: midday minus awakening	Regression models adjusting for age, race, BMI and worksite	Education did not have a significant effect, but modified the inverse relationship between b2 and BMI	Hence, chronic stress is not related to education, but the strength of the association between chronic stress and BMI varied according to education

Cohen 2006 [19]	Educational level Income level Composite SES by z scores of education and income	Design: C-S No.: 193 m/w: 95/98 Age: 21-55 Group: responders to advertisements in news papers Excl: pregnant or chronic disease P rate: n.a.	Days: 3 Samples per day: 7 Times for sampling:Awakening and 1, 2, 4, 7, 9, 11 h after wake up Setting: Ambulatory at home	ELISA	Cortisol data: continuous, corrected for wake up time by residual scores Measurement(s): a1-a4. Mean over time points for each of the 3 days b1: 1h minus awakning b3: regression on all time points c1. AUC for all samples with respect to ground c3. Mean over day as the log of AUC for the 3 days	Linear regressions adjusting for age, sex, race and log BMI. Multilevel regressions used to assess daily slope	High SES as measured by educational level is associated with lower levels of cortisol throughout the day (c3), but not with single time points. The SES composite index showed a similar significant relation, whereas income showed a similar but not significant relation	The overall difference in cortisol concentration is attributable to small differences throughout the day, and not to a particular time point
Cohen 2006 [20]	Educational level Income Ethnicity	Design: C-S No.: 781 m/w: 328/453 Age: 15-year follow- up of 18–30 year old people at inclusion Group: white/black Excl: 25 participants who woke up after 11:00 h P rate: 62.6%	Days: 1 Samples per day: 6 Times of sampling: at awakening, 45 min, 2.5 h, 8 and 12 h and at bed time, preset (to normal wake up time) alarm watches. Setting: Weekday, refrain from eating, drinking, smoking, or tooth brushing 15 min before sampling	Time-resolved immunoassay with fluorometric end point detection. Salivette™	Cortisol data: continuous, Measurement(s): b3, diurnal slope, using all samples	Multiple regression: race, sex, SES, covariates (health behaviors, mental health, social network etc.) + mediational analyses in multilevel model	The lower education or income, the flatter the curve. Black men and women showed flat curves not only because of education and income. No interaction between SES and race	The overall difference in cortisol concentration is attributable to small differences throughout the day, and not to a particular time point
Neupert 2006 [21]	Educational level Age	Design: C-S No.: 74 m/w: 58/26 Age: 45 (SD 12) Group: Population based sample Excl: stroke, diabetes, neurological disorders P rate: 29%	Days: 1 Samples per day: 5-7 Times for sampling: Varies over day from morning to afternoon. First sample taken in beginning of session Setting: Laboratory stress test, with several stress trials (cognitive tasks)	Participants were instructed not to eat four hours prior to laboratory stress test session	Cortisol data: Measurement(s):	Multilevel modeling comparing education differences in cortisol trajectories. Time of testing included as co-variates in all comparisons a2, a3 (cortisol levels) and b4 (cortisol response to stress test) were evaluated	The final model accounted for 4% of the between-person variance in cortisol levels, and demonstrated that age and education were not related to cortisol level (a2, a3), but were important for reactivity (b4)	It is important to note that the age differences in reactivity were qualified by the role of SES. Older adults with lower SES did not experience heightened reactivity, but older adults with high SES did.
Dockray 2008 [22]	Educational level Income Age Sex	Design: C-S No.: 83 m/w: 55/28 Age: 61 (SD 9) Group: Referred to hospital with acute chest pain.	Days:1 Samples per day: 3 Times for sampling: Awakening, + 15 mins, + 30 mins Setting: Ambulatory at home	High sensitive chemiluminiscenc. Participants were asked to refrain from smoking, not brush their teeth and not to eay and drink prior to sampling.e	Cortisol data: Continuous Measurement(s): c1: area under curve with respect to increase based on all three samples	c1: Participants with a negative value were regarded as non-responders and were compared with the other group.	The one factor that was related to non-repsonse in c1 was high income. High educational level was associated with marginal significance (p=0.097). No associations with	The notion that nonreponders were more likely to be of higher SES than responders are consistent with other own studies, defining SES as occupational class2004 [27] or subjective social rating [15]

		Excl: Steroid medication P rate: 94%					ethnicity, age or sex.	
Garcia 2008 [23]	Education level: Income:	Design: C-S No.: 86 m/w: 35/51 Age: 20–45 years Group: Volunteers in Brazil Excl: Any acute or chronic medical condition P rate: n.a.	Days:1 Samples per day: 5 Times for sampling: awakening, +30 min, 07:00 h, 12:00 h, 20:00 h Setting: Ambulatory, instructed to be assessed on a typical working day	EIA. No eating, smoking or brushing teeth 30 min prior to each sample	Cortisol data: continuous Measurement(s): a1. at awakening, a2. +30 min and 07:00h a3. at 12:00 h a4:at 20:00 h b1. Deviation between 30 min after awakening and awakening	Mean comparisons between SES groups. Correlations with monthly income. No adjustments presented in analysis	High educational level and high monthly income associated with lower levels at a2, a3 and a4 (all $p<0.05$) but not with a1 or b1.	These two groups present a huge difference in the SES and correspond to the two extremes of the social pyramid in Brazil
Hong 2009 [24]	Education level:	Design: C-S No.: 26 m/w: 0/26 Age: 20–39 years Group: Volunteers in Korea Excl: Any acute or chronical medical condition P rate: not stated	Days:1 Samples per day: 6 Times for sampling: 07:00 h, 08:00 h, 10:30 h, 12:00 h, 17:00 h, 22.30 h Setting: Ambulatory, instructed to be assessed on a typical (working) day	ELISA. Subjects were instructed to go to sleep between 22.30 h and 23.00 h the day before saliva collection. No eating or drinking 1 h prior to each sample	Cortisol data: continuous Measurement(s): a1: at 07:00 h a2: at08:00 h a3: at 10:30 h, 12:00 h and 17:30 h a4:. at 22.30 h	Mean comparisons between groups	University degree associated with lower a4 ($p = 0.030$) nut no relation with a1, a2 or a3	We suggest that the proper sampling time for female workers is 07:00 h, 08:00 h, 17:30 h and 22:30 h
Rosmond 2000 [25]	Occupational level	Design: C-S No.: 284 m/w: 284/0 Age: 51 Group: substudy in population sample, selected on highest vs lowest waist-hip ratio Excl: P rate: 63%	Days: 2 Samples per day: 7+3 Times for sampling: 8.00 to 9.00, 11.45, 30, 40 and 60 minutes after standardized lunch, 5.00 PM and just before goind to bed. Two noninhibited cortisol morning values and one sample for dexamethasone test the next day. Setting: Ambulatory, random working day	RIA	Cortisol data: continuous, Measurement(s): a5: arithmetic mean of all measures b3: variability throughout the day using all samples d: mean of noninhibited values minus Cortisol level after dexamethasone administration	Occupational status divided into three ordinal groups based on professional title. Mean comparisons between groups	a5: was not related to occupational status. b3: Slope throughout the day increases with occupational status d: Dexamethasone inhibition was less efficient among participants with low occupational status	
Steptoe 2003 [26]	Occupational level	Design: C-S No.: 163 m/w: 87/76 Age: 45-58 years Group: Subgroup of Whitehall II study cohort. Day workers. Excl: history of heart disease, treatment for	Days: 1 Samples per day: 10 Times for sampling: Awakening, + 30 min, + 8.00-8.30h + Sample every two hours until 22.00-22.30 Setting: Ambulatory	Immunoassay. Instructions for saliva sampling not described	Cortisol data: continuous, omitted outliers. Omitted if being more than 10 minutes from described time windows. Measurement(s): a2 Morning (7.50-10.50), a3 Midday (11.00-	High and medium grade of employment was combined and compared to lower employment grade. Combined and separate analyses for men and women.	Cortisol did not differ by grade of employment when studying entire population. There were a significant interaction between time and sex, suggesting different patterns for men and women.	The results for cortisol output over the day present a conflicting picture. A lack of significant difference between employment grades may be a direct result of an insufficient sample size. The results for women were opposite to prediction, with elevated cortisol in higher status individuals. The explanation for the different patterns for

		hypertension, and premenopausal women P rate: 55%			14.00) + afternoon (14.00-17.00) a4 Evening (17.00-22.30) a5: average of all samples b1: Difference +30 mins – awakening. b3: Repeated measure of variance including all measures.			men and women regarding cortisol and SES is not clear.
Kunz-Ebrecht 2004 [27]	Occupational status Sex	Design: Exp No.: 128 m/w: 69/59 Age: 52(SD 2) Group: Whitehall II Excl: current psychiatric illness, neuroendocrine disorder, cancer, cardiovascular disease P rate:55%	Days: 2 Samples per day: 2 samples on a workday and also on a weekend Times of sampling: immediately after waking and 30 min later, Setting: Ambulatory	Analyzed with a time-resolved immunoassay with fluorescence detection. Participants were instructed not to brush teeth, eat, drink, smoke before 2nd sample	Cortisol data: continuous, excluding outliers over 75nmol/l. Measurement(s): a1: awakening b1: Sample 30 mins after awakening minus sample at awakening	Mean comparisons and regressions	a1: No relations to occupational status or sex b1: CAR was greater in groups with low occupational status. Women had higher CAR than men on a workday but not at the weekend.	A difference in CAR over occupational grade might indicate a disturbance in cortisol regulation due to chronic stress. A difference in CAR over sex is not an intrinsic characteristic of women since the differences were only on workdays.
Kraft 2009 [28]	Income	Design: C-S No.:94 m/w: 37/57 Age: 20 Group: students at US University Excl: serious or acure illness, medication that influence hormonal function P rate: not stated	Days:1 Samples per day:5 Times for sampling: Between 1.00 and 5.30 PM, different for different participants. Baseline, after stress test + 15 mns, + 30 mins, +45 mins after task. Setting: Laboratory stress test	High sensitive immunoassay Participants were instructed to abstain from alcohol, medication, smoking, exercising eating and intake of caffeine and enrgy drinks two hours prior to the experimental session	Cortisol data: Continuous Measurement(s): b4: reactivity to stress test c4:levels across the stress test	Multilevel models to evaluate group differences on cortisol levels, with income, family conflict, parental divorce, depression, anxiety and time of day as covariates.	Lower family income was significantly associated with lower cortiol levels across the task (c4). Family income did not predict reactivity (b4)	These results are consistent with literature on hypocrtisolism following chronic stress, which suggests a physiological process by which initial exaggerated cortisol stress response may result in lower overall cortisol levels.
Decker 2000 [29]	SES (composite education x income x material wealth) Age	Design: C-S No.: 31 m/w: 31/0 Age: 17-49 Group: Men living in a selected village in Dominican Republic Excl: No known health problems P rate: 75%	Days:20 Samples per day: 6 to 25 Times for sampling: Spanning from sunrise to about 20:00h Setting: Ambulatory, a researcher walked a route through the community, starting at a randomly picked spot visiting all participants to collect a sample. This was performed several times per day.	Radioimmunoassay.	Cortisol data: continuous Measurement(s): a5: all samples from each individual were converted to a time-standardized residual	All 490 samples were used and converted to z-scores in reference to mean levels at any particular time of day. SES was testes in stepwise regressions along with smoking, perceived stress, number of dependents, present father and social conduct	Neither SES or age were associated with mean cortisol level.	More cross-cultural studies are needed to confirm the lack of association between cortisol and SES
Wrosch	SES (composite	Design: C-S	Days: 3	Flourenscence	Cortisol data:	All values 3 SD or	No significant	The relation between cortisol

2007 [30]	education x income x self perceived SES) Age Sex	No.: 215 m/w: 103/112 Age: 63-94 Group: Montreal aging and health study, recruited by nadvertisement in ewspapers Excl: P rate: n.a.	Samples per day: 5 Times for sampling: Awakening, +30 mins, 2 p.m., 4 p.m. and before bedtime Setting: Ambulatory, during days of normal activity	immunoassay Participants were instructed not to brush teeth or eat prior to sampling. A timer was provided to facilitate compliance for second sample	Continuous and log-transformed Measurement(s): All are means over three days a1: awakening b1: 30-mins minus awakening b3: regression not including peak (+30-mins) C3: AUC with respect to ground	more over mean were excluded. Regression analyses including age, sex, SES and reported intense regret	associations between SES and cortisol. Women had steeper slope (b3) and higher AUC (c3) than men, but no difference in a1 and b1. Age were positively associated with steeper slope (b3) but not a1, b1 or c3.	and reported regrets were not confounded by SES.
Ranjit 2005 [31]	Material hardship	Design: C-S No.: 188 m/w: 0/188 Age: 18-54 Group: Survey of a sample of poor women (receiving cash benefits) Excl: not stated. P rate: 63%	Days:2 Samples per day: 1 day 1. 3 day 2 Times for sampling: Day 1: Visit at clinic Day 2: Awakening, +30 mins, bedtime Setting:Ambulatory	Cout-a count DPC. Participants were instructed to record time of sample and delay breakfast until after the second sample was collected	Cortisol data: Continuous Measurement(s): All estimated with spline regressions with fixed inflections points: a1):Awakening b1: Slope between second and first sample b3: .Slope over day	All models adjusted for age, waking time, smoking, BMI and ethnicity.	Low level of hardship is associated with a higher b1. No associations between level of hardship and awakening level (a1) or slope over the day (b3)	The data supports that women that are economically stressed have a lower cortisol level peak. The sample is already defined as poor, limiting range of exposure regarding material hardship.
Steptoe 2005 [32]	Changes in financial strain	Design: Pros. No.: 114 m/w: 63/51 Age: 55 Group: Part of Whitehall II study Excl: No history of CHD, diabetes or hypertension P rate: 75%	Days:1 Samples per day: 4 Times for sampling: At awakening, + 30 minutes, between 10.00-10.30, between 16:00-16:30 and 20:00-20.30 Setting: Ambulatory, taken a normal day.	Immunoassay. Instructed not to eat, drink, smoke or brush teeth before sample, otherwise follow daily routine.	Cortisol data: Continuous Measurement(s): Comparison to cortisol levels in same study pop three years earlier. a1: Awakening a2: + 30 mins b1: +30 mins minus awakening	Linear regressions with age, sex, employment grade and time of waking as covariates.	No associations between changes in financial strain and cortisol, with exception of subanalysis on men where a lower financial strain were associated with a lower CAR (b1)	This study gives support to earlier cross-sectional studies indicating a positive association between magnitude of CAR and chronic stress.
Wilcox 2005 [33]	Ethnicity	Design: Exp No.: 28 m/w: 0/28 Age: Caucasian: 65.7 (10.5) years. African American: 62.0 (10.2) years. Group: postmenopausal caregivers, recruited from advertisement in newspapers	Days: 1 Samples per day: 2 Times of sampling: after 6 min of baseline-rest and 15 min after a more than 6 min stressing interview about negative aspects of being a caregiver between 09:00 and 10:00 h Setting: Morning laboratory session	RIA	Measurement(s): a2: Baseline values b4: before and after test Samples used: both	Cortisol data: Divided into two groups, dichotomy based on 50% or higher increase in cortisol levels after stress test χ^2 test on groups	a2: no differences. b3: More African Americans (58%) than Caucasians (14%) showed cortisol reactivity (>50% increase during test)	Caregiving burden were similar across race. African-American women however, reported higher greater personal meaning associated with caregiving than did Caucasian women. This might result in lower perceptions of control over the caregiving situation, and thus result in greater cortisol secretion during greater cortisol secretion during the interview.

		Excl: Not being caregiver of family member with Alzheimers P rate: n.a.						
McCallum 2006 [34]	Ethnicity	Design: C-S No.: 117 m/w: 0/117 Age: Group: Pilot study on caregivers and non-caregivers Excl: P rate:	Days: 2 Samples per day: 5 Times for sampling: Througout the day Setting: Ambulatory	Not known.	Cortisol data: Not known Measurement(s): b3: Slope over day	Regression analyses on slope with age, ethnicity, caregiving status, and depressive symptoms as predictor	African Americans had flatter slopes than the European Americans sampled	Findings highlight the role of cultural beliefs and of ethnicity in explaining cortisol function
DeSantis 2007 [35]	Ethnicity	Design: C-S No.: 257 m/w 67/190 Age: 17.1 years Group: African American, Hispanic, Asian and Pacific Islander, multi-racial, Caucasian Excl: medication containing corticosteroids and psychosis P rate: 74 %	Days: 3 Samples per day: 6 Times of sampling: awakening, 40 min and before going to sleep plus 3 semi-random times across the day and early evening: Setting: Ambulatory	DELFIA Participants were instructed to refrain from eating, drinking, or tooth brushing 30 min before sampling. If so report in a diary Alarm watches were administered for semi-random samples, preset at approx. 2.5, 8 and 12 h after awakening	Cortisol data: Natural logarithmically transformed. Measurement(s): b3: slopes were estimated over 3 days Samples used: all	Hierarchical multiple regression analyses, including SES variables adjustment for age, sex, depression, nicotine, sleep hours and time	African Americans had lower wake up values and higher bed time values = a flatter curve than the others. Hispanics had a flatter slope than Caucasians, but stepper than Afro-Americans.	It seems likely that the found differences at least in part are environmental. For both African-Americans and Hispanics, it seems likely that commonly used measures of episodic and chronic stress fail to capture certain aspects of being a part of minority groups.
Gallagher-Thompson 2006 [36]	Ethnicity	Design: C-S No.: 83 m/w: 0/83 Age: range 40–47 years Group: 39 Hispanic/44 non-Hispanic whites Excl: n.s P rate: n.s	Days: 3 Samples per day: 3 Times of sampling: 08:00, 17:00, 21:00 h Setting: Ambulatory	RIA and. EIA for some samples. Correlation between the two methods was 0.97, conversion from EIA to RIA was EIA = –0.0160 + 0.518 RIA	Cortisol data: log transformed. Measurement(s): b3: slope based on all samples	Two way ANOVAs and regression analysis, centered values for ethnicity, caregiver status and their interaction	Hispanics, regardless of caregiving status, had flatter daytime slopes than non-Hispanics	The findings may be indicative of sociocultural factors (i.e., financial stress) and cultural values (i.e., not telling about problems in family)
Kirschbaum 1992 [37]	Sex	Design: Exp Data from 4 separate studies; a, b, c, d No.: 153 m/w: 73/80 Age: 22.6 years Group: Excl: Medication except from	Days: 1 Samples per day: a: 9; b: 9; c: 11; d: 6 Times of sampling: a and d in the morning and late afternoon, and b and c in the late afternoon Setting: Before, under and after stress test.	Time-resolved fluorescence immunoassay Participants were instructed to refrain from smoking, physical exercise, meals, alcoholic beverages and soft drinks with low pH –1 h prior to testing	Measurement(s): a3: baseline cortisol concentration b4: reactivity during stress test	ANOVA repeated measures. Student's t test for dependent measures	No differences in baseline levels (a3), but males responded to the task with larger hormone increase than women (b4)	Psychological phenomenon: cognitive and/or emotional stimuli can alter the HPA axis with different patterns among men and women. Men and women use different coping strategies

		contraceptive drugs P rate: n.s.						
Kirschbaum 1999 [38]	Impact of sex, menstrual cycle phase, and oral contraceptives (OC) on the activity of the HPA	Design: Exp No.: 81 m/w: 20/61 Age: 18–32 Group: smokers, subjects suffer from allergies, women with irregular menstrual cycles or using contraceptives excluded Excl: P rate:	Days: 3 Samples per day: day 1 (basal levels), 5; day 2 (the exp. day), 6; day 3 (circadian rhythm): 24 Times of sampling: day 1, wake up, 15, 30, 45, 60 min thereafter; day 2, 0, 15, 30, 45, 60 min; day 3: 09:00 h to 21:00 h at 30-min intervals Setting: basal levels and daytime circadian rhythm at home, before and after TSST test in laboratory	Saliva. Time-resolved immunoassay, fluorometric detection	Measurement(s): b4: Reactivity in stress test	Cortisol data: ANOVA - repeated measures. Hormone samples obtained before TSST test were treated as covariates. Correlation by Pearson product–moment correlations. Bonferroni corrections	Men had higher levels of cortisol than women (follicular phase) at 10, 20 and 30 min after TSST and higher than OC users 1, 10, 20 and 30 min after TSST. No differences between men and women in the luteal phase.	The consistent differences between salivary free and total plasma cortisol levels in response to psychosocial stress in the present study may explain the discrepant results on sex response differences described in the literature
Stroud 2002 [39]	Sex	Design: Exp No.: 50 m/w: 24/26 Age: Mean age 19.1 years (SD = 1.13) Group: Excl: on oral or injected prescription medications, smoking, exercise more than 7 h per week P rate:	Days: 1 Samples per day: 6 Times of sampling: 2 samples approximately 7 min apart at baseline, 2 during the test and 2 after the stress test (15-min interval) Setting: laboratory. Refrain from food and drink 2 h before stress session, and caffeine the evening before stress session as well as exercise and alcohol 24 h before stress session	Salivette. RIA	Cortisol data: log transformed (base 10) Measurement(s): B4. Reactivity, responses to different stressors	Cortisol data: ANOVA repeated measure. ANCOVA with baseline as a covariate. Huynh–Feldt correction applied for all repeated measures	Men showed significantly greater increase in cortisol in response to mathematical and verbal challenges than women and women showed greater increase in response to social rejection challenges	Our study suggests that women are indeed more physiologically reactive to negative interpersonal events than men
Kudielka 2003 [40]	Sex Age	Design: C-S No.: 166 adults/ 13 children M/W: 67/99 Age: 4–75 years Group: Excl: P rate:	Days: 1 Samples per day: 5 Times of sampling: waking, 15, 30, 45, 60 min thereafter Setting: Morning cortisol profile at home. Avoid brushing teeth, refrain from food, alcohol, caffeine, fruit juice, smoking	Salivette. Assayed with a time-resolved immunoassay with fluorometric detection (DELFIA)	Measurement(s): increase AUC Samples used:	Cortisol data: ANOVA repeated measure, Greenhouse–Geisser correction ANCOVA. Cluster analysis. Student's t test. Pearson's correlation	Age was correlated with the cortisol levels immediately after awakening ($r = 0.2$, $p = 0.04$), the area under the cortisol curve ($r = –0.20$, $p = 0.05$), and with time of awakening ($r = –0.21$, $p = 0.04$), respectively.	Correlation analysis revealed 4% explained variance due to age
Hansen 2003 [41]	Sex Age	Design: C-S No.: 120 m/w: 37/83 Age: 30–59 years Group: Excl: hypertension and diabetes P rate:	Days: 1 Samples per day: 4 Times of sampling: wake up, then 20 and 60 min thereafter + 18:00 h Setting: ambulatory at workday	Salivette (polyester). RIA	Cortiisol data: log transformed Measurement(s): A1, awakening A2. +20 and +60 minutes A3: 18.00h B1: +20 mins minus awakening.	A variance component model with backwards selection was used to estimate effects of age, sex, BMI, alcohol, and smoking.	A significant sex difference could only be demonstrated in the concentrations 60 min after awakening. Noeffects of age could be found	The relatively young study population may explain why there are no signifcant association swith age.

Therrien 2007 [42]	Sex	Design: Pros No.: 82 m/w: 51/31 Age: 23–51 years Group: 37 lean and 54 obese participants Excl: depression or psychiatric disorders, cardiovascular problems, smoking or regular alcohol consumption, medication P rate: n.a.	Days: 3 Samples per day: 2 Times of sampling: awakening and 30 min after awakening Setting: Ambulatory No alcohol, training or caffeine during a study day. Three different occasions within a period of 2 months	Salivette. RIA. Refrain from food and drink between the two morning samples. Allowed to drink water between the two samples but not during the 5 min before sampling.	Cortisol data: Continuous Measurement(s): b1: increase in cortisol levels between time of awakening (time 0) and 30 min thereafter (time 30).	Cortisol values were adjusted for estradiol levels Multivariate ANOVA to analyze slope of morning cortisol. Tukey–Kramer post hoc test	Overall, there was no sex difference in cortisol awakening response (b1). There were however significant interactions between sex and obesity	From this observation, one can argue that a great part of the sex difference between the obese and reduced obese groups came from a different pattern of body fat distribution
Steptoe 2007 [43]	Sex	Design: C-S No.: 2873 m/w: 2126/747 Age: 60 SD 6 Group: Whitehall II study cohort. Day workers. Excl: history of heart disease, treatment for hypertension, and premenopausal women P rate: 55%	Days: 1 Samples per day: 6 Times of sampling: Awakening, 30 minutes after waking, 2.5 hours, 8 hours, and 12 hours after waking; and bedtime Setting: Ambulatory	high-sensitivity chemiluminescence assay Participants were asked to record their time of waking and the time of each sample collection and not to drink caffeinated beverages before the waking and +30 mins sample.	Cortisol data: Continuous. Measurement(s): a5: cortisol over day using the last four samples	χ^2-test. Logistic regression using covariates of age, sex, ethnicity, income, waist hip ratio smoking, employment and time of waking	Cortisol over the day (a5) was greater in women ($p<0.005$).	Our study was carried out with a large, well-characterized sample, with careful exclusion of persons with clinical conditions or those using medications
van Stegeren 2008 [44]	Sex	Design: Exp No.: 80 m/w: 21/59 Mean age: 20.7 years (SD = 3.2) Group: healthy students Excl: prescription medication and use or experience with drugs P rate: n.s.	Days: 1 Samples per day: 5 Times of sampling: the test period (task 1 and 2) was from 12:00 to 18:00 h. First sample just before the picture presentation started (after an acclimatization period of 15 min) and the second was taken immediately after. The next 3 samples were taken +10, +20, +60 min after the start of the cold pressor task Setting: mixed design, task 1 (all) consisted of watching neutral and emotional pictures and in task 2 subjects were randomly assigned to either a CPT procedure versus control condition	Commercially available immunoassay (IBL, Hamburg)	Measurement(s): a3: baseline level b4: changes in levels (reactivity) during stress test	Cortisol data: T-test, ANOVA, GLM with time as repeated measure and stress task and sex as between subject variables	No main effect of sex or interaction effect of sex by stress task	For cortisol, only the cold pressor task leads to a significant response. A Cortisol response can be expected only in more challenging stress tasks than the first task in this study.

O'Donell 2008 [45]	Sex	Design: C-S No.: 2873 m/w: 2126/747 Age: 60 SD 6 Group: Subsample of Whitehall II study cohort. Day workers. Excl: history of heart disease, treatment for hypertension, and premenopausal women P rate: 87%	Days: 1 Samples per day: 6 Times of sampling: Awakening, 30 minutes after waking, 2.5 hours, 8 hours, and 12 hours after waking; and bedtime Setting: Ambulatory	high-sensitivity chemiluminescence assay Participants were asked to record their time of waking and the time of each sample collection and not to drink caffeinated beverages before the waking and +30 mins sample.	Cortisol data: Continuous. Measurement(s): b1: +30 mins minus awakening b3: slope over day awakening minus bedtime c2: Area under curve over day using the last four samples	Multiple linear regression using covariates of age, sex, body mass index, smoking, time of awakening, income, depression and self rated health.	Cortisol over the day (c2) was lower in women . No sex associations with b1 or b3.	Cortisol output over the day may be related to the ongoing demands and experiences during the day.
Bouma 2009 [46]	Sex	Design: C-S No.: 644 m/w: 352/292 Age: 15-17 Group: Prospective study on dutch adolescents Excl: P rate: 81%	Days: 1 Samples per day: 2+4 Times of sampling: Awakening, +30 mins, baseline at stress test, starting in the morning or after lunch plus three samples during stress test Setting: Ambulatory in the morning plus Laboratory session	Stress tests were performed capturing orthostatic stress, spatial orienting task, gambling task, startle reflex task and a social stress test. 16 test assistants were trained together to ensure standradization	Cortisol data: continuous Measurement(s): b1: 30 mins minus awakening. b4: Reactivity during stress tests, using the highest of three concentrations during the test minus baseline	χ^2-test and ANOVA and trend test with repeated mesures GLM	No relation between CAR (b1) and sex. Men had higher reactivity tin the laboratory stress tests than women (b4)	Our findings is consistent with previous reports, where men showing a higher reactivity in laboratory stress tests.
Filiaire 2009 [47]	Sex	Design: C-S No.:52 m/w: 26/26 Age: mean about 40 years Group: Professors Excl: cardiovascular disease P rate:n.a.	Days: 2 Samples per day: 4+5 Times of sampling: 30 mins after awakening, 10am, 12 am, 8 pm day 1 and 2 plus one sample 30 mins after lecture day 2. Setting: One resting day One working day	EIA	Cortisol data: Continuous Measurement(s): a2:30 mins after awakening a3: 10am and 12 am a4: 8pm b4: sample after lecture minus sample before lecture	Mann Whitney U	The only significant finding was that women had significantly higher cortiol than men in the morning sample (a2) during a teaching day.	The difference may be due to differences in subjective interpretation of upcoming stressful events.
Seeman 2001 [48]	Sex differences in age-related changes in HPA axis reactivity (mathematical and a verbal challenge)	Design: Exp No.: 40 m/w: 16/24 Age: Young men and women: 22–36 years and older men and women: 67–88 years Group: Excl: diabetes, heart disease, hypertension or endocrine disorder and medication and	Days: 1 Samples per day: 7 Times of sampling: baseline mean of sample at 15:50 h and 16:00 h. Samples collected immediately after challenge, then 15, 30, 60, 90, 120 min post challenge Setting: stress task 30 min starting at 16:00 h	RIA	Cortisol data: Measurement(s): AUC. Average baseline	ANOVA with age-by-sex interaction. Covariate: depression. Derived measures: average baseline, max increase in response to test and AUC with and without baseline levels included	No significant sex differences among the younger subjects at baseline, but younger men exhibited greater cortisol response to challenge compared with younger women. Older women exhibited greater response to challenge compared with older men. Younger men greater overall response	NB! Only two subjects in the group of elderly female responders

		16+ on CESD scale (depression) P rate:						
Ahn 2007 [49]	Examine the changes of basal cortisol and DHEA levels present in saliva and serum with age, and to determine the correlation coefficients of steroid concentration between saliva and serum	Design: C-S Part 1: No.: 359 M/W: 167/192 Age: 21–69 years Part 2: No.: 78 m/w:42/36 Age: 20–40 years Group: Excl: diabetes, hypo- or hypertension, hormone replacement or sleeping pills P rate:	Days: 1 Samples per day: Part 1: 1 Part 2: 4 Times of sampling: part 1, between 10:00 and 11:00 h; part 2, 20–30 min after waking, 11:00 h, 16:00 h, bedtime Setting: no food or drink (coffee or tea) 30 min prior to sampling	1 ml saliva directly by expectorating into a collecting tube. Immunoassay based on a liquid phased-double antibody method. RIA	Measurement(s): changes in levels Samples used: all	Mean, SD, Student's t-test and ANOVA. Linear regression to determine the relationship between hormone levels and age. Pearson's correlation for correlations of hormone levels between saliva and serum	Salivary cortisol levels did not change with age. However serum cortisol levels declined significantly with age	

Abbreviations: ACR, Awakening cortisol response ANCOVA, analysis of covariance; ANOVA, analysis of variance; BMI, body mass index; CAR, cortisol awakening response; C-C, case–control; CESD, Centers for Epidemiological Studies Depression; CPT, cold pressure test; C-S, cross-sectional; DEXA, dexamethasone; DHEA, dehydroepiandrosterone; EIA, enzyme immunoassay; ELISA, enzyme linked ; Excl, exclusion; Exp, Experimental; GLM, general linear model; HPA, hypothalamo-pituitary-adrenal; MDD, major depressive disorder; Pros, Prospective; RIA, radioimmunoassay; SES, socioeconomic status; TSST, Trier Social Stress Test.

SES, Similarities and Differences Between Different Measures

It should be acknowledged that income, education, and occupational status are different entities that are not easily interchangeable [50]. In this context, however, all 3 may be aggregated as proxies for SES, as there is generally a high correlation between SES measures [13], to see if the associations with salivary cortisol follows a general pattern. The number of studies is still low even after such an aggregation.

The general pattern that emerges is hinting at an association between higher SES and a higher deviation throughout the day, a lower AUC with respect to ground throughout the day, and a higher capacity to react with an increase in cortisol following a laboratory stress test.

However, the differences in SES are consistent but small, in particular for diurnal deviation and AUC throughout the day.

In one study by Cohen *et al.* [19], the overall difference in total concentration throughout the day is attributed to small differences (non-significant for each time point) throughout the day that accumulate to a significant difference in total concentration. In a larger study, Cohen *et al.* [20] demonstrate significantly lower levels in the later part of the day but non-significant differences earlier, consequently with significant diurnal variation due to lower evening levels.

It seems that other measures than income, occupational status and education has a lower frequency of significant findings than studies using the more conventional three measures. This may have to do with the validity of other composite or other proxies for SES.

Of the measures tested, income had the highest proportion of significant findings (50%). This might be explained by a supposedly high correlation between actual income and social status, whereas use of ordinal data of occupational grade and education attainment may suffer from higher variability at individual level, thus diluting the associations with social status. Moreover, data on income may be more feasible for linear analyses (regardless on correlation with social status) than occupational grade and educational attainment. Indeed, most studies using income had significant associations with at least one of the cortisol measurements used in the different studies.

There are several factors that add to the complexity when studying SES and cortisol: One factor of plausible explanatory value is the level of physical challenges at work. For example, Steptoe *et al.* [26] report differences between men and women, where women with higher grades of employment tend to have somewhat higher levels of cortisol throughout the day compared with women with lower grades, whereas men with high grades of employment tend to have somewhat lower cortisol levels throughout the day compared with men with lower grades. The higher levels among women with high grade may partly be driven by psychological challenges and high job demands, whereas the higher levels among men with lower grade may be driven more by physiological challenges. In line with psychological challenges at work, Kunz-Ebrecht *et al.* [51] suggest that there is an interaction between SES and job demands, where mean CAR is high in groups with low SES and high job demands, and low in groups with low SES and low job demands.

Another factor may be the commonly used explanation that individuals in low SES more often are exposed for stressful episodes. Several research groups support their findings by a theoretical assumption that stressful episodes triggers a physiological process by which initial exaggerated cortisol stress response may result in lower overall cortisol levels over time. However, in cross-sectional studies, stressful events and its perceptions naturally vary in duration, intensity and temporality amongst different individuals. Thus it might be a simplistic tool to evaluate solely hyper- or solely hypo-secretion of cortisol, or to study cortisol levels in a linear fashion in regards to SES. In a study using an unconventional statistical approach, Li *et al.* suggests that subjects with low SES are overrepresented in the extremes in both ends, eliciting both high and low cortisol values [52]. If this pattern is correct, it would generally dilute the associations between cortisol levels and SES in statistical analyses assuming linear or ordinal structure in data.

Further, there is an emerging discussion on the biological mechanisms behind the detrimental effects of low SES. In addition to the ongoing discussions on possible epigenetic effects [53], a growing literature suggests that at least part of these associations may be explained by exposure *in utero* or in the first years in life. As examples, Lupien *et al.* has suggested that young children are affected by the parental socioeconomic situation [54, 55]. In concordance, Gustafsson *et al.* have suggested that SES in early life seem to be more important than current SES for cortisol levels [56].

Ethnicity

When studying ethnicity and salivary cortisol, a complex web of potential covariates unfolds. In the studies that we have reviewed, we cannot know if the results found are a product of genetic differences, behavioral differences, or if ethnicity is a proxy for SES. However, the studies on ethnicity yielded the highest proportion in this chapter (58%), and it may be of relevance that the studies on ethnicity in this overview follow a clear trend: Caucasian study populations have a higher diurnal variation than African American study populations. It is also suggested that Caucasians have higher diurnal variation than Hispanics, who, in turn, have higher diurnal variation than African Americans. This ethnicity ladder is in congruence with a translation to a socioeconomic ladder, where Caucasians in many societal contexts have higher status than African Americans and Hispanics. If so, these results give further support to the findings on SES, emphasizing that a higher status seem to be associated with a higher diurnal variation. Importantly, a recent large population study (n=935) using cortisol samples from three days by Hajat *et al.* [57] confirmed the presence of a flatter diurnal deviation in African-Americans and Hispanics compared to Caucasians, and also confirmed a more flat deviation in groups with low SES compared to groups with high SES. The flatter deviations, mainly an effect of lower cortisol levels in the morning, are coherent with other large scale populations studying ethnicity and or/SES in diverse populations using a well standardized cortisol sampling throughout the day [12, 20, 35, 58]. Thus, it seems likely that the found differences over ethnicity at least in part are explained by environmental factors.

Sex

Based on the studies that we have reviewed, it can be stated that there are no large differences in cortisol levels between men and women. It may however be noted that if anything at all, the five significant findings on single time points all hint at a somewhat higher level amongst women in comparison to men. The absence of significant findings for single points in this investigation does not provide support to any great extent for biological differences between men and women. It has however been reported that menstrual cycle might have a substantial impact on cortisol levels for premenopausal women [38]. Thus, there might be a larger variation comparing women over the menstrual cycle than it is comparing women and men.

The most pronounced finding on sex differences hints at a higher reactivity in laboratory stress test settings for men [37-39, 46]. When explaining differences in laboratory stress tests between men and women, focus should, as pointed out and discussed by Stroud *et al.* [39] and Kirschbaum *et al.* [38], be focused on the anticipation and interpretation of the stressors used. If men and women are likely to differ in their interpretation of the situation, then differences in stress tests are psychological rather than biological phenomena. This is further supported by Kirschbaum *et al.* [38] who reported that pharmacological stimulation yields similar patterns with peaks in the same range and at the same level for both men and women.

Age

There is some evidence that cortisol levels increase somewhat with increasing age. Even though the number of studies is low, the differences that follow an age gradient are higher in the later part of the day than in the morning. This is further supported by Deuschle *et al.* [59], who suggested that plasma cortisol does not differ in the daytime, but that cortisol levels in the evening increase with age. Thus, they suggest that higher age is associated with a flatter diurnal slope [59]. In concordance, Van Cauter *et al.* [60] have also suggested that the diurnal slope becomes lower as age increases. Still, it should be remembered that the results hint at a relatively small increase in cortisol levels with increasing age.

CONCLUSIONS

The number of studies with significant findings is relatively low. However, there is a fair degree of consistency in that high SES, regardless of how it is measured, is associated with a higher deviation throughout the day, and a higher capacity to react with an increase in cortisol following a laboratory stress test. Regarding ethnicity, results consistently hint at a higher deviation throughout the day amongst Caucasians in comparison to Hispanics and Afro-Americans.

There are no apparent consistent large differences between men and women. There is a tendency that women in general have somewhat higher levels than men. There is also a tendency that men have a higher cortisol response than women in laboratory stress test settings, but these responses are dependent on the stressors used.

The studies on age are few but provide some support for a small increase in salivary cortisol with increasing age.

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CHAPTER 3

Psychosocial Work Stressors and Salivary Cortisol

Björn Karlson[1,*], Petra Lindfors[2], Roberto Riva[3], Christin Mellner[4], Töres Theorell[5] and Ulf Lundberg[6]

[1]Professor at the Department of Psychology, Lund University, Box 117, 221 00 Lund, Sweden; [2]Associate professor in psychology at the Department of Psychology, Stockholm University, Sweden; [3]Student at the Department of Psychology, Stockholm University, Sweden; [4]Researcher at the Department of Psychology, Stockholm University, Sweden; [5]Professor at the Stress Research Institute, Stockholm University, Stockholm, Sweden and [6]Professor in Psychology at the Department of Psychology and Centre for Health Equity Studies (CHESS), Stockholm University, Stockholm University, Sweden

Abstract: This chapter systematically reviews how different measures of salivary cortisol are related to different measures of psychosocial work stress. Divergent findings were scrutinized with respect to study quality and the methods used. Measures of work stress included concepts reflecting those included in the demand-control-support model or the effort-reward-imbalance model. General bibliographic databases (PsychINFO and PubMed) were searched up to September 30, 2009. Two reviewers extracted data on study characteristics and study quality. In total 27 articles fulfilled the inclusion criteria. Cortisol measures were grouped into single time points at different times during the day, deviations at different time periods during the day, reactivity and recovery after a standardized laboratory test, area under the curve from deviations and reactivity measures. A large proportion of the analyses of the associations between cortisol and psychosocial work stressors showed nonsignificant findings. However, of the significant findings, most results showed that a high work stress was associated with high cortisol levels. Significant relationships were evenly distributed across different measures of psychosocial work stress. As regards salivary sampling or statistical analysis, no strategy seemed superior but some strategies have only been used in the past few years. Typically, older studies were of lower quality. Low quality studies tended to have a higher proportion of significant findings which is a reason for concern. The relatively few significant findings may be because many psychosocial work stressors were of mild or moderate intensity and the study groups were rather small and fairly homogeneous, thus variability was too small to reveal any effects. The results indicate a normal, healthy response to work stress in most workers, according to CATS and the Allostatic Load Models.

Keywords: Salivary cortisol, working adults, psychosocial work stress, work load, job strain, job demands, job control, effort, reward, social support.

INTRODUCTION

It has been a popular idea among stress researchers and those organizing work to use salivary cortisol as an index reflecting work stress. This is partly associated with the hope that salivary cortisol would be an easily administered objective measure of work stress. More specifically, its exponents hoped that salivary cortisol would prove useful for employers who may want or require objective evidence in order to accept subjective claims of a work environment being harmfully stressful. Similarly, salivary cortisol could be useful for employees wanting to use high salivary cortisol concentrations as an argument for claiming that they are exposed to a highly stressful environment or when arguing for the beneficial effects of interventions aimed at reducing work stress. However, the interpretation of the results of salivary cortisol concentration analyses in relation to work conditions is more complicated than was initially expected. Working populations differ from patient populations or from populations of retired individuals in the sense that those who are working are healthier and younger than patients and retired persons. Thus, cortisol regulation in working individuals may show a healthier pattern than in other groups. The study of cortisol regulation at work should be more closely linked to the assessment of stressors than cortisol regulation in patient groups.

***Address correspondence to Björn Karlson:** Professor at the Department of Psychology, Lund University, Box 117, 221 00 Lund, Sweden; Tel: +46 46 222 9117; Fax: +46 46 222 4209; E-mail: bjorn.karlson@med.lu.se

Margareta Kristenson, Peter Garvin and Ulf Lundberg (Eds)

However, this is only partly true; working individuals can also be exhausted and depressed although they still manage to carry out their work tasks.

In this context, increased cortisol excretion when mobilizing resources to endure a situation and successfully cope with it plays an important role in the organism's response to increased demands. The expected effects of a high cortisol level include a decrease in the negative psychological reactions to the stressor, such as feeling uneasy in an adverse psychosocial work environment. When this mechanism functions adequately, a working individual feels less uneasy in a poor psychosocial work environment than would be the case if cortisol levels stayed low. At the group level, this effect decreases the association between feelings of uneasiness and high cortisol excretion as reflected in blood and salivary cortisol concentrations.

With regard to psychosocial work stressors, most of these daily stressors are of mild or moderate intensity. Irritations caused by lazy colleagues, a constant lack of time to do satisfactory work or poor leadership are milder stressors than natural disasters, emergency situations, giving birth to children, and exposure to unexpected violence. Even in occupations where violence or natural disasters may be expected to be part of the work (police officers, prison staff, and fire fighters), strongly stressful situations are relatively rare and may not be captured in a few measurements of salivary cortisol during a single work day. In addition, successful coping with work stressors during ordinary work days is according to the CATS model inducing positive expectancies and a successive attenuation of the stress response and rapid return to baseline.

In the systematic analysis of research findings on salivary cortisol in relation to psychosocial work conditions, the bodily responses in terms of cortisol excretion to milder stressors must be considered. Most of the published research within the field has investigated psychosocial work conditions through the widely accepted theoretic models of work stressors such as the Demand-Control-Support (DCS) model [1, 2], or the model of Effort-Reward-Imbalance (ERI) [3]. This means that the stressors investigated have indeed been mild in character. However, long-lasting levels of mild or moderate stress may also be harmful.

AIM

This chapter examines how different measures of salivary cortisol are related to different measures of psychosocial work stress by systematically reviewing the literature. In addition, divergent findings have been scrutinized with respect to study quality and methods used.

METHODS

Search Strategies

Procedure

Literature searches were performed in two general bibliographic databases: PsychInfo (until September 2009) and PubMed (until September 2009). Articles were identified by combining different sets of search terms in separate searches ("cortisol" AND "job demands" OR "work demands" OR "job strain" OR "work strain" OR "job control" OR "work control" OR "social support" OR "job stress" OR "work stress"), ("cortisol" AND "work" OR "job" AND "ERI" OR "effort" OR "reward" OR "effort reward"). The searches were limited to published articles written in English and including adult study participants (age 19+ years). Reference lists from relevant review articles were also scrutinized. The search was completed with articles known by the authors of this chapter but not identified through the search terms. This resulted in 957 hits, including duplicates (*e.g.*, articles found in both databases and across different searches). Further evaluation of articles was performed according to specific inclusion criteria.

Inclusion Criteria

For an article to be included, cortisol had to be analyzed from salivary samples. This means that studies including parallel examination of, for instance, salivary and urinary cortisol were included. The exposure variable had to include psychosocial work stress, mainly including concepts relating to the DCS model or the ERI model. However, use of the original measures of these models was not required because this would

have restricted the number of articles unnecessarily. The researched measures had to be conceptually similar to the psychosocial work stressors included in the DCS model or the ERI model. The exposure variable had to be either self-reported by study participants or objectively defined. In the latter case it had to be defined as some kind of continuous measure of workload that could vary over time. This means that psychosocial work stress defined in terms of occupation, job title, or work day in contrast to a day off was considered insufficient. The study participants had to be in gainful employment (*e.g.*, excluding students and charity workers), and the exposure studied had to involve work life (*e.g.*, excluding laboratory studies).

The first selection of articles based on publication titles and abstracts resulted in 103 articles. All of these articles were retrieved and read by at least two of the authors of this chapter. Assessment of study inclusion criteria was made independently by each author. Disputes were settled by consensus. This evaluation resulted in a final selection of 27 articles to be included in the systematic review (Fig. **1**).

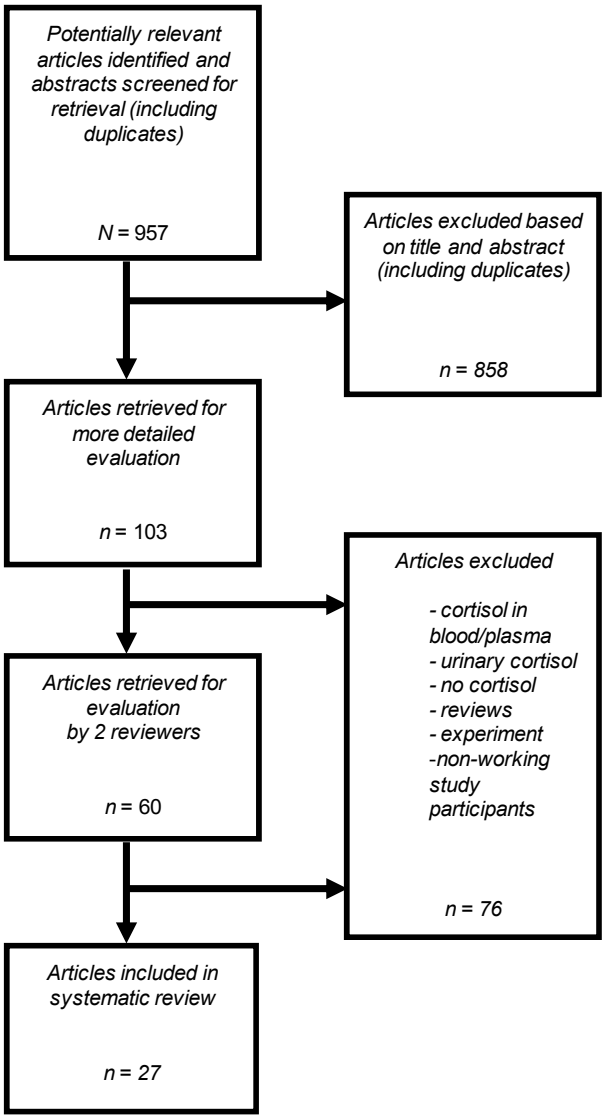

Figure 1: Brief description of the retrieval of publications included in the review.

Evaluation Process

In addition to summarizing the articles in line with the matrix shown in Table **1**, the authors of this chapter also rated the quality of the articles.

Table 1: Descriptives of the included articles on salivary cortisol parameters and exposure sorted by year of publication

References	Exposure	Study design/group characteristics	Sampling	Laboratory method	Statistical approach for cortisol measure	Statistical analysis, cortisol measure in relation to exposure	Results	Discussion
Fox 1993 [5]	*Subjective* DC model *Objective* Objective behavioral + subjective measures of work load. Perceived control	Design: C-S No.: 136 M/W: 0/136 Age (years): Type of job: Nurses Analyzed subgroups: High/low C (vs home + work cortisol); high/low D (work cortisol); high/low patient contact time (home cortisol)	Days: 2 Samples per day: 3 Times for sampling: Day 1: +0, +2/3 h after working, +2/3 h after return from work Day 2: before leaving for work, +2/3 h after working, 2/3 after return from work Condition: 2 consecutive days at work and at home	RIA	Measurement(s): a3. Average of work and home cortisol respectively Cortisol data: Continuous data	Hierarchical regression analysis	Interaction effect. Low control combined with subjective high work load associated with high cortisol. Objectively assessed job demands associated with high cortisol	Support for the DC model. Personal control important to reduce health risk
Zeier 1996 [6]	*Objective* Objective work load *Subjective* Perceived work load	Design: R-M No.: 126 M/W: 126/0 Age (years): Type of job: Air traffic controllers Analyzed subgroups: Pre-/post working sessions High vs low job demands working sessions	Days: 2 Samples per day: 2 Times for sampling: Before and after a working session (08:00-12:00 h) Condition: 2 100-min working sessions: Feb: low traffic, low demands May: high traffic, high demands	RIA	Measurement(s): b2. Deviation between samples before and after work sessions Cortisol data: Continuous data	Repeated measures ANOVA and ANCOVA Pearson's correlations	Higher b2 after higher objective work load session. Positive correlation between b2 and objective as well as perceived high work load	Increase in job demands is associated with increased cortisol excretion
Fujigaki 1997 [7]	*Subjective* Effects of high stress (self-reported stressful events) on psychological problems	Design: L No. 10 M/W: 10/0 Age (years): Type of job: Information System engineers Analyzed subgroups: BUSY (n=3) vs non-BUSY (n=7)	Days: 19 (n=6), 17 (n=2) Samples per day: 1 Times for sampling: 12:00 h Condition: Every 2 weeks for 5 months and every week the following 2 months	RIA	Measurement(s): a3. At 12:00 h b2. Increases from the preceding cortisol value. > 1 SD higher than the individual's average value Cortisol data: Number of cortisol increase points	ANOVA between cortisol increase points and type of job events Time series analysis	Cortisol increased after continuation of busy state, finish of big project, and getting used to a job. Supported by certain events preceding cortisol increase	Cortisol captures chronic work stress reaction
Steptoe 1998 [8]	*Subjective* DC model Social support	Design: L No.: 71 (61 for analyses) M/W: 27/44 Age (years): Type of job: Employees of a large department store Analyzed subgroups: High vs low job strain	Days: 4 Samples per day: 1 Times for sampling: Not described Condition: Work days at 4 sessions: Nov, Dec, Jan, Mar	Time resolved immunoassay fluorescence detection	Measurement(s): a3. Single measures Cortisol data: Continuous data	Repeated measures ANOVA Correlations	No relation to job support. High strain related to low a3. Also negative correlation between job strain and a3	Unexpected direction of results. May be due to the participants being well adapted to their job, tolerating wide variations in work load
Fischer 2000 [9]	*Objective* Crowded environment Time pressure	Design: R-M, L No. T1=64; T2=60 M/W: T1=60/4; T2=58/2 Age (years): not included	Days: 3 Samples per day: 5 scheduled + x unscheduled Times for sampling:	Fluorescence detection	Measurement(s): b2. Mean of several measurements throughout the day	Wilcoxon ANOVA Multiple regression analysis with adjustment for	Mean cortisol was reduced at the late shift 1 year after organization change aiming to reduce objective work load	

	Conflicting tasks	Type of job: Nurses in a pediatric intensive care unit divided into 2 teams: - Team A (high staff turnover) - Team B (low staff turnover) Analyzed subgroups: Period 1 (1997) stressful late shift vs Period 2 (1998) non-stressful late shift due to an organizational change	Start of work and every 2 h (scheduled samples) 15-20 min after a potentially stressful event (unscheduled samples) Condition: 3 shifts: early shift (control), late shift (experimental condition), night shift		Cortisol data: Log transformed	confounders		
Fischer 2000 [10]	*Subjective* Perceived stress and workload *Objective* Workload as ratio of available nurses to required nurses	Design: C-S, L No. 138 M/W: 20/118 Age (years): Type of job: 111 nurses and 27 physicians in a neonatal and pediatric critical care unitAnalyzed subgroups: 5 groups based on their work experience: 0-2 years (*n*=65); 3 years (*n*=15); 3-5 years (*n*=22); 5-8 years (*n*=13); >8 years (*n*=24) High vs low stressed workers Nurses vs physicians	Days: 3 × 2 times with 1-year time lag Samples per day: 5 scheduled for nurses and 6 for physicians + unscheduled Times for sampling: Start of work and every 2 h scheduled + 15-20 min after potentially stressful event Condition: 3 shifts: early shift (control), late shift (experimental condition), night shift 12-day periods in Feb 1997 and Jan 1998	Fluorescence detection	Measurement(s): a5. Mean of several measurements throughout the day b2. Deviation between two samples; baseline vs response after event, *i.e.*, endocrine response Cortisol data: Log transformed	Kruskall-Wallis One-way ANOVA Logistic and linear regression analysis	Higher mean cortisol and more frequent ERs in shifts with higher objective work load Lower mean cortisol and fewer ERs in those with >8 years experience of the job	
Hanson 2000 [11]	*Subjective* ERI model Need for C Momentary Demand-Satisfaction Ratio (MD-SR), momentary negative mood	Design: C-S, R-M No.: 77 M/W: 43/344 Age (years): Type of job: 36 health professionals (nurses); 41 office clerks	Days: 2 Samples per day: 6 for health professionals, 10 for clerks Times for sampling: For health professionals from 08:00 to 22:30 h, every 140 min; for clerks: from 08:00 to 22:30 h, every 90 min Condition: Work day vs day off	Two different analyses combined: 1. Time-resolved immunoassay 2. Radioimmunoassay using polyclonal anticortisol antibody	Measurement(s): a3. Single measurements throughout the day Cortisol data: 5th root transformed data	Multi-level analysis; two-level linear model (random coefficient model)	No effect on cortisol of ERI, need for control, MD-SR, but higher cortisol at moments of more negative mood	Momentary negative affect, rather than ongoing (minor) work-related stress, is related to cortisol. 20-min time lag between rating and cortisol might be needed
Steptoe 2000 [12]	*Subjective* DC model Demand Control Skill utilization Job strain	Design: C-S No.: 105 M/W: 41/64 Age (years): Type of job: School teachers Analyzed subgroups: M vs W High (28 M, 52W) vs low (32 M, 50 W) job	Days: 1 Samples per day: 8 Times for sampling: 08:00,10:00,12:00,14:00, 16:00,18:00,20.00 h (22:00 h) (30-min time windows) Condition: 1 work day	Time-resolved immunoassay fluorescence detection	Measurement(s): a2, a3, a4. Single measurements (8/day) b3. Deviation from morning to evening Cortisol data: Continuous data Samples used: The last sample not analyzed, missing for	Repeated measures ANOVA	High job strain had higher a2 at 08:00 h High job strain and low C related to greater decline in b3	Job strain reflects anticipatory psychobiological responses. No expected flatter profile in high job strain

		strain scores			some subjects due to sleep			
Evans 2001 [13]	*Subjective* DC model Job support Perceived stress	Design: C-S No.: 93 M/W:40/53 Age (years): Type of job: Nurses (*n*=61), accountants (*n*=32) Analyzed subgroups: High (*n*=54) vs low (*n*=39) social support	Days: 5 Samples per day: 2 Times for sampling: 12:30-14:00 h, 19:00-21:30 h No precise time of the day for samplings Condition: 3 working days, 2 days off	Fluorescence detection	Measurement(s): b2. Means of day time, and evening samples respectively for work days and weekend days separately Cortisol data: Continuous	Repeated measures ANOVA	High cortisol day and evening on leisure days in the group with high social support	
Yang 2001 [14]	*Subjective* Perceived work-related stress (PSS)	Design: C-S No.: 73 M/W: 0/73 Age (years): Type of job: Nurses: 23 emergency department (ED), 50 general ward (GW) Analyzed subgroups: ED vs GW	Days: 1 Samples per day: 2 Times for sampling: Start of morning shift (06:50-07:20 h) and end of morning shift (13:00-14:00 h) Condition: Work day	Salimetrics HS-Cort kit (Salimetrics LLC)	Measurement(s): a2. Single measurement in the morning a3. Single measurement in the evening b2. Deviation between morning and evening samples Cortisol data: Log transformed data	Two independent samples t-test Paired t-test GLM Partial correlations	ED had lower a2 and lower absolute and relative b2 than GW. Also high scores on 3 PSS subscales negative related to a2 and b2. No effect on a3	The flat profile related to high strain indicates a long-term over-activation of the HPA axis
Fujiwara 2004 [15]	*Subjective* DC model (JCQ) High vs low strain	Design: C-S No.: 16 M/W: 0/16 Age (years): Type of job: Health care providers Analyzed subgroups: High (*n*=8) vs low (*n*=8) job strain group	Days: 3 Samples per day:3 Times for sampling: Day off: 09:00, 13:00, 19:00 h Day shift: 07:00 or 08:00 h or 09:00 h (start shift), 13:00, 17:30 h (end shift) Night shift: 17:00 h (start shift), 05:00, 09:00 or 09:30 h (end shift) Condition: 1 day off, 1 day shift, 1 night shift	RIA	Measurement(s): a2, a3, a4. Single measurements a5. Mean of several measurements throughout the day Cortisol data: Continuous	Repeated measures analysis	Cortisol concentration on day shift marginally higher in high-strain compared to low-strain group	Job strain may cause a disturbance in the circadian rhythm of cortisol
Kunz-Ebrecht 2004 [16]	*Subjective* DC model Demand Control	Design: C-S No.: 181 M/W: 97/84 Age (years): Type of job: Administrative and professionals; senior and higher executive officer; clerical, office supporter Analyzed subgroups: High (70 M, 56 W) vs low 27 M, 28 W) SES; high vs low D and C	Days: 1 Samples per day: 10 Times for sampling: +0,+30 min, 08:00,10:00, 12:00, 14:00, 16:00, 18:00, 20:00, 22:00 h (30-min time windows) Condition: 1 work day	Biotin-strepta fluorescence-immunoassay	Measurement(s): a1. Morning measurement a3. Mean of the remaining samples of the day b1. Deviation from wakeup to +30 min Cortisol data: Continuous data	ANCOVA	High b1 in low SES men + women with high D High a3 in women with high D High a3 in men with low C	Women of low SES more responsive to high demands than men; in men, low control has more influence on cortisol Exposure and response to work stress determines SES differences in psychobiological responses
Schlotz 2004 [17]	*Subjective* Perceived chronic stress (TICS work	Design: C-S, R-M n: 219	Days: 6 Samples per day: 4	Time-resolved immunoassay	Measurement(s): b1. Mean increase	Repeated measures ANOVA t-test	High work overload related to high b1	

	overload and worries)	M/W: 102/219 Age (years): Type of job: No precise info on the type of job; 140 employed, 6 unemployed, 72 retired, 1 missing Analyzed subgroups: M vs W Work days vs days off Low (*n*=64) vs average (*n*=83) vs high (*n*=72) work load scores Low (*n*=73) vs average (*n*=78) vs high (*n*=68) worries scores	Times for sampling: +0,+30,+45,+60 min Condition: 6 consecutive days (4 work days + 2 days off)	fluorescence detection	(CAR) based on 4 work days Cortisol data: Continuous data		Similar for worry	
Steptoe 2004 [18]	*Subjective* ERI and OC	Design: C-S No.: 197 M/W: 105/92 Age (years): Type of job: Administrative and professionals; senior and higher executive officer; clerical, office support Analyzed subgroups: M vs W High (41 M, 53 W) vs low (64 M, 39 W) OC	Days: 1 Samples per day: 10 Times for sampling: +0,+30 min,08:00, 10:00, 12:00, 14:00, 16:00, 18:00, 20:00, 22:00 h (30-min time windows) Condition: 1 work day	Biotin-streptavidin fluorescence immunoassay	Measurement(s): b1. Deviation from awakening to +30 min sample (CAR) b2. Slope over the day Cortisol data: Continuous data Percentage showing a CAR (≥2.49 nmol/l) Samples used: CAR and rest of the day separately	Repeated measures ANOVA Multiple logistic regression analysis	No associations with ERI. Men with high OC had a higher b1. Higher b2 average cortisol over the day in men with high OC. No OC effects in women	CAR is sensitive to chronic psychosocial stress
Dahlgren 2005 [19]	*Objective* Predicted high stress week *Subjective* Perceived high stress	Design: R-M No.: 34 M/W: 11/23 Age (years): Type of job: Office workers: 17 administration, 16 union representatives, 1 supervisor	Days: 2 Samples per day: 5 Times for sampling: +15 min, 10:10, 13:00, 16:00 h, bedtime Condition: High stress work week vs low stress week Workday and day off	RIA	Measurement(s): a2, a4, a5. Single measurements b3. Deviation morning to evening Cortisol data: Continuous data Samples used: All samples	Repeated measures ANOVA Pair-wise t-test for post hoc analysis	Higher a2 in high stress condition, and tendency for higher a4, resulting in a flatter profile over the day	
Aasa 2006 [20]	*Subjective* D (JCQ by Karasek and Theorell, 1990) Worry about work conditions (Brulin *et al.* 1998)	Design: C-S No.: 26 M/W: 24/2 Age (years): Type of job: Ambulance personnel Analyzed subgroups: Few health complaints (*n*=14) vs Many health complaints (*n*=12)	Days: 3 Samples per day: 5 Times for sampling: 19:00, 21:00,07:00, 11:00 and 15:00 h Condition: A 24-h work shift (17:00-17:00 h) and the next 2 work free days	Spectria cortisol RIA	Measurement(s): a1, a2, a3, a4. Single measurements Cortisol data: Continuous Samples used: All	Spearman correlations of cortisol vs exposure measures Repeated measures ANCOVA for comparing shift to work free days same hours. Also with group (few/many complaints) × time of day with age as covariate. The few women (n=2) did not make any difference and were therefore included	Morning cortisol was positively correlated to worry about work but not to job demands The RM ANCOVA and paired t-test showed significantly higher saliva cortisol at 07:00 h in "many complaints" group during shift than during free days. No difference between shift day and other days in "few	The only positive finding was in the subgroup with many health complaints in which saliva cortisol was increased at 07:00 h during the shift compared with same hour on a work free day. Small samples and non-dramatic study days

						Paired t-test for post hoc analyses	complaints" group	
Alderling 2006 [21]	*Subjective* DC model (JCQ)	Design: C-S No.: 529 M/W: 181/348 Age (years): Type of job Actively working population, but type of work not included Analyzed subgroups: M vs W separately 4 DC-categories: low strain: -D +C (64 W, 50 M) vs passive; -D -C (69 W, 28 M) vs active; +D +C (110 W, 66 M) vs high strain: +D -C (105 W, 37 M)	Days: 3 Samples per day: 4 Times for sampling: +0, +30 min, lunch time, before sleep Condition: Work days	RIA	Measurement(s): a1. Single measurement on awakening a2. Single measurement +30 min a3. Single measurement at lunch time a4. Single measurement at bed time b1. Deviation from awakening to+30 min b2. +30 min to lunch time b3. Lunch time to bed time Cortisol data: Log transformed	Mixed models	Low strain women had lower a1 +30 min than women in other quadrants	
Eller 2006 [22]	*Subjective* DC model Job support ERI and OC	Design: C-S No.: 83 M/W: 55/28 Age (years): Type of job: W: 66% non-manual jobs (secretary, laboratory workers, nurses, or midwives) M: 50% academics (physicians) or high non-manual job (policemen, engineers, teachers, computer workers); 42% skilled workers (carpenter, electrician) Analyzed subgroups: M and W separately High/low DC, social support, ERI and OC scores and high/low social status of jobs	Days: 1 Samples per day: 6 Times for sampling: +0, +20, +30, +60 min, +8 h, 18:00 h Condition: Working day	RIA	Measurement(s): a1. Single measurement on awakening a5. Measurements throughout the day b1. Deviation from awakening to +30 min Cortisol data: Continuous data	GLM univariate ANOVA GLM repeated measures ANOVA	Men with high ERI or OC had higher cortisol over the day a5 Men with high support had a low b1 Women with a high ERI and men with a high OC had a high b1	
Harris 2007 [23]	*Subjective* ERI model (effort, reward, ERI) DC model (demand, control, skill discretion, decision authority, social support, D/C)	Design: C-S No.: 44 M/W: 0/44 Age (years): Type of job: Nurses Analyzed subgroups: High/low score in decision authority	Days: 2 Samples per day: 5 Times for sampling: +0, +30, +45 min, 15:00 h, 22:00 h Condition: 2 consecutive work days	RIA	Measurement(s): a1. Single measurement on awakening a4. Single measurement in the evening (22:00 h) b3. Deviation +45 min from wakeup to evening (22:00 h) c1. AUC for wakeup	Pearson product-moment correlations Multiple linear regression analyses	Low decision authority score associated with high a4	The results may indicate insufficient recovery and restitution due to lack of control. Limited by small sample

					to +30 min (trapezoidal model) Cortisol data: Log transformed (log 10) data			
Bellingrath 2008 [24]	*Subjective* ERI and OC	Design: C-S, R-M? No.: 135 M/W: 40/95 Age (years): Type of job: School teachers Analyzed subgroups: Work days vs day off (*n*=101) High vs low R after dexamethasone	Days: 2 Samples per day: 7 Times for sampling: +0, +30, +45, +60 min, 11:00, 15:00, 20:00 h Condition: 2 consecutive work days, 1 day off, 1 day after dexamethasone	DELFIA	Measurement(s): a5. Measurements throughout the day Cortisol data: Log transformed Samples used: All single measurements	Repeated measures ANOVA	No effects of exposure on basal cortisol on any day type, but higher dexamethasone suppression in those with low reward	
Maina 2008 [25]	*Subjective* DC model Demand Control Job strain	Design: C-S No.: 68 M/W: 12/56 Age (years): Type of job: Call centre operators Analyzed subgroups: M and W separately	Days: 3 Samples per day: 7 Times for sampling: +0, +30, +60 min, +3 h, +6 h, +9 h,+12 h Condition: 2 work days, 1 day off	Solid phase RIA	Measurement(s): a1, a2, a3, a4. Single measurements b1. Deviation from wakeup to mean of +30 and +60 min samples c1. AUC of first 3 morning samples c2. AUC work from samples 4-7 c3. AUC diurnal from samples 1-7 Cortisol data: Log transformed	Non-parametric analyses: Friedman Wilcoxon Spearman rank correlation GLM	High D related to high a2 and a3 and to c1 in women. Low C related to high a2 and c1 in men. No relations between work stress measures and cortisol post morning	CAR is the most sensitive measure of physiologic response to psychosocial variables. Need for development of measures of mental stress involving psychological components Limitations: few men, single occupation
Rydstedt 2008 [26]	*Subjective* DCS model Demand Control Support Iso-strain	Design: C-S, R-M No.: 77 M/W: 53/24 Age (years): Type of job: Participants working in health care, government, technology, or consultant Analyzed subgroups: M and W separately	Days: 7 Samples per day: 2 Times for sampling: +0, 22:00 h Condition: 7 consecutive days	Not clearly indicated ("sent samples to Dresden")	Measurement(s): a1, a4. Single measurements Cortisol data: Continuous data Samples used:	Correlations MANCOVA	High D related to high morning a1 in women	
Chandola 2008 [27]	*Subjective* DCS model (JCQ by Karasek & Theorell, 1990)	Design: C-S, L No.: 3490 M/W: not included Age (years): Type of job: Civil servants Analyzed subgroups: 0, 1 or 2 reports of work stress	Days: 1 Samples per day: 1 Times for sampling: +0, +30 min Condition: No info	Not clearly indicated ("Kirschbaum's lab")	Measurement(s): bl. Deviation from awakening to +30 min Cortisol data: Difference between the 2 samples on raw data Samples used: All	Logistic and linear regression	Work stress (Iso-strain) associated with higher cortisol awakening response	

Wright 2008 [28]	*Subjective* DCS model (JCQ by Karasek, 1985). Job strain = (ratio>1) D + C D + C + S	Design: C-S No.: 98 M/W: 43/55 Age (years): mean 37.23, SD 9.93 Type of job: Direct-care disability workers Analyzed subgroups: Not clear	Days: No info Samples per day: 2 Times for sampling: +0, +30 min Condition: No info	Enzyme-linked immunoabsorbent assay 96, DRG-kit, Marburg	Measurement(s): a1. Awakening b1. Difference between the morning measurements Cortisol data: Log transformed Samples used: All	Pearson bivariate correlation Linear regression SEM	The exposure measures used in isolation or in combinations (DC vs DCS) did not predict cortisol. Cortisol and SIgA used as a combined physiologic outcome predicted DCS	Cortisol data not related to the demand-control model. Indices of multiple physiologic measures may reveal stronger relationships with job stress measures
Berset 2009 [29]	*Subjective* JDC model with Instrument for Stress Oriented Task analysis (Semmer *et al.*, 1995)	Design: C-S No.: 69 M/W: 39/30 Age (years): Type of job: Employees from 3 departments of a large Swiss service provider. Blue- and white-collar workers not in a supervisory position Analyzed subgroups: Not clear	Days: 3 Samples per day: 1 Times for sampling: 12.00 h Condition: 2 work days and 1 day off (Sunday)	Not indicated	Measurement(s): a3. At 12:00 h during 2 work days and a rest day Cortisol data: (average value for work days analyzed)	Multiple regression analysis	Low control related to high a3 on rest day High demands and high strain not related to high cortisol No effect of sex	Lack of control during work days leads to lower recovery during a rest day (Sunday) Limitations: only 1 cortisol sample, no control over awakening time during work and rest days
Maina 2009 [30]	*Subjective* DC model (JCQ Italian version)	Design: C-S No.: 36 M/W: 16/20 Age (years): Type of job: Call handlers Employees Analyzed subgroups: 4 job strain categories: high strain (3 W, 6M); active work (9 W, 7 M); passive work (4 W, 3 M); low strain (4 W, 0 M)	Days: 3 or 4 (unclear concerning weekend) Samples per day: 7 Times for sampling: +0, +30, +60 min, at work shift start, +3 h, +6 h, +9 h Condition: 2 work days and weekend	RIA	Measurement(s): b1. MinINC (deviation from 1st to mean of 2nd and 3rd samples) c1. AUC 1st, 2nd, 3rd cortisol samples c2. AUC 4th, 5th, 6th, 7th samples c3. AUC diurnal cycle 1st, 4th, 5th, 6th, 7th samples Cortisol data: Square root transformation	t-test Spearman rank test Generalized estimating equation	High strain related to high c1 Higher cortisol during week days than during week-end CAR higher in women than men	High strain participants have high cortisol. Sex is an important factor, with higher cortisol among women Limitations: no strict control over compliance
Metzenthin 2009 [31]	*Subjective* Self-reported stress level at each sample; retrospective ratings of stress in the shift *Objective* Hospital's LEP Nursing workload and management system	Design: R-M, L No.: 82 M/W: 0/82 Age (years): Type of job: Pediatric critical care nurses Analyzed subgroups: 3 time points; Sep 2004, Dec 2004, Mar 2005	Days: 3×9=27 Samples per day: 5 every shift in each session Times for sampling: Start of shift, +2 h, +4 h,+6 h,+8 h Condition: Three 9-days sessions: Sep and Dec 2004, and Mar 2005 3 shifts: 07:00-16:00, 14:00-23:00, 22:30-07:30 h	Luminometric immunoabsorbent assay	Measurement(s): c2. AUC Cortisol data: Log transformed Samples used: From +2 h	Correlations Multivariate regression analysis Multilevel analysis	Unadjusted correlation to 2 objective workload indicators, but not to subjective load. In fully adjusted model, relation to subjective load concurrently but not retrospectively rated	In line with previous findings. Lacking association with retrospect. Ratings may be due to recall bias Limitations: objective load assessed at unit level

Table 2: Brief summary of main findings of associations between salivary cortisol parameters and exposure/outcome/biological marker in studies sorted by type of exposure and year of publication (arrows up/down or 0 indicate results)

References	Year	Exposure	Design	No. (cortisol)	M/W	Single time points (or sum/mean of 2 or more time points)					Deviation (difference/slope between 2 or more time points)			AUC					
						a1	a2	a3	a4	a5	b1	b2	b3	c1		c2		c3	
IMBALANCE INDICES																			
High job strain																			
Fox [5]	1993	Subjective high job strain	C-S	136	0/136			0											
Steptoe [8]	1998		C-S	61	27/44			↓					↑						
Steptoe [12]	2000		C-S	105	41/64		↑	0	0				↑						
Hanson [11]	2000	MD-SR	C-S, R-M	77	43/59			0											
Fujiwara [15]	2004		C-S	16	16/0		0	0	0	0									
Alderling [21]	2006		C-S	529	181/341	0/↑	0/↑	0	0		0	0	0						
Harris [23]	2007		C-S	44	0/44	0			0				0	0					
Maina [25]	2008		C-S	68	12/56	0/0	0/0	0/0	0/0		0/0			0/0	0/0	0/0	0/0	0/0	0/0
Berset [29]	2009		C-S	69	39/30			0											
Maina [30]	2009		C-S	36	16/20						0			0/↑	0/↑		0		0
High iso-strain																			
Chandola [27]	2008		C-S, L	3490							↑								
High effort-reward imbalance																			
Hanson [11]	2000		C-S, R-M	77	43/59			0											
Steptoe [18]	2004		C-S	165	86/79						0	0							
Eller [22]	2006		C-S	83	55/28	0				↑/0	↑								
Harris [23]	2007		C-S	44	0/44	0			0				0	0					
Bellingrath [24]	2008		C-S, R-M	135	40/95					0									
DEMAND DIMENSIONS																			
High demands																			
Steptoe [12]	2000		C-S	105	41/64								0						
Kunz-Ebrecht [16]	2004		C-S	181	97/84	0		0/↑			0								
Aasa [20]	2006		C-S	26	24/2	0	0	0	0										
Eller [22]	2006		C-S	83	55/28	0				0	0								
Harris [23]	2007		C-S	44	0/44	0			0				0	0					
Maina [25]	2008		C-S	68	12/56	0/0	0/↑	0/0	0/0		0/↑			0/↑	0/↑		0/0		0/0
Rydstedt [26]	2008		C-S, R-M	77	53/24	0/↑			0/0										
Wright [28]	2008		C-S	98	43/55	0					0								
Maina [30]	2009		C-S	36	16/20						0			0/0	0/0		0		0
Berset [29]	2009		C-S	69	39/30			↓											
High effort																			
Eller [22]	2006		C-S	83	55/28	0				↑/0	↑/0								
Harris [23]	2007		C-S	44	0/44	0			0				0	0					

Bellingrath [24]	2008		C-S, R-M	135	40/95					0									
Subjective high workload																			
Fox [5]	1993		C-S	136	0/136			0											
Fischer [10]	2000		C-S, L	138	20/118					0		0							
Schlotz [17]	2004	TICS work overload	C-S, R-M	219	102/117						↑								
Harris [23]	2007		C-S	44	0/44	0			0				0	0					
Metzenthin [31]	2009	Concurrent (1 item)	R-M, L	82	0/82												↑		
Metzenthin [31]	2009	Retrospective (1 item)	R-M, L	82	0/82												0		
Objective high work load																			
Fox [5]	1993	Patient load, patient contact hours, deaths	C-S	136	0/136			0											
Zeier [6]	1996	High air traffic load	R-M	126	126/0							↑							
Fischer [10]	2000	Patient events	C-S, L	138	20/118					0		↑							
Metzenthin [31]	2009	Patient load index	R-M, L	82	0/82												0		
RESOURCE DIMENSIONS																			
Low control																			
Steptoe [12]	2000		C-S	105	41/64								↑						
Kunz-Ebrecht [16]	2004		C-S	181	97/84	0		↑/0			0								
Eller [22]	2006		C-S	83	55/28	0				0	0								
Harris [23]	2007		C-S	44	0/44	↓			0				0	0					
Maina [25]	2008		C-S	68	12/56	0/0	↓/0	0/0	0/0		0/0			0/0	↓/0		0/0		0/0
Rydstedt [26]	2008		C-S, R-M	77	53/24	0/0			0/0										
Wright [28]	2008		C-S	98	43/55	0					0								
Maina [30]	2009		C-S	36	16/20						0			0	0		0		0
Berset [29]	2009		C-S	69	39/30			0											
Other control-related dimensions																			
Fox [5]	1993	Subjective low control	C-S	136	0/136			0											
Hanson [11]	2000	Need for control	C-S, R-M	77	43/59			0											
Steptoe [12]	2000	Low skill discretion	C-S	105	41/64								0						
Harris [23]	2007	Low decision authority	C-S	44	0/44	↓			↑				↓	↑					
Harris [23]	2007	Low skill discretion	C-S	44	0/44	0			0				0	0					
Low social support at work																			
Evans [13]	2001		C-S	93	40/53							0							
Eller [22]	2006		C-S	83	55/28	0				↓/0	↓/0								
Harris [23]	2007		C-S	44	0/44	0			0				0	↓					
Wright [28]	2008		C-S	98	43/55	0					0								
Rydstedt [26]	2008		C-S, R-M	77	53/24	0/0			0/0										

Low reward																			
Eller [22]	2006		C-S	83	55/28	0				0	0								
Harris [23]	2007		C-S	44	0/44	0			0				0	0					
Bellingrath [24]	2008		C-S, R-M	135	40/95					0									
OTHER STRESS MEASURES																			
Fujigaki [7]	1997	Subjective high stress	L	10	10/0			↑				↑							
Fischer [10]	2000	High perceived stress	C-S, L	138	20/118					0		0							
Fischer [9]	2000	High perceived stress	R-M, L	64								0							
Yang [14]	2001	High perceived stress	C-S	73	0/73		↓	0				↓							
Dahlgren [19]	2005	Stressful week	R-M	34	11/23		↑		0	↑			↓						
Harris [23]	2007	High job stress	C-S	44	0/44	0			0				0	0					

Exposures are expressed in terms of the assumed stressful pole of the concept, so an upward arrow will always indicate a higher cortisol measure under the conditions of a higher stress exposure. a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground.

Quality Rating

A separate matrix (Table **3**) was used to rate the quality of each study included with respect to the criteria used by Chida and Steptoe [4]. These criteria include whether or not relevant confounders were addressed or controlled for. Specifically, the following eight confounders were evaluated: age, sex, smoking status, participants' adherence to procedures for salivary sampling (*e.g.*, by electronic monitoring, self-reported time of sampling), steroid medication, time of awakening, sampling days (number of days and type of sampling day; week day or weekend), and clear and standardized instructions to study participants regarding the sampling procedure.

Three levels of judgments for each of the criteria were used giving 0, 1 or 2 points. In general, 2 points involved statistically controlling for a confounder (*e.g.*, as a covariate) or analyzing group differences and taking the results into consideration. One point typically included addressing the confounder in the text and indicating awareness of its potential role as a confounder and/or discussing why a confounder was not measured or statistically controlled for and the potential consequences of not including a confounder among the study variables. As regards standardized instructions, a maximum of 1 point was given when study participants were reported to have received any instructions, irrespective of the levels of detail. When no report of instructions was included, the rating was 0 points. Six additional criteria were rated from 0 to 2 points and one from 1-2 points, giving a possible total score range of 1 to 15.

The principles for evaluating the other 7 confounders are specified below:

Age - included in statistical analysis, controlled for or otherwise accounted for (2 points); discussed but not statistically analyzed or accounted for (1 point); not analyzed or discussed (0 points).

Sex - sex was taken into account in the analyses, *e.g.*, separate analyses of women and men or included as a between group factor (2 points); if possible sex effects were discussed but not analyzed, even when the study include one sex only (1 point); only one sex was included in a study and/or possible sex effects were not discussed, or women and men were not studied separately in any way (0 points).

Smoking status - evaluated in the same way as age.

Adherence - adherence was monitored technically with electronic devices, or analyzed based on other data and treated by exclusion of non-adherent participants or otherwise treated in a stringent manner (2 points); only addressed or discussed (1 point); not addressed at all (0 points).

Steroid medication - exclusion of study participants taking some kind of medication or of post-menopausal women (2 points); potential effects of steroid medication were discussed (1 point); such medication not mentioned or discussed at all (0 points).

Waking time - controlled or in some way accounted for or handled (2 points); discussed but not measured or accounted for (1 point); not mentioned or discussed (0 points).

Sampling days - cortisol sampling on more than 1 day analyzed and accounted for (2 points); one sampling day or possible effects or sampling days discussed (1 point); when it was unclear on which days sampling had occurred the article was excluded from further analyses.

Global Quality Evaluation

A quality rating based on scoring a set of variables may give an indication of many quality aspects of a study, but there is also a risk that the pre-defined scoring principles result in an over- or underevaluation of a study. For instance, in an article with a high score it is still possible that poor biochemical analysis or inadequate statistical methods influenced the study findings and result in an overevaluation of its quality. Similarly, a low quality score may following from not statistically controlling or not discussing specific confounders that were of minor importance in the study setting. This means that a well-designed study failing to meet the quality criteria scored lower, which may result in underevaluation of the study quality. To adjust for this bias in quality scores relating to the predefined criteria, a global quality evaluation was performed, in which a study was classified as being of high, moderate, or low quality. This assessment included taking into consideration the sample size in relation to the study design and statistical analyses, clarity in defining the exposure measure(s), specificity of the exposure measure(s), reliability with regard to the biochemical analyses, restrictions with respect to generalizing the results (including details on drop-out rates, selection bias, and heterogeneity of the study participants with respect to sex, age, occupation, and socioeconomic status).

Procedure

Initially, all 6 authors summarized and evaluated one of the articles in accordance with the summarizing matrix, in order to fine tune their inter-rater concordance and the design of the matrix. Each of the 60 articles selected from the abstract, was then read, briefly summarized, and preliminarily evaluated according to the matrix independently by 2 of the authors in varying pair-wise groupings with each author reading about 20 articles.

In the next step, all groups of authors went through the summaries and preliminary evaluations of the articles read, which resulted in another 33 studies being found not to fulfill the inclusion criteria because only urinary or plasma cortisol was used, because they did not fulfill the criteria for exposure (*e.g.*, not having used or sufficiently described a measure for self-reported job stress), resulting in 27 articles remaining for in-depth review. These articles were read once more by all authors with each author independently scoring the articles in accordance with the quality criteria matrix. These second scorings for each article were discussed among the authors and disputes were settled by re-evaluating the article until consensus was reached. Each study was also given a global quality evaluation score by the author groups. The remaining 27 articles [5-31] were re-read once more with each article being read by two authors in varying groupings in order to summarize the study and relevant findings (Tables **1** and **2**).

RESULTS

Quality Assessment

The quantitative summary of the quality score had a possible range from 1 to 15 and the quality scores among the 27 articles ranged from 5 to 14 (median=10) (Table **3**). Fig. **2** shows that the quantitative quality scores increased over time, which means that recent studies were of higher quality than the earlier ones. According to the global quality evaluation summarized in Table **3**, 11 articles were of high quality, another 11 articles were of moderate quality, and 5 articles were of low quality. As shown in Fig. **3**, articles of high

or moderate global quality had similar quantitative quality scores. In contrast, articles of a low global quality had lower quantitative quality scores than the others.

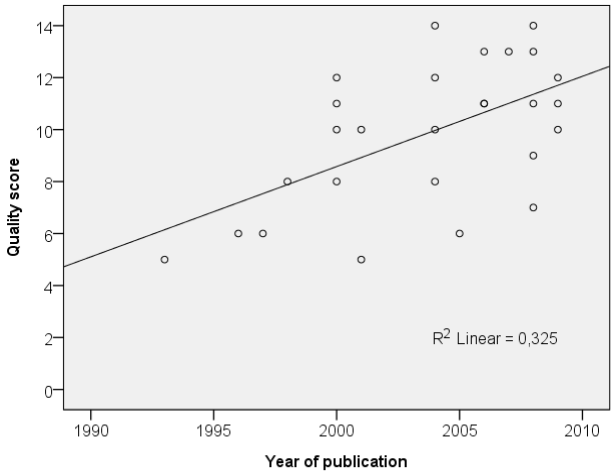

Figure 2: Quality scores of articles by year of publication.

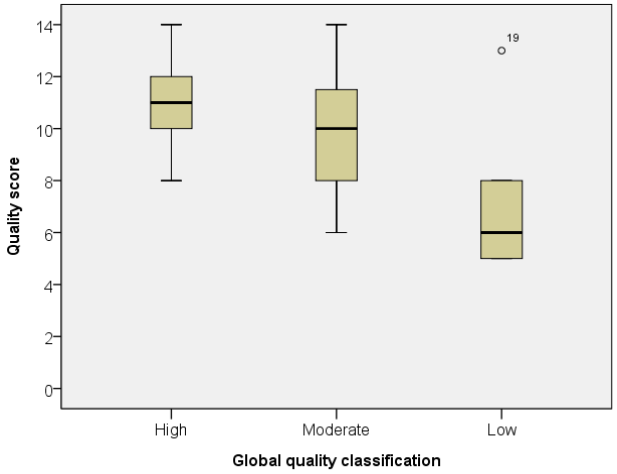

Figure 3: Quality scores of articles by global quality classification.

Design and Study Participants

Fifteen studies used a cross-sectional design, 2 used a repeated-measures design, 2 used a longitudinal design, and 8 used a combination of these designs. Four of the studies that used a mixed design were found to combine cross-sectional and repeated-measures designs; another four used a longitudinal design combined with either a cross-sectional (n = 1), case-control (n = 1) or a repeated-measures design (n = 2).

With regard to the study participants, the 27 articles included blue-collar and white-collar workers of different socioeconomic status.

Exposure Measures

Sixteen of the articles investigated associations between salivary cortisol and one exposure measure; 4 articles investigated associations between salivary cortisol and two or three exposure measures; 3 articles investigated associations between salivary cortisol and 4, 5, and 10 exposure measures, respectively. Of the 27 articles, 21 included subjective exposure measures only and 2 included only objective exposure

measures. Four articles included both subjective and objective exposure measures. Of the 27 articles, 15 included subjective exposure measures that were related to the DCS model, assessing one or more of the dimensions, usually using some version of the Job Content Questionnaire [32]. Thirteen articles focused exclusively on the dimensions included in the DCS model; 4 articles focused exclusively on dimensions included in the ERI model [3]. However, 2 articles included measures relating to both the DCS model and to the ERI model. Six articles used some other subjective exposure measure. Seven articles included an objective exposure measure, and 4 of these articles combined the objective exposure measure with a subjective measure. Often, the objective exposure measures were based on some kind of administrative assessment of workload, such as time pressure, conflicting tasks or insufficient number of nurses in an emergency ward.

Cortisol in Relation to Work Stress Exposure

In the 27 articles, 185 analyses of associations between a cortisol measure and a psychosocial exposure measure were done. However, this number includes overall analyses only; the total number of analyses increased if, for instance, sex specific analyses were included. Of these 185 analyses, the distribution of statistically significant associations with respect to the 3 main categories of cortisol measures evaluated in Table **2** were as follows: 20/97=21% of the single time point measures, 14/48=29% of the deviation measures, and 8/40=20% of the Area Under the Curve (AUC) measures. The proportions for the different subcategories of cortisol measures included in Table **2** were as follows: single time point measures a1 (4/30=13%), a2 (6/9=67%), a3 (5/22=23%), a4 (1/21=7%), a5 (4/14=29%), deviation measures b1 (6/21=29%), b2 (4/10=40%), b3 (4/17=24%), AUC measures (increase/ground) c1 (4/17=24%; 3/6=50%), c2 (0/1; 1/9=11%), c3 (0/1; 0/6). In total, 42 (23%) of the 185 analyses showed statistically significant findings. Of these significant results, 29 (69%) were positive with a higher cortisol value being associated with a higher stressor exposure, and 13 (31%) were negative. The negative relationships were evenly scattered across the different categories of cortisol measures.

With regard to the different types of cortisol measures, Table **1** makes it clear that some ways of statistically analyzing salivary cortisol are new to the area, focusing on salivary cortisol and exposure to psychosocial work stressors. For instance, research including the deviation in the morning, that is, the Cortisol Awakening Response (CAR), was published in 2004; research investigating the AUC was published in 2007. In addition, studies on other cortisol measures, such as suppression of cortisol after intake of dexamethasone, are limited. Such limitations may be due to practical reasons and restrictions associated with field studies. Of the 27 articles, only 1 article included a dexamethasone test. The findings showed a higher suppression related to high work stress (lower reward, higher burnout and vital exhaustion), which according to the authors was interpreted as a heightened HPA axis negative feedback.

Table **2** was structured to include the main dimensions in the 2 predominant models of work stress (DCS and ERI) that formed the basis for the literature searches. Table **2** also shows the results for different dimensions of psychosocial work stress. The proportion of significant relationships between different salivary cortisol measures and measures of psychosocial exposure were as follows: 10/51 (20%) for imbalance indices, 13/57 (23%) for demand dimensions and 12/61 (20%) for resource dimensions. Some measures of work stressors (*e.g.*, referring to high levels of perceived stress at work) did not obviously fit into 1 of these 3 main dimensions and these were labeled “other stress measures”. Of these other stress measures, 7/16 (44%) were significant.

Effects of Design

Study design is a factor that may be related to the number of significant findings. For instance, compared with cross-sectional designs, repeated-measures designs are likely to yield more robust measures and increase statistical power even with smaller sample sizes, which in turn increases the chances of detecting significant effects. Considering this, an initial intention was to investigate the number of statistically significant findings in relation to study design. However, few articles included repeated-measures designs or longitudinal designs; of these, the number of significant findings was limited.

Table 3: Quality criteria according to Chida and Steptoe [4] and own additional criteria

References	Chida and Steptoe criteria: confounders addressed (A), controlled for (C) or examined (E)[a] Mark with A, C, E combinations of these (*e.g.*, AC) or 0 (not considered at all)									Own quality criteria: for sample size mark OK or NOK (not OK); for exposure definition O (Obj), S (Subj) or both. Comments may be given. For generalizability, add comment			Total quality assessment	
	Age	Sex	Smoking	Compliance	Steroid medication	Clear instructions	Biochemical analysis	Waking time	Sampling days	Sample size (total W/M)	Exposure definition	Generalizability	Quantitative score	Qualitative assessment
Fox 1993 [5]	C	A	0	0	0	OK	OK	0	A	OK 136 W136/M0	OK SO (pat load, contact hrs, deaths; D 7 items, C 22 items) OK	Restricted to women Uncommon S and O measures Poor cortisol sampling and biochemical analysis	5	Low
Zeier 1996 [6]	A	0	C	0	0	OK	OK	0	C	OK 126 W0/M126	OK Objective load	Restricted to men, air-traffic controllers or similar	6	Moderate
Fujigaki 1997 [7]	A	A	A	0	0	NOK	OK	A	C	NOK 10 W0/M10	OK S (job events item diary; overwork; semi-structured interview)	Restricted cortisol sampling, sex and number, but long series; uncommon not previously validated measures	6	Low
Steptoe 1998 [8]	A	C	C	0	0	OK	OK	0	C	OK 71 W44/M27	OK S Job social support (6 items) job demands (3 items) skill (4 items) job strain	Representative for retail	8	Low
Fischer 2000 [9]	A	A	C	A	C	OK	OK	A	A	OK 64 T1: W4/M60 T2: W2/M58	OK O (shift type)	Restricted due to changed team composition, high turnover	10	High
Fischer 2000 [10]	C	C	C	A	C	OK	OK	A	A	OK 138 W118/M20	OK SO (shift type; nurse/patient ratio; subjective work load 17 items)	Restricted occupation (health care) limits generalizability, as does uncommon exposure measure	12	High
Hanson 2000 [11]	A	C	C	C	0	OK	OK	A	C	OK 77 W34/M43	OK S (Dutch ERI, E 6, R 12 items; momentary D 3 items diary)	Good, some restriction to occupation and work content similar to that studied (*e.g.*, call centers). Homogeneous, low exposure contrast. Biochemical analyses	11	High
Steptoe 2000 [12]	C	C	C	0	0	OK	OK	0	A	OK 105 W64/M41	OK S Skill (4 items), demands (3 items), control (3 items)	Restricted to teachers No waking time	8	High
Evans 2001 [13]	A	C	A	0	C	OK	OK	A	C	OK 93 W53/M40	OK S (D 3 items, C 7 items; S 5 items Undén-91)	Restricted measure (only social support). Effects of sex and occupation mixed and not separable	10	Moderate
Yang 2001 [14]	A (but not related to cortisol)	0	0?	0	C	OK	OK	0	A	OK W31+56/M0	OK S Cushway *et al.* self-reported work stress in health care	Restricted to women in health care	5	Low
Fujiwara 2004 [15]	C	A	A	0	0	OK	OK	A	C	OK 16 W16/M0	OK S (JCQ D 5, C 9, S 4+4, Physical exertion 3 items	Restricted to women	8	High
Kunz-Ebrecht 2004 [16]	C	C	C	C	E	OK	OK	C	A	OK 181 (W56 high SES/M70 high SES; W28 low SES/M27 low SES	OK S (D 4; C 9 items)	High SES white collar workers (Whitehall 45-58 years healthy postmenopausal if women). Power problems low SES	14	Moderate

Schlotz 2004 [17]	A (not in analyses of job stress)	A (not in analyses of job stress)	0	C	C	OK	OK	A	C	OK 321 W219/M102 140 employed, 6 unemployed, 72 retired	OK S TICS (work overload, 8 items)	Problem with group. How valid are assessments of work overload among the retired, who constitute a large % of the sample Overall = MI Sampling procedure? Exposure measure? Age not considered	10	Moderate
Steptoe 2004 [18]	C	C	C	C	0	OK	OK	C	A	OK 165 W79/M86	OK S ERI (5 items Effort, 7 items Reward, Over commitment) Job demands/control	Restricted to 45-55 year old white collar workers	12	High
Dahlgren 2005 [19]	A	A	0	0	0	OK	OK	A	C	OK 34 W23/M11	OK SO Predicted high stress week, S-E rating	Representative for white collar workers. Uncommon S measure. Strength: varying conditions	6	Moderate
Aasa 2006 [20]	C	E	A	0	C (no subjects with steroids included?)	OK	OK	A	C	OK 26 W2/M24	OK Kjellberg (stress and energy) Demand dimension of DCQ Worry about work (Brulin adapted to ambulance staff)	Ambulance staff only	11	Moderate
Alderling 2006 [21]	C	C	C	C	C	OK	OK	A	A	OK 529 W348/M181	OK S (JCQ) only 4-field strain category analyzed	Strength: population-based but high drop outs; selection bias? Few men	13	High
Eller 2006 [22]	C	C	C	A	0	OK	OK	C	A	OK 83 W28/M55	OK S (D 2 items, C 12 items, S 4 items; ERI questionnaire)	Biased selection base (intima media study) restricts representativity NOK	11	High
Harris 2007 [23]	C	A	C	C	A	OK	OK	C	C	NOK 44 W44/M0	OK	Health care. Selection bias (dropout). Too many statistical analyses on a small sample	13	Low
Bellingrath 2008 [24]	C	C	C	C	C	OK	OK	A	C	OK 135 W 95//M40)	OK S (ERI 6+11 item). E+R+ERI analyzed	Restricted occupation. Representative for teachers. Homogeneous, low contrast	14	High
Maina 2008 [25]	C	C	A	C	C (excluded)	OK	OK	A	C	OK 68 W56/M12	OK S (JCQ; D5, C 6 items). Median split 4 dimensions + job strain	Restricted to women. Volunteers from single occupation. 26% participation rate, small subgroups	13	Moderate
Rydstedt 2008 [26]	C	C	0	A	0	OK	NOK	A	C	OK 77 W24/M53	OK S (JCQ; D 4, C 16, S 7 items Job strain, Iso-strain (high/low)	Few women: power problems. Difficulties recruiting participants	9	Moderate
Chandola 2008 [27]	C	C	C	A	C	OK	OK	A	0	OK 3490 W/M?	OK Whitehall DCS questionnaire. Median splits defining Iso-strain Phase 1 85-88 added to phase 2 89-90, thus cumulative assessment	Large sample. 10,308 in total cohort. Half of this sample was clinically examined. 90% of those gave saliva (4609). Exclusion of those with late first samples and on steroids	11	High
Wright 2008 [28]	C	C	0	0	0	OK	OK	A	A	OK 98 W55/M43	OK (S) JCQ (version unclear). Decision latitude and demands, and job strain score, dichotomized by cut-off >1	Restricted to direct-care disability workers. Moderate sample size	7	Moderate

Berset 2009 [29]	A	C	A	A	A	OK	NOK missing	A	C	OK 69 W30/M39	OK Job DC model	Larger sample and more types of jobs to enhance the generalization	10	Moderate
Maina 2009 [30]	C	C	0	C	A	OK	OK	C	C	NOK 36 W20/M16	Job strain model (Italian version of JCQ; Cesana *et al.*, 2003)	Small sample of call handlers with low response rate	12	Moderate
Metzenthin 2009 [31]	C	A	A	0	C	OK	OK	C	C	OK 82 W82/W0	(O+S) Objective workload (hospitals LEP) in 3 parameters on unit level; perceived work stress intensity at times of sampling	Restricted to female nurses in the studied ward units. Moderate sample size	11	High

A (1 p) = addressed (*e.g.*, mentioned in text but not included in statistical analyses).

C (2 p) = controlled statistically (covariate).

E (2 p) = examined group differences (*e.g.*, *t*-test differences in smoking between 2 groups but confounder not included as covariate in subsequent analyses).

O = objective (exposure definition).

S = subjective (exposure definition).

Effects of Quality

With regard to the relationships between quality and significant findings, the proportion of significant findings varied somewhat depending on the quality of the study. In articles with a quality score below the median score of 10, 13/48 (34%) of the associations between salivary cortisol measures and any psychosocial exposure measure were significant. The corresponding figure for articles with a quality rating above the median score was 29/147 (20%). Similar analyses with respect to the global quality evaluations showed that of the high quality articles, 15/61 (25%) of the associations were significant. For the moderate and low quality articles, the proportions of significant findings were fairly similar to that of the high quality articles 16/70 (23%) for moderate quality and 11/54 (20%) for low quality.

DISCUSSION

The findings from this systematic review of 27 articles including 185 analyses investigating linkages between different measures of salivary cortisol and psychosocial work stressors showed that most of the published findings were nonsignificant. This pattern of findings suggests that there are no consistent associations between salivary cortisol and exposure to psychosocial work stressors among working individuals. However, along with the nonsignificant results, a number of significant findings showing an association between salivary cortisol and psychosocial work stressors was identified. Most of these significant relationships were in a positive direction showing that higher cortisol levels were associated with a higher degree of psychosocial work stress. These findings suggest that, in most cases, exposure to psychosocial work stress is far from severe and the increase in cortisol levels represents a normal, healthy activation response. According to CATS and the Allostatic Load Model, this response pattern should not induce any health problems. More specifically, it seems that most working individuals manage to cope with psychosocial work stressors or recover from the strain associated with psychosocial work stressors before the strain has any measureable effects on salivary cortisol levels resulting in changes in salivary cortisol patterns. With regard to different psychosocial work stressors, deviations in salivary cortisol are perhaps more sensitive to some types of psychosocial exposure than others. The present review of psychosocial work stressors focused on a specific and limited number of exposures. As a result of this focus, a limited number of articles was reviewed and the data obtained from these articles did not allow for a fine-tuned analysis to differentiate between different work stressors. The psychosocial work stressors were roughly categorized into three different groups including concepts relating to either demands, resources, or imbalance. At this level of analysis there were no differences with regard to the ratio of significant relationships. In addition, there was a fourth category labeled “other stress measures”. In contrast to the other 3 categories, which mainly assessed perceived stressors, this fourth category included measures assessing perceived work stress in terms of responses to perceived stressors. This fourth category including other stress measures had a higher ratio of significant relationships than the other categories.

The overall findings and lack of robust significant associations may also be related to methodological and statistical strategies. For instance, strategies for sampling saliva and methods of statistically analyzing derived cortisol measures, such as CAR and AUC, can be hypothesized to influence the results. Of the different methods of statistically analyzing salivary cortisol measures, single points in time at waking, midday, and evening were the most common measures in the 27 articles reviewed, followed by deviation measures in the morning (*i.e.*, CAR); the other methods of statistically analyzing cortisol were less frequent. There was a fairly high ratio of significant findings for a few of the cortisol measures, including single measures in the morning and AUC_{ground}. But these measures were less frequently used and cannot be concluded to be more successful than any other measure. Moreover, as the overall ratio of significant relationship was quite low, the number of analyses for each cortisol measure was too small to reveal any clear pattern with regard to the more successful strategies. In addition, the distribution of significant relationships was comparable across the main categories of cortisol measures applied (*i.e.*, single time point measures, deviation measures, and AUC measures), which indicates that no clear pattern emerged with regard to comparisons between static and dynamic cortisol measures. However, some of the cortisol measures, such as the dynamic CAR and AUC measures, have not been used until recently, which means that there are far fewer published articles including these measures compared with articles including single

points in time. The quality of the published articles has increased over the years. Recent publications are of higher quality than older articles. Perhaps some of the findings reported in earlier research are related to inadequate sampling procedures or to not controlling for potential confounders. This reasoning is supported by the quality evaluation in this systematic review, which showed that the proportion of significant associations between psychosocial work stressors and salivary cortisol was higher in low quality articles.

Apart from methodological and statistical strategies, the nonsignificant findings presented in this review may be related to the homogeneity of the groups studied. Many of the field studies focus on individuals working in a specific organization (*e.g.*, health care), at a specific location (*e.g.*, a hospital) and often include only one group of employees (*e.g.*, nurses). This means that the study participants were homogeneous with respect to occupation but also in terms of psychosocial exposure. For instance, study participants working in the same organization and performing similar work tasks can be classified as exposed to high or low job strain. Typically, this classification is empirically based, which means that the contrasts between individuals in homogeneous groups are too small to allow small group differences in cortisol excretion to be detected. This means that for some of the articles, the many nonsignificant findings may be attributable to a lack of statistical power when comparing subgroups of the full sample studied; in other cases, small groups may have produced significant chance findings. Also, with regard to cortisol, the interindividual variation is very high, which further reduces the power to identify significant associations with psychosocial factors such as work stress. In addition, a certain degree of interindividual variation in the self-report measures of psychosocial stressors will contribute to reduce the ability to detect associations. It may be noted that there is usually a lack of temporal match between cortisol and self-report data, the latter mostly being a global rating based on the individual's mental representations of a longer period of time. However, the findings from the present review are in line with research on work stress and urinary cortisol [37].

From a biological perspective, cortisol responses seem to be mobilized during situations characterized by extreme demands and efforts such as child birth [33, 34] and sailing around Cape Horn [35]. However, the psychosocial work stressors included in this systematic review are not of extreme intensity. Instead, psychosocial work stressors can be considered to be of mild to moderate intensity. Ambulance drivers, policemen, and fire fighters do experience intensive work stress during emergency situations. The present review includes studies on intensive care nurses [10, 31], another group that has to deal with intensive bouts of psychosocial work stress. The articles on intensive care nurses examined unexpected emergency situations and these situations did elicit clear cortisol responses. In contrast, the milder psychosocial work stressors experienced daily by most working individuals are unlikely to elicit any clear cortisol responses. The effects are likely to reflect the relatively small and inconsistent effects found in this review. In addition to daily psychosocial work stressors being of mild to moderate intensity, there is also a potential habituation effect. Specifically, working individuals who are repeatedly exposed to mild to moderate psychosocial work stressors often adapt to the stress. From a biological perspective, this adaptation involves a successive reduction of the cortisol response. This line of reasoning draws on findings from experimental stress provocation research [36] showing habituation effects on cortisol secretion. According to CATS this means that most workers have adapted positive expectancies regarding the outcome from exposure to their work demands. Another factor to keep in mind when trying to explain associations between psychosocial work stressors and cortisol is that cortisol, along with other corticosteroids, reduces the negative feelings associated with a stressor. Accordingly, study participants experiencing intensive psychosocial work stress may actually underreport stress levels due to biological mechanisms. This can be illustrated by the study of intensive care nurses in which the objectively recorded emergency situations were associated with increased concentrations of salivary cortisol but not to self-reports of stress [10]. Similar findings have been reported among ambulance service personnel [38]. These findings were based on a repeated measures design, which is often assumed to be more sensitive in detecting effects than cross-sectional designs. However, on the basis of this review it is not possible to draw any conclusions on the effects of study design.

In view of previous research showing clear linkages between exposure to psychosocial work stressors and various cardiovascular disorders, it is perhaps surprising that there are no consistent associations between

psychosocial stress at work and salivary cortisol. But compared with the research on cardiovascular disorders, which is based on large samples to allow the effects of psychosocial work stressors on cardiovascular events and mortality to be delineated, the research on salivary cortisol is based on smaller samples and often of a different character. For instance, the studies reviewed here included working individuals with no severe health problems. Most articles are based on young and middle-aged working individuals. This means that longitudinal studies of salivary cortisol, psychosocial work stress and cardiovascular events and mortality are needed before discarding salivary cortisol as one of the key bodily mechanisms involved in the development of work-related cardiovascular disorders. The findings from the present systematic review show no consistent support for cortisol as one of the key bodily mechanisms involved in the development of various disorders related to psychosocial work stress. Perhaps this is due to cortisol not only being related to the exposure to psychosocial stressors but also the fact that the acute and long-term responses of the Hypothalamo-Pituitary-Adrenal (HPA) axis and the associated secretion of cortisol differ. Such differences relating to habituation are seldom discussed in field studies of working individuals. Most of this research focuses on examining the linkages between various aspects of psychosocial work stress and different cortisol measures without taking into account habituation effects other than years in employment or similar factors providing secondary measures of physiologic habituation. However, some significant associations were found. Exposure to high work stress was more often associated with high cortisol levels than low cortisol values. This indicates that when found, the linkages between psychosocial work stress and cortisol levels were associated with a physiologic activation rather than with a downregulation of the HPA axis activity. According to CATS and the Allostatic Load Models physiologic activation represents a normal, healthy response if followed by deactivation after work.

The number of studies reviewed was fairly small (n = 27), which means that additional research is needed before drawing any firm conclusions on associations between salivary cortisol and psychosocial work stressors. However, future studies would benefit from a careful evaluation of the positive and negative effects of the study design, issues relating to statistical power, relevant confounders, instructions given to study participants, the timing of salivary sampling, and the number of samples a study participant is asked to collect. All these factors are likely to influence the findings and quality of the research.

CONCLUSIONS

The present systematic review of 27 articles investigating the associations between cortisol and psychosocial work stressors showed that there was a large proportion of nonsignificant findings, with no strategy for sampling saliva or statistically analyzing the data that was superior to others. Some significant associations were found and these showed that exposure to high work stress was more often associated with high cortisol values than low values. This indicates that the linkages between psychosocial work stress and cortisol levels are more likely to be associated with normal physiologic activation than with a downregulation of HPA axis activity. With regard to the nonsignificant findings, it is possible that most of the articles reviewed examined exposure to stress of mild to moderate intensity and that the groups contrasted were too homogeneous, thus giving too little variation to reveal effects.

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CHAPTER 4

Perceived Stress, Psychological Resources and Salivary Cortisol

Christina Halford[1,*], Ingibjörg H. Jonsdottir[2] and Frida Eek[3]

[1]Researcher at the Department of Public Health and Caring Sciences, Uppsala University, Box 564, 751 22 Uppsala, Sweden; [2]Associate professor at the Institute of Stress Medicine, Gothenburg, Sweden and [3]Associate professor at the Division of Occupational and Environmental Medicine, Lund University, Sweden.

Abstract: The aim of this chapter was to analyze associations between measures of cortisol in saliva with measures of perceived stress, using the Perceived Stress Scale (PSS), and of psychological resources in terms of mastery, locus of control, self-esteem and sense of coherence. Only studies on healthy individuals were included and cortisol measures were grouped into single time point measures, deviation measures, Area Under the Curve (AUC), laboratory test responses, and dexamethasone suppression. For both Perceived Stress Scale (PSS) and for psychological resources, most results of associations with saliva cortisol were nonsignificant particularly for single measures and for cortisol awakening response. For PSS the largest proportion of significant findings (38%) was seen for morning AUC, however with conflicting results. For psychological resource constructs, mastery and sense of coherence were related to lower cortisol level at baseline in standardized rest and high mastery was related to steeper diurnal slope in two studies. For self-esteem, no associations showed significant results. Differences in findings may to a large extent be dependent on theoretical assumptions made and methods used.

Keywords: Salivary cortisol, perceived stress, mastery, locus of control, sense of coherence, single time point measures, deviations measures, area under the curve, laboratory test, dexamethasone.

INTRODUCTION

Since the 1960s it has been virtually unanimously acknowledged that psychological stressors are among the most potent stimuli of the Hypothalamic-Pituitary-Adrenal (HPA) axis [1]. Although the importance of psychological factors for HPA axis activity is undisputed, research on associations between HPA axis activity and subjectively perceived stress as well as associations between HPA axis activity and different psychological resource constructs is inconclusive, and knowledge is still very limited.

The Perceived Stress Scale (PSS) [2] is a widely used instrument for measuring the perception of stress. It measures the degree to which situations in one's life were appraised as stressful during the last month. The purpose is to assess how unpredictable, uncontrollable, and overloaded respondents find their lives, by asking how often the respondent has experienced certain feelings and thoughts during the last month. Some items also cover queries about current levels of stress experienced. The questions are of a general nature and hence not specific to any subgroup population.

The concept of psychological resources refers to psychological factors perceived as potentially protective of well-being and health in the face of stressor exposure. The present literature study takes its departure point from four well-established psychological resource constructs: self-esteem, mastery, locus of control, and sense of coherence [3]. Self-esteem refers to a relatively stable sense of overall self-worth; a sense of being a person of value, and an acceptance of personal strengths and weaknesses [4]. The Concept of Locus of Control (LoC) refers to an individual's general beliefs regarding their ability to influence events [5]. Mastery refers to generalised beliefs of control in terms of "the extent to which people see themselves as being in control of the forces that importantly affect their lives" p. 340 [6]. Sense of Coherence (SOC) refers to a construct based on stress theory developed by Antonovsky, according to whom SOC represents a global orientation that reflects the extent to which stressors in the internal and external environment are

***Address correspondence to Christina Halford:** Researcher at the Department of Public Health and Caring Sciences, Uppsala University, Box 564, 751 22 Uppsala, Sweden; Tel: +46 18 471 6572; Fax: +46 18 471 6675; E-mail: christina.halford@pubcare.uu.se

Margareta Kristenson, Peter Garvin and Ulf Lundberg (Eds)

perceived as (1) structured, predictable and comprehensible, (2) challenges worthy of engagement and investment, and (3) the extent to which internal or external resources needed to handle stressors are perceived as available [7].

AIM

The aim of the present chapter was to review existing literature on association between measures of cortisol in saliva and psychological constructs in terms of perceived stress and psychological resources (self-esteem, mastery, LoC, SOC). The evaluation against different measures of saliva cortisol is based on the question of whether seemingly divergent results may be functions of differences in methods used and theoretical assumptions made.

METHOD

Electronic searches were performed in PubMed and PsychInfo data bases, covering the period up to October 1, 2009. Searches on perceived stress were based on the following search terms: (cortisol AND saliva* AND "perceived stress"). The search terms used for psychological resources were (cortisol AND saliva* AND self-esteem, "locus of control", mastery, "sense of coherence"). English-language, full-length articles, published in peer-reviewed journals, based on adult study populations, reporting direct statistical analyses of associations between cortisol in saliva and measures of perceived stress or psychological resources were included. Results based on patient populations, or with the primary aim of investigating associations in pregnant women, were excluded. When studies included analyses of healthy control groups, results from analyses relating to the healthy control groups were included.

Searches based on the search terms (cortisol AND saliva* AND "perceived stress") generated 95 papers. For perceived stress, only papers measuring perceived stress based on the PSS [2] were included. Based on a review of the titles and abstracts, and when relevant by reading the full-length article, 18 papers were finally included.

Searches on associations between cortisol in saliva and psychological resource constructs based on the search terms (cortisol AND saliva* AND self-esteem, "locus of control", mastery, "sense of coherence", respectively) generated 54 papers. Based on a review of the titles and abstracts, and when relevant by reading the full-length article, based on the inclusion and exclusion criteria, 11 papers were finally included.

In the following analyses, findings were considered significant if p values were <0.05. As most studies involved small numbers of participants and thus seemingly low statistical power, we also included marginally significant results ($0.05<p<0.10$) if they were reported; these are denoted by arrows in parentheses in the tables.

RESULTS

Perceived Stress

In total, 18 articles on possible associations between cortisol in saliva and the PSS fulfilled inclusion criteria [8-25]. A brief summary of the results (indicated as a positive association, a negative association, or a nonsignificant finding) is presented in Table **1a**. Study design, statistical approach, main results and discussion for each of the 18 articles are presented in Table **2a**.

Generally, few significant associations were found between PSS and cortisol in the papers examined. Of the 18 articles, six found significant associations, three articles reported trends towards significant associations ($0.05<p<0.10$) and nine failed to find any significant associations between PSS and cortisol measures. Most studies reported more than one measure of cortisol, ranging from one to four.

For single time points, 1 out of 14 analyses showed significant results. The significant finding was seen for samples taken at midday, whereas three other studies showed nonsignificant findings, *i.e.*, 1/4 (25%) of midday single measures showed significant results. Among deviation measures, all of which measured diurnal deviation, 2/13 results were significant and another two marginally significant. Thus, among the

nine measures of diurnal variation, four or 44% did report significant or marginally significant results. For measures of Area Under the Curve (AUC), 3/8 (38%) analyses showed significant results; morning (increase), 1/2 (50%); morning (ground), 2/2 (100%); morning-evening (increase), 0/2 (0%); and laboratory (increase), 0/1 (0%). Only one study was included with results from a dexamethasone challenge test, reporting 1/1 (100%) significant result.

Table 1a: Summary of main findings of associations between measures salivary cortisol and Perceived Stress Scale (PSS) sorted by year of publication

References	Year	Expo	Design	No. cortisol	m/w	Single time points (or sum/mean of two or more time points)					Deviation (difference/ slope between two or more time points)					AUC								Dexamethasone Suppression test
						a1	a2	a3	a4	a5	b1	b2	b3	b4		c1		c2		c3		c4		d
Perceived stress																								
Van Eck [8]	1996	PSS10	Exp	87	87/0									0								0		
Van Eck [9]	1996	PSS10	C-S	87	87/0								0											
Pruessner [10]	1999	PSS	C-S	66	24/42						0													↑
Nicholson [11]	2000	PSS10	C-S	59	59/0		0			0														
Edwards [12]	2003	PSS	C-S	36	10/26						0		0				↑				0			
Schwarz [13]	2003	PSS14	C-S	75	20/55		0																	
Abercrombie [14]	2004	PSS	C-S	31	0/31					0			(↑)											
Tull [15]	2005	PSS10	C-S	53	0/53								↓[a]											
Thorn [16]	2006	PSS14	C-S	48	8/40						0						↓							
Putterman [17]	2006	PSS14	C-S	170	0/170		0	0					0											
Gallagher-Thompson [18]	2006	PSS	C-S	45	0/45		0	0	0				0											
Wahbeh [19]	2008	PSS	C-S	15+15	11/19		(↑)																	
Faraq [20]	2008	PSS	C-S	78	0/78								↓											
Lasikiewicz [21]	2008	PSS	C-S	147	68/79					0			0							0				
Simpson [22]	2008	PSS	C-S	41	23/18			0	0															
O'Connor [23]	2009	PSS	C-S	118	0/118			↓[b]								↓								
Schulze [24]	2009	PSS	C-S	21	12/19						0		(↓)											
Mondeli [25]	2009	PSS10	C-S	36												0				0				

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory test reactivity (first column)/recovery (second column); c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory test increase/ground; d, C-S, cross-sectional. Arrows in parentheses denoting marginally significant findings.

[a] Significant effect only for women with high internalized racism.

[b] Marginal effect (p=0.053), and significant (0.02 when suspected non-adherents were excluded).

Table 2a: Studies on PSS sorted by year of publication

References	Outcome	Study design/group characteristics	Sampling	Laboratory method and standardization in cortisol sampling	Statistical approach for cortisol measure	Statistical analysis, cortisol in relation to outcome	Results	Discussion
Van Eck 1996 [8]	PSS10 Upper (≥16, HS) vs lower (≤ 10, LS) tertile of PSS10 compared	Design: C-S/Exp No.:87 (42 HS, 45 LS) M/W: 87/0 Age: 42.1 years (27-57 years) Group: Male white-collar workers Excl: Serious chronic illness, endocrine disorder, or medications known to affect cortisol levels (*n*=316). 92 recruited, 5 excluded due to missing data	Days: 1 (exp) Samples per day: 4 (5) Times for sampling: Exp: (T1) upon arrival, (T2) after the 10 min preparation, (T3) after the 5-min presentation, and (T4) after 15-min relaxation. For 49 subjects a fifth saliva sample (T5) was taken 50 min after the first assessment Setting: Response to exp speech test (SIST)	Direct RIA	Measurement(s): b4. Repeated measures MANOVA with interaction c4. AUC response to speech task, baseline=T1 Cortisol data: Fifth root transformation (cortiso1 0.2)	Repeated measures MANOVA; time/group/time×group interaction Confounders: Baseline cortisol was included as covariate	No difference in response to speech test between high and low stress groups	Individual differences in current distress (especially anticipator distress) may be more important determinants of cortisol secretion than perceived stress level, which is a measure of more chronic distress
Van Eck 1996 [9]	PSS10 Upper and lower tertile (<10 or >16) of PSS10 compared	Design: C-S No.: 87 M/W: 87/0 Age: 42.1 years (27-57 years) Group: Male white-collar workers; 316 completed questionnaire. *n*=87 (41+46) subjects were recruited Excl: History of serious chronic illness, endocrine disorder, medications known to affect cortisol, treatment (past or current) for mental health problem	Days: 5 Samples per day: 10 Times for sampling: semi-random intervals of approx. 90 min, between 08:00 h and 22:00 h	Direct RIA	Measurement(s): b3. Slope from third-degree polynomial fitted curve Cortisol data: Five extreme cortisol values (>1200 ng/dl) were deleted before analysis	Multilevel model/hierarchical linear model with nesting levels: measurement level and person level Matched for age, marital status, household composition. Adjustment for alcohol, coffee, food intake, smoking, physical exertion, trait anger, psychosomatic symptoms, life events, chronic difficult, positive affect	The effect of perceived stress on cortisol slope was not significant. PSS by time of day interaction term was also non-significant	
Pruessner 1999 [10]	PSS14 High vs low stress according to median split in PSS	Design: No.: 66 M/W: 24/42 Age: 43.6 years (SD 9.5) Group: Teachers Excl: Medication except OC	Days: 2+1 dexamethasone 0.5 mg Samples per day: 4 Times for sampling: awakening and +15, 30, and 60 min	Time-resolved fluorescence immunoassay	Measurement(s): b1. Increase after awakening d. Dexamethasone	High vs low stress groups entered in ANOVAs. Three-way (group by day by time) within-subject ANOVAs with repeated measurements on two factors (day by time) Confounders: Tested: OC, alcohol, total hours of sleep, time of awakening, and self-report of health status (acute stress) sex, age, height, and body weight	High levels of perceived stress were associated with stronger increase of cortisol levels after awakening day after dexamethasone. No associations during days without dexamethasone	We suggest that a key factor for differentiation between burnout scale and PSS is the chronicity and exhaustion. The PSS asks for the ability to cope with the current stress load, and does not cover feelings of exhaustion. However, the larger increases of cortisol levels after dexamethasone seen in teachers who reported high levels of stress extends the recent

								findings to suggest a decreased feedback sensitivity in these subjects
Nicholson 2000 [11]	PSS10	Design: C-S No.: (29+30)=59 M/W: 59/0 Age: 51.1 (4.5)/52.2 (5.1) years Group: VE subjects + controls Excl: smokers, several diseases P rate: selected from 577/1600 (36.1%)	Days:2 Samples per day: 2/6 Times for sampling: Day 1: 21:30 h and 22:35 h Day 2: 06:55 h, 07:15 h,11:00 h, 16:00 h, 17:40 h and 19:00 h Setting: Day 1 SIST speech test, however analyses of cortisol-PSS did not include SIST response sample	Direct RIA, using an HPLC purified preparation of cortisol-3-CMO-histamine	Measurement(s): a5. All 7 samples (2 days) except second day 2 sample a2. Day 2 Cortisol data: Log-transformed	Multiple linear regression analysis Confounders: Introduced in model: VE, perceived stress, sleep quality, and current fatigue. (BMI, alcohol, coffee, and number of recent life events were unrelated to hormonal measures in preliminary analyses, hence not included in analyses)	PSS did not predict mean level of all measures or morning cortisol levels	Not discussed (not primary aim)
Edwards 2003 [12]	PSS	Design: C-S No.: 36 (34 included in analyses) M/W: 10/26 Age: 34 years (range 23-52 years) Group: Healthy students Excl: Psychiatric, neuroendocrine, eating disorder, medication that may have affected cortisol concentrations	Days: 2 Samples per day: 8 Times for sampling: awakening (0), +15, 30, and 45 min + 4 samples collected at 3-h intervals through the day	Instruction: Nothing by mouth other than water, not brush teeth, until after the fourth sample (45 min post awakening). For the remaining samples participants were instructed not to eat, drink, or smoke for at least 30 min before collection of samples	Measurement(s): b1. Mean increase (2-4)-1) b3. Slope 3-12 h post awakening c1. Morning (1-4) c3. 12-h excluding morning peak (1, 5-8) Median split of AUC/DAUC and mean increase Cortisol data: Square root transformed. Data +2 SD were excluded. Participants whose "difference between days" scores were +1 SD above the mean difference were excluded from further analyses	*t*-test, Pearson correlation, chi squared analyses undertaken to check that effect sizes were not sensitive to inclusion or exclusion of males, smokers, OC takers. No such sensitivity was found, thus results reported for total group	High morning AUC group showed higher PSS than low AUC group. No associations between cortisol and DAUC (AUC during 12-h), mean increase or slope	Not primary aim. The effect (AUC-PSS) was not apparent for groupings based on DAUC, suggesting the possible importance of absolute waking period values of cortisol for identifying individual differences in stress experience. Note that similar slope statistics, like any summary measure, can be the result of very different profiles
Schwarz 2003 [13]	PSS14	Design: No.: 75 M/W: 20/55 Age: 63 (15.3) years Group: Caregivers of family members with heart failure and non-caregivers Incl: English-speaking, no hospice client or cognitive impairment	Days:1 Samples per day: 1 Times for sampling: Mid-morning Setting: Caregivers and non-caregivers	Clinical assays gamma coat cortisol ^{125}I Kit (INCSTAR, Stillwater, MN). No smoking for 2 h before collection, rinse their mouths with tap water 10 min prior to sample collection	Measurement(s): a2. Cortisol data: Continuous or categorical (5 categories)	Correlation	No correlation between PSS and cortisol	PSS may not reflect current stressful events. One's ability to adjust to chronic stress is affected by its appraisal, and with adjustment to chronic stress, cortisol secretion no longer increases. Individuals who are socially supported may appraise stress as less threatening than those who are unsupported. Limitations: only one sample

Abercrombie 2004 [14]**	PSS	Design: C-S No.: 31 M/W: 0/31 Age: 56 years (SD 13) Group: Healthy controls (from case-control study of breast cancer) Excl: Incl: Age >30 years, not pregnant, no psychopathology, not treated with systemic corticosteroids, no prior history of cancer P rate: 9 excluded because of unwillingness	Days: 3 Samples per day: 4 Times for sampling: waking, 12:00 h, 17:00 h, and 21:00 h	EIA kits from Salimetrics	Measurement(s): a5. All 3×4 samples; 4×4?? b3. Diurnal slope Cortisol data: Sample time points >4 SD from the mean time for the respective time point were excluded. Because all raw values were in the physiological range (0.01-2.54 µg/dl) no data were excluded as outliers. Log-transformed cortisol data	Correlation cortisol-PSS	A tendency (r=0.32, p=0.07) of significant, positive, relationship between slope and PSS. No significant correlations with mean cortisol	These findings suggest that the cortisol diurnal slope may have important but different correlates in healthy women versus those with breast cancer. Among patients, no correlations were found between cortisol and PSS among patients
Tull 2005 [15]	PSS10	Design: C-S No.: 53 M/W: 0/53 Age: 36.9 (9.2)/38.3(9.3) years Group: African-Caribbean women with high or low levels of INR Excl: Diabetes Incl: 25-60 years P rate: Recruited from 244/317 (77%)	Days: 1 Samples per day: 2 Times for sampling: 08:00 h, 22.30 h	Not mentioned ("reference laboratory")	Measurement(s): b3. Morning-evening	Spearman correlation. Partial correlations adjusted for: age + education, and coping (BDC)	Zero order Rs = -0.32, p = 0.022 (higher stress = flatter curve). Stratified analyses (high vs low level of INR) showed sign only for women with high INR. Partial correlation also significant	Only discussed difference among women with high or low INR. Metabolic abnormalities develop over time with continued exposure to an environment that affects cortisol secretion. It is likely that a high level of INR over an extended period of time is needed for the significant relationships among PSS and cortisol to occur
Thorn 2006 [16]	PSS14	Design: C-S No.: 48 (2 dropouts) M/W: 8/40 Age: 20.5 years (SD 3.9) (range 18-36 years) Group: Psychology students Excl: Medications, acute or chronic illness	Days: 4 (2 work days, 2 weekend days) Samples per day: 4 Times for sampling: awakening, +15, 30, 45 min	ELISA. No food, only water, no smoke no brush of teeth	Measurement(s): b1. Mean increase (morning) c1. Cortisol data: 4 day mean of each measure. Square root transformed	Correlation. Multiple regression Confounders: Awakening time, state stress? (not described in detail)	PSS and mean cortisol AUC correlated inversely (r = -0.382, p = 0.011). PSS significant independent predictor of cortisol AUC in multiple regression analyses (negative sign). Mean increase did not correlate with PSS scores	Not primary aim. Negative correlation (as opposed to positive) depending on more females? Previous studies have shown negative associations among women and results for women and men may differ. The unequal number of men/women did not allow for stratified analyses. Supplementary analyses excluding non-responders (suspected non-adherence) showed

								similar effect sizes
Putterman 2006 [17]	PSS14	Design: C-S No.: 170 M/W: 0/170 Age: 20.4 (3.2) years Group: Undergraduate students Excl: Medication that may affect cortisol, night shift	Days:1 Samples per day:2 Times for sampling: 30 min post awakening, +6-8 h Setting: Undergraduate students examined concerning dietary restraint and stress	Time-resolved fluorescence immunoassay with a cortisol-biotin conjugate as a tracer Instructions: Refrain from brushing teeth, eating or drinking prior to sampling. Asked if they had deviated from instructions, and whether there were any unusual circumstances surrounding sampling	Measurement(s): a2. a3. +6-8 h b3. Cortisol data: >+6 SD from mean excluded	Correlation	No significant correlation between cortisol and PSS	Not primary aim. Given that the PSS assesses stress levels over the previous month, while the cortisol sampling may reflect changes in HPA activation over the last half hour or so, the PSS may tap stress more generically, and may not be sensitive to the more subtle, insidious demands associated with cognitive dietary restraint.
Gallagher-Thompson 2006 [18]	PSS14	Design: C-S No.: 45 M/W: 0/45 Age: 40-70 years Group: Caregivers and non-caregivers Incl: At least high school education P rate: Selected (see inclusion) from *n*=83	Days: 3 Samples per day: 3 Times for sampling: 08:00 h, 17:00 h, 21:00 h	RIA + EIA	Measurement(s): a2. a3. a4. b3. Slope Cortisol data: Log-transformed	Regression	PSS did not predict log cortisol values at any time point, or the slope over day	Not primary aim, association between PSS and cortisol not discussed Limitations: Small sample, no information about overall health status, lifestyle factors. etc
Wahbeh 2008 [19]**	PSS	Design: C-S No.: 15+15 M/W: Age: Group: Caregivers for Alzheimer disease patients and non-caregivers Excl: Non-caregivers, medication known to affect central nervous system function, significant psychiatric disease, insulin-dependent diabetes, uncontrolled hypertension, and other significant medical illnesses. Four caregivers excluded due to diabetes	Days: Samples per day: 5 Times for sampling: <5 min post awakening, +30 min, before lunch, 1 h after lunch, 23:00 h	Commercial enzyme-linked immunoassay kit (Active Cortisol EIA). No food, citrus, alcohol, or tobacco for 1 h before sampling	Measurement(s): a2. Only +30 min values presented Cortisol data:	Correlations	At 30 min after waking time, trend for significant, positive, relationship between PSS and cortisol among caregivers; $r = 0.44$, $p = 0.10$	The PSS examines perceptions of stress rather than actual stress events
Faraq 2008 [20]	PSS	Design: C-S No.: 78 M/W: 0/78 Age: 46 years (range 24-72 years) Group: Employees at	Days: 1 Samples per day: 7 Times for sampling: Wakeup, wakeup + 40 min, 11:00 h, 14:00 h, 18:00 h, 21:00 h, and	Competitive protein-binding enzyme immunoassay	Measurement(s): b3. Morning peak (awakening + 40) - mean evening (21:00 h and bedtime) Cortisol data: Log-	Linear regression Confounders: BMI, waist circumference, age, SES included in model, medication use and comorbidities (as present vs absent)	Perceived stress predicted impairment of normal diurnal cortisol rhythm, seen in a reduced morning to evening cortisol difference in the sample	Chronic stress, with its characteristic repeated and prolonged cortisol peaks, causes a rigid cortisol secretion pattern with reduced daily variation. Thus,

		primary school P rate: From larger study sample (*n*=202)	bedtime		transformed		as a whole. Stress predicted approximately 11% of the variability in diurnal cortisol variation	the peak nadir difference in cortisol levels is smaller in individuals exposed to high levels of chronic stress. The resultant loss of the magnitude of the peak nadir variation is seen as a degraded signal and an indicator of HPA axis dysfunction
Lasikiewicz 2008 [21]	PSS10	Design: C-S No.: 147 M/W: 68/79 Age: 46.2±7.18 years Group: Healthy adult volunteers Excl: Prescribed medication, smokers P rate: 180 recruited, 33 failed	Days: 1 (*n* = 64) or 3 (*n* = 83) days Samples per day: 8 Times for sampling: 0, +15, 30 and 45 min and at +3, 6, 9 and 12 h post awakening	Non-commercial time-resolved fluorescence (DELFIA) immunoassay	Measurement(s): a5. Mean of measures over the day from sample 3 (45 min post awakening) b3. Slope from sample 3 (45 min post awakening) c3. Cortisol data: Log-transformed	K-means cluster to extract profiles. ANOVA for PSS between clusters. Linear regression Confounders: Age, gender included in model	No sign associations between PSS and cortisol	Participants were not selected on the basis of their perceived stress score and as a result, the sample were not suffering extreme stress levels compared with those of previous studies
Simpson 2008 [22]	PSS	Design: C-S No.: 41 M/W: 23/18 Age: 61.8 years (SD 4.8) (range 55-69 years) Group: Recruited from community organizations serving older adults in Northern Ireland Excl: Smokers (>10/d), medication that might affect cortisol or mood, BMI >35, physical or mental health problems, depression or dementia P rate: 50 asked to participate, 3 declined, 6 did not leave sufficient number of samples	Days: 7 consecutive days Samples per day: 2 Times for sampling: 02:30 h and 22.30 h	ELISA, sampling at least 1 h after eating	Measurement(s): a3. a4. Cortisol data: 10 log-transformed. Cortisol normal reference ranges was taken as between 4 and 28 nmol/L and extreme cortisol concentrations were assumed to be those > 44 nmol/L	Pearson's bivariate correlations Confounders: See exclusion criteria (none excluded)	There was no association between salivary cortisol levels and perceived stress	Results from analysis PSS-cortisol mentioned only in abstract
O'Connor 2008 [23]	PSS10. High (PSS >14) or low (PSS <14) stress, were compared	Design: C-S No.: 118 M/W: 0/118 Age: 49.4 (5.8) years Group: HS (*n* = 70) vs LS (*n* = 48) stress Excl: Significant current or past medical history; hormonal disorders; steroid-based medication or recent users of	Days: 2 Samples per day: 8 Times for sampling: awakening, + 15, 30, and 45 min, and 3, 6, 9 and 12 h post awakening	Auto DELFIA (PerkinElmer)	Measurement(s): a3. 3, 6, 9 and 12 h c1. Cortisol data: Log-transformed, mean of 2 days for each sampling time. Suspected non-adherence. No increase in cortisol between awakening and +15 or 30 min, on any of the days	Linear regression Confounders: Age, waist/hip ratio and educational attainment included as covariates. Menopause status did not differ between groups	Significant group difference. AURC measure ($p = 0.023$); HS secreted lower levels of cortisol compared with LS (largely dependent on +30 min sampling). Indication of a main effect for the cortisol diurnal mean ($p = 0.053$), HS group	Psychological stress may influence aspects of HPA axis regulation leading to a reduction in the release of cortisol throughout the day. These alterations may be determined by improved sensitivity to the negative feedback of glucocorticoids or by a reduction in the

		recreational drugs, psychiatric history			(12 LS, 17 HS)		tended to secrete less cortisol throughout the day. When suspected non-adherent excluded, effect sign ($p = 0.02$)	release of key hormones and releasing factors such as corticotrophin releasing factor from the hypothalamus. Limitations: HS and LS groups recruited separately, may have resulted in samples from populations that may differ in unknown way
Schulze 2009 [24]	PSS	Design: C-S No.: 21 M/W: 12/9 Age: 53 years (SD 14.6) Group: Colorado ranchers P rate: 105 invited	Days: 9 (3 days per 2 week period), 3×2 weeks (representing high, medium, and low stress) Samples per day: 3 Times for sampling: Awakening, +30 min, before retiring	Laboratory method: high-sensitivity commercial EIA kit (Salimetrics) Sampling using the Saliva Procurement and Integrated Testing (SPIT Book). Trident Original Flavor gum for stimulating saliva flow	Measurement(s): b1. b3. Daytime cortisol decline	Correlation, Bonferroni adjustment	No significant associations. Marginal significant effect of smaller daytime cortisol decline and higher PSS scores ($r = -0.37$, $p = 0.053$)	Not primary aim, and no discussion about this. General discussion: the small sample size, low response rate. most of the participants lived in one town
Mondeli 2009 [25]**	PSS10	Design: C-S No.: 36 M/W: 26/10 Age: 27.3 years (SD 0.8) Group: Healthy controls	Days: 1 Samples per day: 5 Times for sampling: Awakening, +15, 30 and 60 min, 12:00 h, 20:00 h	Immulite DPC's immunoassay analyzer Instructions: To wake up before 10:00 h, first sample while still in bed, no breakfast or brushing teeth during the first hour of awakening, and in the 30 min before taking the sample at 12:00 h and 20:00 h	Measurement(s): c1. c3.	Correlation Pearson's *r*	No significant correlations were found between perceived stress and cortisol levels, either diurnal or in the awakening response	

Analyses/methods/study design described in relation to relevance for examinations of association between PSS and cortisol only. May differ from main study presentation.

* Association between cortisol and PSS was not the primary aim; the results are presented only for analyses regarding this association.

** Analyses/results only presented for healthy subgroups, and regarding relation between PSS and cortisol.

Abbreviations: AURC, Area under response curve; BMI, body mass index; DAUC, Day(time) area under curve (12 hours); EIA,enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; HPLC, high performance liquid chromatography; HS, high stress; INR, internalized racism; LS, low stress; MANOVA, multivariate analysis of variance; OC, oral contraceptives; RIA, radioimmunoassay; SES, socioeconomic status; SIST, Stress Inducing Speech Task; TBS,:VE, vital exhaustion.

The specific findings among significant results were as follows. The only significant finding for a single time point was when groups with high and low PSS were compared; a lower mean cortisol level in the afternoon was found for the high PSS group [23]. With regard to diurnal deviation measures, in a study of school employees and a study including African-Caribbean women, high PSS levels were associated with a flatter cortisol curve [15, 20]. In line with these results, in a study of ranchers high PSS was related to smaller daytime decline [24]. However, this finding had marginal statistical significance (p=0.053), and the instrument used was not the typical PSS scale. In contrast with these results, in a study on a group of healthy women (control study group), a tendency (p=0.07) to a positive relationship between slope and PSS was seen [14].

In a study of psychology students [16], morning AUC with respect to ground was inversely and independently related to PSS. Similarly, in a normal population sample, the high PSS group had lower cortisol secretion during the morning (AUC with respect to increase) compared with a group with low PSS [23]. In contrast, in a group of healthy students, persons with higher AUC during morning hours reported significantly more stress on the PSS measure [12]. In a group of teachers, high levels of perceived stress were associated with higher cortisol levels after awakening on the day after dexamethasone intake, but no associations during days without previous dexamethasone intake [10].

Psychological Resources

Eleven articles fulfilled the inclusion criteria [26-36]. The proportion of significant associations (in any direction) observed in the 11 papers investigating associations between different psychological resource construct variables and cortisol was 1/9 (11%) for single measures, 2/4 (50%) for standardized baseline measures, 4/22 (18%) for deviation measures, and 3/8 (37.5%) for AUC measures. A brief summary of the results (indicated as a positive association, a negative association, or a nonsignificant finding) are presented in Table **1b**. Study design, statistical approach, main results and discussion for each of the 11 articles are briefly presented in Table **2b**.

Questionnaire for Competence and Control

Associations between combined measures of self-esteem and locus of control (SEC) were investigated in three studies, based on study populations comprising 16 men, 20 men, and 52 women and men, respectively [26, 27, 31].

One study examined associations between SEC and cortisol AUC in relation to a laboratory stress test in healthy students. An inverse association between cortisol AUC and the subscale "self-concept" (r=-0.69, p=0.003), and a positive association between cortisol AUC and the subscale "chance" (r=0.51, p<0.05) were observed. There were no significant associations between cortisol AUC and the subscales "internality" or "powerful others" [26].

Two studies investigated deviations in cortisol levels during a laboratory stress test in relation to SEC in healthy young men [27, 31]. One study reported a significant inverse effect of SEC on cortisol reactivity, *i.e.*, low SEC was associated with a stronger cortisol response [31]. In the other study, a significant negative effect of self-esteem on cortisol levels was observed only in a low self-esteem/failure condition subgroup [27].

Table 1b: Summary of main findings of associations between measures salivary cortisol and psychological resources sorted by year of publication. Note; For mastery and self esteem, first column under b4 denotes baseline levels before stress

References	Year	Expo	Design	No. cortisol	m/w	Single time points (or sum/mean of two or more time points)					Deviation (difference/slope between two or more time points)						AUC								Suppression test
						a1	a2	a3	a4	a5	b1	b2	b3	b4			c1	c2	c3	c4					d
Self-esteem and locus of control (questionnaire of competence and control)																								
Pruessner [26]	1997	SEC scales	C-S	20	M																			
		SC																						↓

		INT																					0	
		POC																					0	
		FAT																					↑	
Pruessner [27]	1999	SEC	C-S/E	52	m/w																			
		Failure												↓										
		Non-failure												0										
Pruessner [31]	2005	SEC	C-S/E	16	M									↓										
Locus of control																								
Gregg [28]	1999	tress test	C-S/E	100	m/w																			
		1. Mental arithmetic												/0										
		2. Cold pressor												/↓										
Bollini [30]	2004	LOC	C-S/E	48	m/w																			
		Control												↑										
		Non contro												0										
Mastery																								
Kristenson [32]	2005	Mastery	C-S	183	M									↓	0									
Cohen [33]	2006	Mastery	C-S	781	m/w			0	0				↑									0		
Sjögren [34]	2006	Mastery	C-S	257	m/w	↑	0		0		0		↑											
Vedhara [35]	2006	Mastery	C-S	59	W						0		0								0	↓		
Self-esteem																								
Wüst [29]	2000	Self-esteem	C-S	208	m/w						0							0						
Kristenson [32]	2005	Self-esteem	C-S/E	183	M									0	0									
Sjögren [34]	2006	Self-esteem	C-S	257	m/w	0	0		0		0		0											
Quirin [36]	2008	Self-esteem	C-S	48	W	0					0			0	0									
Sense of coherence																								
Kristenson [32]	2005	SOC	C-S/E	183	M									↓	0									

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory test reactivity (first column)/recovery (second column); c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory test increase/ground; d, C-S, cross-sectional; E Experimental; SC; Positive self-concept; INT internality, POC Powerful others; FAT Chance.

Table 2b: Studies on psychological resources sorted by year of publication

References	Outcome	Study design/group characteristics	Sampling	Laboratory method and standardization in cortisol sampling	Statistical approach for cortisol measure	Statistical analysis, cortisol in relation to outcome	Results	Discussion
Pruessner 1997 [26]	SEC (questionnaire of competence and control; Krampen 1991) Subscales: 1. Positive self-concept (SC) 2. Internality (INT) 3. Powerful others control (POC) 4. Chance (FAT)	Design: C-S No.: 20 M/W: 20/0 Age: 22.4 years (SE 2.1) Group: Non-smoking, drug-free students, who reported good health Excl: - P rate: -	Days: 5 Samples/day: 6 Times for sampling: Late afternoon at 10-min intervals starting after an initial 10-min rest period Setting: Laboratory, stress test TSST, 1 h	Salivette (Sarstedt) time-resolved fluorescence immunoassay (DELFIA) Instructions: abstain from physical exercise ≥1 h before session, and refrain from drinks with low pH or larger meals immediately before start of session	Measurements: c4. Single AUC, and mean aggregated AUC Cortisol data: - Samples used: All	Spearman rank correlations between psychological variables and single and aggregated cortisol AUC. Cortisol AUCs were aggregated by summation of single cortisol AUCs	c4. There was a significant negative association between subscale SC ($r = -0.69$, $p = 0.003$) and FAT ($r = 0.51$, $p < 0.05$) and mean AUC days 2-5. There were no significant associations between subscales INT, or POC and cortisol AUC	Results suggest an association between certain psychological variables and cortisol responses after repeated exposure to psychosocial stress. Data aggregation may have led to the identification of a trait component of the state variable stress response
Pruessner 1999 [27]	SEC	Design: C-S No.: 52 M/W: 23/29 Age: 22.9 years (SD 2.8) Group: Students. Subgroups: Success/high SE (n = 14) Success/low SE (n = 12) Failure/high SE (n = 14) Failure/low SE (n = 12) Excl: Current infection, medical treatment (not OC)	Days: 1 Samples/day: 5 Times for sampling: Session start between 15:00 h and 17:00 h. Baseline (after rest), +15, 25, 35, 45 min after start of stress test Setting: Laboratory, two versions of the Trier Mental Challenge Test: (1) success and (2) failure condition	Salivette (Sarstedt) time-resolved fluorescence immunoassay (DELFIA) Instructions: not to consume large meals or low pH drinks 1 h prior to session	Measurements: b4. Cortisol data: - Samples used: All	Scores from 4 subscales aggregated to one scale of self-esteem and locus of control. Scores higher/lower than population average assigned to high/low SEC group, Spearman rank correlation 3-way ANCOVA, test of interaction effects Covariates: gender, smoking	b4. There was no significant effect of SEC on cortisol stress levels. Interaction between version of TNCT and SEC on cortisol was tested: SEC was inversely related to cortisol stress levels if the subject was in the failure condition	Main aim was to investigate the interaction of a personality scale assessing SEC and a success or failure condition on cortisol response to stress
Gregg 1999 [28]	Locus of control [5]	Design: C-S No.: 100 M/W: 50/50 Age: Mean 20 years, range 17-46 years Group: Healthy, normotensive volunteers Excl: Smoking, use of OCs, history of CVD or circulatory disorders P rate: -	Days: 1 Samples/ day: 5 Times for sampling: 40 min before stressor (baseline) +25 min after each of the two stressors (stimulation measure) and 45 min after each of the two stressors (recovery measure) Setting: Laboratory, stress test: mental	RIA (Coat.A-Count)	Measurements: b4. Change score in relation to stressor exposure, for two different stress tests Cortisol data: - Samples used: All five	Correlational analyses. Change scores obtained by subtracting mean baseline score from mean observed stimulated cortisol score for each stressor	b4. There was no association between locus of control and changes in cortisol levels induced by the mental arithmetic test. There was a significant positive association between locus of control and cortisol change score in response to cold pressor test, with higher scores for external locus of control associated with larger cortisol changes ($r = 0.22$, $p < 0.05$)	Main aim was to investigate hemodynamic profile during both active and passive tasks

			arithmetic and cold pressor test					
Wüst 2000 [29]	Self-esteem (Rosenberg)	Design: C-S No.: 208 M/W: 94/114 Age: 19.6 (8-64) years Group: Medication-free (except for OCs) adult and child twins Subgroup: High vs low self-esteem Excl: - P rate: -	Days: 2 Samples/day: 8 Times for sampling: 1. Cortisol response to awakening: awake, +30, 45, 60 min 2. Day-time cortisol: 08:00 h, 11:00 h, 15:00 h, 20:00 h Setting: Ambulatory, weekend	Salivette (Sarstedt) time-resolved immunoassay with fluorescence detection (DELFIA) Instructions: not to brush teeth before having completed morning sampling. Food intake 10 min before sampling and smoking on test days was not allowed	Measurements: b1. Mean increase in cortisol after awakening c1. AUC awakening response Cortisol data: - Samples used: Morning samples	Pearson correlations. 3-way ANOVA. High vs low self-esteem groups based on median split (ANOVA)	b1, c1. No significant associations between self-esteem and mean increase in cortisol after awakening or AUC b1. No significant interaction: time × self-esteem group (high vs low)	Aim was to investigate genetic influence on cortisol response to awakening, and the relationship between several psychological variables and early morning cortisol levels
Bollini 2004 [30]	Locus of control. ANSIE	Design: C-S No.: 48 M/W: 7/41 Age: 19.4 years (SD 1.3) Group: Students Subgroup: Categorized as believers/non-believers based on experience of control in PC condition/belief that they were effective in reducing aversive noise Excl: - P rate: -	Days: 1 Samples/day: 12. 4 baseline samples, +1 baseline sample before and 3 stress induction samples +6, 12, 18 min after each stress induction Times for sampling: 09:00 h Setting: Laboratory stress test × 2: noise + math test (PASAT) with (PC), and without (NC), control option	Lemon crystals to induce saliva. Cotton ball, placed in specimen tube. Material and procedures by Incstar Corp. Asked to refrain from alcohol, caffeine, smoking and exercising rigorously beginning from night before participation	Measurement: b4. Reactivity: change in cortisol from baseline to post-stress-induction task Cortisol data: - Samples used:	Correlations	b4. Participants did not differ in cortisol levels as a function of their LoC orientation ($r = -0.28$, $p = 0.08$). When only believers ($n = 31$) were included in analyses, individuals with more internal LOC had a more pronounced cortisol decline in the PC condition ($r = 0.29$, $p < 0.01$). In NC condition there were no differences in cortisol related to LOC scores. Those with more internal LOC appeared to be less biologically stressed in the PC condition	Main aim to investigate influence of perceived control on biological and subjective stress responses. LOC perceived as a potential moderator
Pruessner 2005 [31]	Self-esteem (Rosenberg), locus of control (Krampen 1991)	Design: C-S No.: 16 M/W: 16/0 Age: 20-26 years Group: healthy Subgroup: High SEC: High self-esteem and locus of control scores Low SEC: vice versa Excl: current depression, history of psychiatric illness or head trauma P rate: -	Days: 1 day Samples per day: 7 Times for sampling: -30, -20, 0, 20, 40, 50, 60 min in relation to onset of MIST Setting: Laboratory, stress test (MIST)	Salivette time-resolved fluorescence immunoassay	Measurements: b4. Cortisol data: - Samples used: All 7 samples	Each sample was split into groups with high or low scores on self-esteem and internal locus of control: referred to as high vs low SEC. k-means cluster analysis. ANOVA	b4. There was a significant main effect of SEC on cortisol stress response. Low SEC individuals showed a significantly larger cortisol response to a stressful situation ($F = 6.53$, $p < 0.05$). Self-esteem correlated highly with internal locus of control ($r = 0.67$, $p < 0.001$), but not with external locus of control ($p = >0.20$)	Low self-esteem and locus of control predicted a higher cortisol response when exposed to a psychosocial stressor
Kristenson 2005 [32]	Mastery, self-esteem (Pearlin 1978), sense of	Design: C-S No.: 183 M/W: 183/0	Days: 1 Samples per day: 2 Times for sampling:	RIA (Coat-A-Count). Participants came fasting after a night's sleep	Measurements: b4. Baseline levels in relation to stress	Partial correlation analyses Confounding/covariates: Residence, starting time of stress	b4. Significant negative correlation between SOC, and mastery with baseline cortisol	Main aim to investigate associations between self rated health and

	coherence (Antonovsky 1984)	Age: 50 years Group: Population-based, random sample Excl: Systolic BP >160 mmHg, diastolic BP >105 mmHg, acute MI and stroke <3 months prior to test, unstable angina pectoris, untreated diabetes P rate: 183/210 included in stress test	Baseline (after pre-test rest) and 40 min after start of stress test Setting: Laboratory, weekday TSST: Stress test start at either 07.30 h or 09.30 h, weekday		test, and arithmetic difference between cortisol stress response and baseline levels Cortisol data: - Samples used: All samples	test, smoking	levels (r = -0.18 and -0.16, p = 0.02 and 0.04, respectively), but no significant association between self-esteem and cortisol at baseline. There were no significant correlations between SOC, mastery or self-esteem, and cortisol change score at +40 min	psychosocial resources, and psychosocial strain
Cohen 2006 [33]	Mastery (Pearlin 1978)	Design: C-S No.: 781 M/W: 328/453 Age: 39.95 years (SD 3.65) Group: Randomly selected Excl: Blind, deaf, mute, retarded, unable to walk on treadmill, pregnant, awakening after 11:00 h P rate: 838/1336 invited agreed to participate (62.6%)	Days: 1 Samples per day: 6 Times for sampling: Awakening, +45 min, 2.5 h, 8 h, 12 h after awakening, and at bedtime Setting: Ambulatory, weekday	Salivette, cotton roll (Sarstedt). Time-resolved immunoassay with fluorometric end point detection Instructions: Not to eat, brush teeth or drink liquid for at least 15 min before sampling. Provided with alarm watches to remind them to collect samples, instructed to record awakening and sampling times	Measurements: a3. 8 h after awakening a4. 12 h after awakening and right before going to bed b3. Diurnal slope calculated by fitting a linear regression line c3. AUC Cortisol data: Log transformed Samples used: 8 h, 12 h, and bedtime	Partial correlations Covariates: Sex, race, age, BMI, awakening time, time since awakening	a3, a4. There were no significant associations between mastery and cortisol sampled 8 h or 12 h after awakening, or just before bedtime b3. There was a significant inverse association (r = -0.12, p < 0.05) between mastery and diurnal slope c3. No significant association between AUC and mastery	Main aim to investigate associations between SES and cortisol
Sjögren 2006 [34]	Mastery, self-esteem (Pearlin 1978)	Design: C-S No.: 257 M/W: 129/128 Age: 30-64 years Group: Random population-based sample Excl: P rate: 61% response rate in initial health survey. Random sample of 400 individuals invited. 64.5% responded (257/400)	Days: 3 Samples per day: 3 Times for sampling: Awakening, +30 min, before going to bed Setting: Ambulatory, workdays	Salivette (Sarstedt) time-resolved fluorescence detection Instructions: Fill in exact time of sampling and awakening. Instructions on fasting	Measurements: a1. Awakening a2. +30 min after awakening a4. Evening b1. Arithmetic difference between awakening, and +30 min samples b3. Arithmetic difference between awakening and +30 min, respectively, and evening values Cortisol data: Log-transformed Samples used: All samples	Partial correlation analyses. Mean values of the three days, for each sampling time Cortisol: Log-transformed Confounders: Age, gender, awakening time, regular medication, smoking, alcohol entered as covariates	Mastery: a1. Significant positive association between mastery and cortisol at awakening (r = 0.13, p < 0.05) a2, a4. No significant associations between mastery and cortisol at +30 min, and evening sample) b1. No significant association between mastery and awakening response b3. Significant positive association between mastery and diurnal deviation measured as difference between awakening and evening (r = 0.13, p < 0.05), but not when measured as +30 min after awakening-evening sample (p < 0.10) Self-esteem**:** a1, a2, a4, b1, b3. No significant	In population-based sample of middle-aged women and men, higher mastery was associated with a steeper diurnal cortisol slope

							association between self-esteem and cortisol in group as a whole b3. In women, but not in men, there was a direct significant correlation between self-esteem and diurnal deviation (awakening-evening) ($r = 0.21$, $p = 0.02$)	
Vedhara 2006 [35]	Mastery (Pearlin 1978)	Design: C-S No.: 59 M/W: 0/59 Age: 53 years (SD 10) Group: Healthy controls randomly sampled Excl: History of cancer, samples collected more than 30 min outside of specified time P rate: 59/64 who agreed to participated	Days: 2 Samples/ day: 4 Times for sampling: Awakening, +30 min, before lunch, late at night (at least 2 h after evening meal) Setting: Ambulatory	Salivette (Sarstedt). RIA. Asked to refrain from eating or drinking within 30 min of sampling and to note on each salivette exact time of sampling	Measurements: b1. Difference cortisol +30 min-awakening b3. Linear slope calculated c3. AUC_{ground}, $AUC_{increase}$ Cortisol data: Log-transformed Samples used: All	Pearson's correlations. Linear regression: a linear slope was calculated for all participants with 3 measures (awake, before lunch, and evening)	b1, b3. There were no significant associations between mastery and awakening cortisol or diurnal cortisol rhythm c3. There was a significant negative association between mastery and AUC_{ground} ($r = -0.29$, $p < 0.05$), but no significant association between $AUC_{increase}$ and mastery	Greater mastery was associated with lower basal cortisol levels over the day (AUC_{ground}). Literature on the HPA axis activity and mastery is limited. Results underscore the differing nature of the selected cortisol indices. There was a trend for trait measures to achieve prominence over mood measures
Quirin 2008 [36]	Self-esteem (Rosenberg)	Design: C-S No.: 48 M/W: 0/48 Age: 33.9 years (SD 8.4) Group: Working non-menopausal women on OCs Excl: Smoking, cortisone or psychotropic medication, psychiatric disorders, alcohol abuse, symptoms of common cold, age <20 or >45 years P rate: -	Days: 2 + 1 Samples per day: (1) 2 days: 5/day (2) Stress test: 2 samples/1 day Sampling times: (1) Awakening, +30, 45, 60, 75 min (2) Stress test start 14:00 h: 20 min before and 25 min after onset of stress test Setting: Ambulatory, workdays, and laboratory stress test (exposure to uncontrollable noise)	Salivette (Sarstedt) time-resolved immunoassay with fluorescence detection. Electronic drug exposure monitor for monitoring sampling time. Asked not to brush teeth, have breakfast during the sampling period, or to rinse mouth after eating or drinking and waiting 5 min before taking a sample	Measurements: a1. Awakening b1. Cortisol response to awakening: (max individual cortisol increase during 1st hour after awakening) b4. Baseline and increase in cortisol response to stress test Cortisol data: Samples used:	Spearman correlation	a1, b1, b4. There were no significant correlations between self-esteem and cortisol at awakening, increase after awakening, at baseline before stress test, or with cortisol increase in response to stress test	Main aim of study was to investigate associations between attachment styles and cortisol

Abbreviations: ANSIE, Adult Nowicki Strickland internal external control scale; BP, blood pressure; MI, myocardial infarction; PC, Perceived control; SEC, self-esteem and locus of control (questionnaire of competence and control; Krampen 1991).

Locus of Control

Analyses of associations between LoC and cortisol were investigated in two studies, based on study populations of 100 and 48 healthy volunteers and students (women and men), respectively [28, 30].

Both studies investigated cortisol response to a laboratory stress test. In one study, LoC was inversely associated with cortisol reactivity to the cold pressor test, *i.e.*, higher scores for internal LoC were associated with poorer cortisol response (r=0.22, p<0.05), but there were no significant associations between LoC and cortisol response to a mental arithmetic test [28]. In the second study, no significant associations were observed between LoC and cortisol levels in relation to a stress induction task based on exposure to aversive noise and a working memory math test (r=-0.28, p=0.08). However, when comparing participants in a perceived control and a non-control condition, internal locus of control in the perceived control condition was associated with a stronger cortisol response (r=0.29, p<0.01) while in non-control condition, cortisol response did not vary as a function of locus of control [30].

Mastery

Associations between mastery and cortisol (single time point measures, deviation measures, and cortisol AUC) were investigated in four studies based on normal populations consisting of 183 men [32], 781 women and men [33], 257 women and men [34], and 59 women [35].

Associations between mastery and single time point measures were investigated in two studies (five measures) [32, 34]. In one of these studies, a significant positive association between mastery and cortisol at awakening was reported (r=0.13, p<0.05) [34]. Single cortisol measurements based on morning, afternoon, and evening samples, were not statistically significantly associated with mastery in either of the two studies [32, 34].

All four studies investigated associations between mastery and deviation measures [32-35]. Two studies examined associations between mastery and morning deviation; none of these were significant [34, 35]. Three studies investigated associations between mastery and diurnal deviation, of which two studies reported significant positive associations between mastery and diurnal slope (r=0.12, p<0.05) [33], (r=0.13, p<0.05) [34], and one study found no significant associations [35].

One study investigated cortisol response in relation to laboratory stress, and reported an inverse association between mastery and baseline cortisol (r=-0.16, p=0.04), with no significant associations observed between mastery and cortisol reactivity [32].

Associations between mastery and cortisol AUC based on several samples over the day were investigated in two studies [33, 35]. One study found no significant associations between mastery and cortisol AUC_{ground} [33]; the other study reported an inverse association between mastery and AUC with respect to ground (r=-0.29, p<0.05) but not to AUC with respect to increase [35].

Self-Esteem

Associations between cortisol and self-esteem were investigated in four studies, based on study populations of 208 women and men [29], 183 men [32], 257 women and men [34], and 48 women [36].

None of the studies reported any significant associations between self-esteem and cortisol. Two studies reported analyses based on single time point measures at awakening, one of which also included an evening measure [34, 36]. All four studies investigated associations between self-esteem and cortisol deviation measures [29, 34, 36], three of which investigated deviations in morning or morning-evening cortisol levels; two studies investigated cortisol deviation in relation to a laboratory stress test. One study reported results based on analyses of associations between self-esteem and awakening cortisol AUC_{ground} levels.

Sense of Coherence

One study, based on a study population of 183 men, investigated associations between sense of coherence and salivary cortisol [32]. Analyses were based on a laboratory stress test. Inverse significant associations

between sense of coherence and baseline levels of cortisol (r=-0.18, p=0.02) before a laboratory stress test, and no significant associations between sense of coherence and cortisol reactivity to the laboratory stressor were reported.

DISCUSSION

Perceived Stress

There is a large proportion (more than half of the studies) of nonsignificant findings among the statistical analyses reported. However, the largest proportion of significant findings was seen for the AUC measure with significant associations for 38% of the studies (3/8 papers); negative associations were found in two studies (morning AUC) and a positive association in one study (morning AUC with respect to ground). For diurnal deviation measures, 44% were significant or marginally significant; three of these had a negative association with diurnal deviation.

The inconsistency found for diurnal deviation arose from one study with marginally significant results, suggesting that stronger diurnal deviation is related to high PSS. Two studies showed the opposite findings. The positive associations were seen among healthy controls in a case-control study of patients with breast cancer (in which the patients showed flat diurnal deviation) [14]; while negative associations were seen among people in a stressful context. Significant findings were reported for employees in a primary school, who the authors characterized as being under "chronic stress" [20] and among African women with internalized racism [15]. Marginally significant findings were reported among Colorado ranchers [24]. These data do underpin the importance of subject's earlier experiences and context when evaluating cortisol response to an acute stress.

This relationship between long-term and acute stress is discussed in several of the papers in which the nature of the PSS scale was discussed as a potential explanation for the lack of significant findings. Van Eck *et al.* [8] suggest that individual differences in current distress, especially anticipatory distress, may be more important determinants of cortisol secretion than PSS level, because the latter is a measure of more long-term distress. This is also in agreement with Schwartz *et al.* [13] and Putterman *et al.* [17], who discuss that PSS may not reflect current stressful events but, rather, stress levels over the previous month; the cortisol sampling may reflect changes in HPA activation over approximately 30 min. Thus, the PSS may tap stress more generically, and may not be so sensitive to the more subtle demands associated with cortisol levels.

Hellhammer *et al.* [37] concluded that a missing or poor association between perceived stress and salivary cortisol is not surprising, considering the complex interplay of neurobiological events that link perceived stress to HPA activation. Methodological difficulties related to the assessment of perceived stress by self-report instruments are also mentioned as possible explanations for a lacking covariance of perceived stress and salivary cortisol. Because several additional variables, such as adrenal sensitivity, capacity, and cortisol binding, also affect salivary cortisol levels, perceived stress can only be expected to be moderately associated with cortisol.

We could not find any apparent similarities for study design or methods, either in the studies that found significant associations or in the studies that failed to show effects. In several of the papers, the analyses of associations between cortisol and PSS were not the primary aim. Hence, the lack of significant results was sometimes not further discussed.

Although the present review gives little empiric support to the hypothesis that PSS is related to HPA activity, earlier studies have demonstrated that chronic stress does affect an individual's ability to respond to acute stress [8].

Psychological Resources

Based on the inclusion and exclusion criteria adopted, 11 studies were identified in which direct associations between cortisol in saliva and psychological resource constructs were investigated.

Most of the statistical analyses on associations between different measures of cortisol and psychological resource constructs were nonsignificant. This was especially so for single time point measures and for the resource construct self-esteem. Associations between psychological resource constructs and a standardized single measurement, in terms of baseline measures before stress testing, were reported in two studies, in one of which mastery and SOC were significantly associated with lower baseline cortisol levels [32]. There were no associations observed between cortisol and self-esteem in either study [32, 36].

Regarding deviation measures, 0/5 measures of Cortisol Awakening Response (CAR) were significant for mastery and self-esteem alike. For diurnal deviation measures, 2/3 studies reported significant associations with mastery. In two studies, higher levels of mastery were associated with a steeper cortisol diurnal slope (one study of 781 participants based on results from one sampling day [33], and one study of 257 participants based on three sampling days [34]) and nonsignificant findings for self-esteem. In laboratory stress testing, 2/9 analyses were significant, both showing an inverse association between psychological resources and cortisol response, one for SEC and one for LoC [28, 31].

Thus, consistencies in results on the one hand relate to nonsignificant results regarding associations between psychological resources and cortisol levels based on single time point measures or CAR. On the other hand, consistencies are seen for significant findings of lower cortisol at standardized rest for baseline, and for steeper diurnal deviation measures.

The present studies highlight a number of methodological issues of potential importance for further investigations on associations between psychological resources and cortisol. Cortisol is secreted in response to the daily life cycle of activity and rest and in response to internal and external stressors. The sensitivity of the HPA axis to internal and external events is reflected in large intra- as well as inter-individual variability of cortisol [37, 38]. Most of the studies identified were based on relatively small study populations. Power calculations were generally not presented, and whether or not nonsignificant results are due to lack of power or lack of effect size can not be determined. All studies on psychological resources were on normal populations, and thus medical conditions of participants did not seem to explain any of observed differences in results.

Aggregation of cortisol data over several days has been demonstrated to lead to increasingly consistent patterns in associations between psychological measures and cortisol, and it is suggested that data aggregation is needed for identification of the trait component of the stress response [26, 38]. In a recent study on the reliability of CAR, the results suggest that measurements during 2 days are necessary for reliable AUC with respect to ground measures, and 6 days are necessary to achieve reliable AUC with respect to increase measures, with state factors biasing data based on single day measures [38]. In parallel, in studies of diurnal deviation, evidence of increased reliability of results was seen comparing mean values over 3 days compared with single day values [26]. Of the six studies using ambulatory saliva sampling, three studies sampled over 1 or 2 days.

In two of the studies, nonsignificant results were reported for associations between psychological resources and cortisol in the study population as a whole. Significant associations were observed in the subgroup analyses [27, 30], in one study, only if the subject was in a failure condition [27], and in another study, only among participants who believed they were in control of the laboratory stressor [30].

CONCLUSIONS

For both PSS and for psychological resources, most results of associations with saliva cortisol were nonsignificant particularly for single measures and for cortisol awakening response. For PSS the largest proportion of significant findings (38%) was seen for morning AUC, however with conflicting results. For psychological resource constructs, mastery and sense of coherence were related to lower cortisol level at baseline in standardized rest and high mastery was related to steeper diurnal slope in two studies. For self-

esteem, no associations showed significant results. Differences in results may to a large extent be dependent on theoretical assumptions made and methods used.

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CHAPTER 5

Biological Markers and Salivary Cortisol

Åse Marie Hansen[1,*], Lars-Gunnar Gunnarsson[2], Anette Harris[3], Nanna Hurwitz Eller[4], Peter Garvin[5] and Anne Helene Garde[6]

[1]Senior reseacher at the National Research Centre for the Working Environment, Lersø Parkallé 105, 2100 København Ø, Copenhagen, Denmark; [2]Medical Doctor in Occupational and Environmental Medicine at Örebro, University Hospital, Örebro, Sweden; [3]Researcher at Uni Health and Department of Psychology, Bergen University, Bergen, Norway; [4]Medical Doctor in Occupational and Environmental Medicine at Bispebjerg Hospital, Copenhagen, Denmark; [5]Post-doc at the Department of Medical and Health Sciences, Linköping University, Sweden and [6]Senior reseacher at the National Research Centre for the Working Environment, Copenhagen, Denmark.

Abstract: This chapter focuses on salivary cortisol in relation to biological markers. Specifically, associations with conventional cardiovascular risk factors and metabolic abnormalities (body mass index, waist circumference, waist/hip ratio, lipid status, glucose, blood pressure, heart rate and heart rate variability), markers related to inflammation (C-reactive protein, cytokines and tumor necrosis factor-alpha) and other stress hormones (adrenaline and noradrenaline) were studied. The focus was on healthy adult populations; studies on patient populations and pregnant women were excluded. Studies on genome variations and pharmacological interventions were also excluded. After meeting all exclusion criteria, 42 papers remained. In total, 273 associations between salivary cortisol and any of the markers mentioned were studied, comprising 241 associations on metabolic abnormalities, 30 on inflammation, and 2 on stress hormones. Of the salivary cortisol measures reported for evaluations of all markers tested were 136 (49%) single time points, 100 (37%) deviations, 36 (13%) AUC, and 1 (1%) dexamethasone test. Of these, 72 (26%) were statistically significant, and 201 (74%) indicated non-significant findings.Several of the markers tested showed low or no association with any of the measurements of salivary cortisol. The number of studies exploring the association between cortisol in saliva and markers for inflammation is low, which limits the possibility of interpretation. The number of studies on adrenaline and noradrenaline is also low. To sum up, the proportion of non-significant findings was considerable. This may be due to a large number of studies with relatively small study populations. This is true for metabolic abnormalities, markers related to inflammation as well as other stress hormones. Further studies on inflammatory markers and approaches designed to study variability in other systems in relation to cortisol variability are required.

Keywords: Salivary cortisol, body mass index, waist circumference, waist/hip ratio, lipid status, glucose, blood pressure, heart rate, C-reactive protein, cytokines, adrenaline, noradrenaline.

INTRODUCTION

In the last decade, the technique of using ambulatory saliva sampling has become increasingly popular in field research and clinical studies. The non-invasive method is easy to administer and analyze, and therefore allows implementation in large-scale study designs. However, as with other biological, behavioral, and psychological measurements, the possibility of answering any research question is dependent on when and how measurements are made. Cortisol has considerable day-to-day and diurnal variation. Therefore, a fair number of saliva samples are needed to illustrate the general capacity of the Hypothalamic-Pituitary-Adrenal (HPA) axis. The HPA axis, a major part of the neuroendocrine system, controls physiologic response to stress and regulates many bodily processes, including digestion, the immune system, mood and emotions, sexuality, and energy storage and expenditure [1, 2].

Allostasis is the process of achieving stability, or homeostasis, through physiologic or behavioral change

***Address correspondence to Åse Marie Hansen:** Senior reseacher at the National Research Centre for the Working Environment, Lersø Parkallé 105, 2100 København Ø, Copenhagen, Denmark; Tel: +45 39 16 52 00; Fax: +45 39 16 52 01; E-mail: aamh@arbejdsmiljoforskning.dk

Margareta Kristenson, Peter Garvin and Ulf Lundberg (Eds)

[3, 4]. This can be carried out by means of alteration in HPA axis hormones, the autonomic nervous system, cytokines, or a number of other systems. A shift in the HPA axis is generally adaptive in the short term [5]. However, it has been suggested that a long-term shift may have deleterious health effects [3, 4]. In particular, alterations in the HPA axis have been suggested as a plausible mechanism linking stress with metabolic abnormalities [6]. It has also been suggested that the immunoregulating effects of cortisol may be diminished by a long-term increase in cortisol levels [7]. Further, it has been suggested that a flat diurnal curve (low decrease from morning to evening and high evening values) may represent alack of recovery or sustained activation that may be associated to negative health outcome [8, 9], whereas a high as well as a low awakening response may reflect the individuals expectations to the upcoming day [8, 10]. The effects of cortisol are well described in several experimental studies, but it is unclear to what extent salivary cortisol in observational studies mirrors other biological measures associated with metabolic abnormalities or inflammation.

This chapter primarily aims to describe associations between measures of cortisol in saliva and other biological markers in non-clinical settings. We have focused on markers related to metabolic abnormalities (with particular interest in cardiovascular risk factors), inflammation, and other stress hormones. The evaluation of the literature was based on single cortisol measurements, the sum or mean over the day, diurnal variability, Cortisol Awakening Response (CAR), Area Under the Curve (AUC), reactivity and recovery from stress tests and the dexamethasone suppression test.

AIM

To examine to what extent associations between cortisol measurements and other biological measures can be found, and which of the measurements are of highest relevance. The evaluation of the literature was based on the following question: is it possible that the seemingly divergent results of the studies involving cortisol assessments and biological markers are functions of differences in the theoretic assumptions made and methods used.

METHOD

Search Strategies

In a first step, an online search of the NCBI PubMed database (National Library of Medicine, National Institutes of Health, Bethesda, MD, USA-http://www.ncbi.nlm.nih.gov/PubMed) was conducted. The search covered the time period up to October 2009 (allowing e-publications if a full paper was published electronically prior to journal publication). Search terms were selected with reference to relevant PubMed terms and key words (see detailed description for each of the biological markers below), in combination with salivary cortisol in its truncated form ("saliva*"). The limitations were set only to include studies matching "Human", "English" and "Adults" (aged 19 years old or more).

In a second step, studies on patient populations were excluded (*e.g.*, cancer, diabetes, and major depressive disorder). Studies on genome variations, pregnant women, and pharmacological interventions were also excluded.

In a third step, all articles retrieved from each search were briefly read. If no direct statistical analysis between salivary cortisol and the explored biomarker were presented in tables, figures, or text, the paper was excluded. Intervention studies (other than pharmacological) were included if associations with the biomarker of interest were present before the intervention. However, the effects on salivary cortisol in response to the intervention are not included in this review. Papers were also excluded if another (prior) publication from the same study material was already included in the evaluation.

Body Mass Index

The term "body mass index" in combination with truncated salivary cortisol yielded 110 hits. In addition, 8 hits were found in the search using "metabolic." After meeting all exclusion criteria, the final number of papers was reduced to 24.

Waist Circumference

The term "waist circumference" in combination with truncated salivary cortisol yielded 13 hits. In addition, 3 hits were found in the search using "metabolic", giving 16 papers. Of these, 7 papers remained after exclusion.

Waist/Hip Ratio

To identify papers on cortisol in relation to cholesterol, the following search terms were used in combination with truncated salivary cortisol: "waist hip ratio" (16 hits), "waist-hip ratio" (16 hits, same as previous), "waist-to-hip" (19 hits, of which all was covered by the previous mentioned), and "WHR" (14 hits, all of which were included in the previous searches). In addition, 3 papers from the search on body mass index were included, as they presented associations between waist/hip ratio and cortisol. In total, 38 unique papers were identified in the first step. After meeting all exclusion criteria, the final number was reduced to 11 papers.

Cholesterol

To identify papers of cortisol in relation to cholesterol, the following search terms were used in combination with truncated salivary cortisol: "cholesterol" (29 hits), "HDL" (18 hits, of which one was not covered by the previous search terms), "LDL" (8 hits, of which one was not covered by the previous search terms), "lipids" (56 hits), , "metabolic" (81 hits), "metabol*" (8 hits) and "metabolite" (12 hits) and "apolipoprotein" (4 hits). In total, 131 unique papers were identified in the first step. After meeting all exclusion criteria, the final number was reduced to 6 papers.

Triglycerides

To identify papers on cortisol in relation to triglycerides, the search term "triglycerides" was used in combination with truncated salivary cortisol (20 hits). "TG" as a search term did not yield any extra papers. In addition, 3 papers from the search on cholesterol were included, as they presented associations between triglycerides and cortisol. In total, 23 unique papers were identified in the first step. After meeting all exclusion criteria, the final number was reduced to 5 papers.

Plasma or Blood Glucose

To identify papers on cortisol in relation to triglycerides, the search term "glucose" was used in combination with truncated salivary cortisol (68 hits); "blood sugar" as a search term did not yield any extra papers. After meeting all exclusion criteria, the final number was reduced to 5 papers.

Blood Pressure

To identify papers on cortisol in relation to blood pressure, the search term "blood pressure" (216 hits) was used in combination with truncated salivary cortisol. "hypertens*" (11 hits) was also used. In total, 224 unique papers were identified in the first step. After meeting all exclusion criteria, the final number was reduced to 14 papers.

Heart Rate

To identify papers on cortisol in relation to heart rate, the search term "heart rate" was used in combination with truncated salivary cortisol (270 hits). Almost all those met the exclusion criteria. The final number was reduced to 3 papers.

For several of the parameters mentioned above, two additional papers were found [11, 12]. These two, conducted within the same research group, used a method of measuring cortisol that could not be fitted into the overview of the cortisol measurements used in this book. For that reason, these are excluded from the overview. However, they are reflected upon in the discussion on cortisol and metabolic abnormalities.

Heart Rate Variability

To identify papers on cortisol in relation to heart rate variability, the search term "heart rate variability" was used in combination with truncated salivary cortisol. In total, 32 unique papers were identified in the first step. After meeting all exclusion criteria, the final number was reduced to 4 papers.

Interleukins and Other Markers Related with Inflammation

To identify papers on cortisol in relation to inflammation, the search term "interleukin" (61 hits), "cytokine" (71 hits), "CRP" (5 hits), and "C-reactive protein" (13 hits) were used in different searches in combination with truncated salivary cortisol. After meeting all exclusion criteria, the final number was reduced to 11 papers.

Adrenaline

To identify papers on cortisol in relation to adrenaline, the search terms "adrenaline" and "epinephrine" were used in combination with truncated salivary cortisol. "The latter did not yield any extra papers. In total, 69 unique papers were identified in the first step. After meeting all exclusion criteria, the final number was reduced to 2 papers.

Noradrenaline

To identify papers on cortisol in relation to adrenaline, the search terms "noradrenaline" and "noraepinephrine" were used in combination with truncated salivary cortisol. "The latter did not yield any extra papers. In total, 72 unique papers were identified in the first step. After meeting all exclusion criteria, the final number was reduced to 2 papers.

RESULTS

After meeting all exclusion criteria, 42 papers were included. In total, 273 associations between salivary cortisol and any of the markers mentioned were studied, comprising 241 associations on metabolic abnormalities, 30 on inflammation, and 2 on stress hormones. Of the salivary cortisol measures reported for evaluations of all markers tested were 136 (49%) single time points, 100 (37%) deviations, 36 (13%) AUC, and 1 (1%) dexamethasone test. Of these, 72 (26%) were statistically significant, and 201 (74%) indicated non-significant findings.

Body Mass Index

Quantitative Analysis on the Evaluated Studies

In the 24 studies [13-36], there were 60 analyses on the relationship with salivary cortisol (see Table **1a**). Of these, 29 were on single time points, 23 on deviations, and 8 on AUC. In total, 14 of the analyses (23%) showed significant associations with salivary cortisol, whereas the other 46 (77%) showed non-significant findings. The significant findings were mainly clustered in the following three categories:

- A lower level in the morning was associated with higher Body Mass Index (BMI) (5/9; 56%).
- A lower deviation between two time points at midday was associated with higher BMI (3/3; 100%).
- AUC at midday was lower in subjects with higher BMI (2/2; 100%).

Consistency of the Material

Regarding a possible association between a lower cortisol level in the morning and BMI, there are no contradictions in the results. However, analyses suggesting such an association is derived from only five studies [20, 22, 23, 32, 34]. These studies point in the same direction, suggesting a lower cortisol level in

the early phase of the diurnal cycle. None of the studies evaluating awakening values found any associations with BMI. The possible association with a lower cortisol level in the early phase of the diurnal cycle seems to be valid regardless of the use of cross-sectional designs [23, 34] or a prospective design with cortisol levels as the outcome [22].

Methodological or Contextual Explanation on Divergent Findings

Overall, only two significant positive associations were found. One might be attributed to a subpopulation analysis [25]. The most striking contradictory finding was found in a large-scale population sample (Whitehall-II, n=2873) [29], in which associations were evaluated using an averaged cortisol level throughout the day based on 7 samples from awakening to bedtime.

Most of the other studies were based on fewer samples. Computing an average of 7 samples devalues the potential impact of lower levels at the early stages of the diurnal cycle. Thus, the seemingly contradictory findings may be accurate, given that values taken at time points later in the diurnal cycle were slightly higher among subjects with higher BMI (not reported in the study). On the other hand, there is no support in the other studies evaluating cortisol levels in the evening; all reported non-significant findings. Coutinho *et al.* reported a marginal significant negative association (p=0.063) between a sample taken at 23:00 h and BMI [27].

Waist Circumference

Quantitative Analysis on the Evaluated Studies

In the 7 studies [13, 21, 26, 27, 31, 37, 38] there were 16 analyses on the relationship with salivary cortisol (see Table **1a**). Of these, were 9 on single time points, 6 on deviations and 1 on AUC. In total, 4 of the analyses (25%) showed negative associations with salivary cortisol, whereas the other 12 (75%) showed non-significant findings. However, the negative findings were clustered in the same category where a lower deviation in a daily slope was associated with higher waist circumference (3/3; 100%), and a lower AUC in the morning was associated with a higher waist circumference (1/1; 100%).

Consistency of the Material

Relatively few associations were found in the material. The low proportion of non-significant findings is explained by the non-significant findings for single time points. The significant findings included points in the same direction as BMI, implying that there may be an association between low values of cortisol in the morning/midday and waist circumference.

Methodological or Contextual Explanation on Divergent Findings

There are no major contradictions in the overview. The reported results are either non-significant findings or negative associations between cortisol levels and waist circumference. However, all studies presented are fairly small, thus increasing the risk for beta errors.

Waist/Hip Ratio

Quantitative Analysis on the Studies Evaluated

In the 11 studies [13, 15-18, 22, 24, 25, 27, 29, 30], there were 31 analyses on the relationship with salivary cortisol (see Table **1a**). Of these, 15 were on single time points, 12 on deviations, and 4 on AUC. Seven of the analyses (31%) showed negative associations with salivary cortisol, 4 (13%) showed positive associations, and the other 20 (65%) showed non-significant findings. The negative significant findings were clustered in measure including morning values, either as single time points (3/4, 75%), low deviation at midday (1/1, 100%) or low AUC including morning values (1/1, 100%).

Consistency of the Material

There were relatively few associations found in the material. Negative associations suggesting a lower cortisol level in the early phase of the diurnal cycle were found in 4 independent studies. The consistency of

positive associations is weaker, partly because they are occur less, partly because the same group presenting positive associations also presented non-significant findings for similar cortisol measurements in different study populations [17, 24, 29].

Methodological or Contextual Explanation on Divergent Findings

A similar pattern to that found for BMI emerged. Only 4 significant positive associations were found, 3 of which might be attributed to a sub-population analyses [17]. The most striking contradictory finding was found in a large-scale population sample (Whitehall-II, *n*=2873) [29], in which associations were evaluated using an averaged cortisol level throughout the day based on 7 samples from awakening to bedtime. Most of the other studies were based on fewer samples. Computing an average of 7 samples devalues the potential impact of lower levels at the early stages of the diurnal cycle. Thus, the seemingly contradictory findings may be accurate, given that values taken at time points later in the diurnal cycle are slightly higher among subjects with higher waist/hip ratio (not reported in the study). As with BMI, there is no support in the other studies evaluating cortisol levels in the evening; all reported non-significant findings [15, 17, 27].

Table 1a: Summary of the main findings of associations between measures of salivary cortisol and body mass index, waist circumference, and waist/hip ratio sorted by year of publication

References	Year	Exposure	Design	No. cortisol	m/w	Single time points (or sum/mean of two/more time points)					Deviation (difference/slope between two or more time points)					AUC							Dexamethasone suppression test
						a1	a2	a3	a4	a5	b1	b2	b3	b4		c1		c2		c3		c4		d
Body mass index																							
Laederach-Hofmann [13]	2000		C-S	42	24/18												0						
Roy [14]	2001		Exp	82	82/0										0								
Eller [15]	2001		C-S	121	37/84	0	0		0														
Kunz-Ebrecht [16]	2003		C-S	160	98/62									0									
Steptoe [17]	2004		C-S	172	89/83	0			0	0	0		0										
Ward [18]	2004		C-S	678	678/0		↓																
Ward [18]	2004		C-S	117	117/0		↓										0						
Patel [19]	2004		C-S	248	128/120	0																	
Weitz [20]	2005		C-C	48	24/24		↓																
Tull [21]	2005		C-S	53	0/53								0										
Power [22]	2006		Pros	6452	3176/ 3276		↓/↓					↓/↓						↓/↓					
Daniel [23]	2006		C-S	129	0/129							↓											
Steptoe [24]	2006		C-S	83	28/55					0	0		0				0						
Therrien [25]	2007		Pros	82	51/31	0/0					↑/0												
Kidambi [26]	2007		C-S	96	46/50		0		0														
Coutinho [27]	2007		C-S	47	0/47				0														
Roberts [28]	2007		Pros	71	0/71															0			
Steptoe [29]	2007		C-S	2873	2126/747					↑													
Lasikiewicz [30]	2008		C-S	147	68/79					0			0			0							
Brydon [31]	2008		Exp	67	0/67			0						0	0								

O'Donnell [32]	2008		C-S	442	350/192						0		0								↑			
Wirtz [33]	2008		Exp	42	42/0			0					0	0										
Farag [34]	2008		C-S	127	0/127	0	0	0	0				0/↓[a]											
Brummett [35]	2009		C-S	328	n.s.						0													
Boyne[36]	2009		C-S	40	0/40		0	0	0				0											
Waist circumference																								
Laederach-Hofmann [13]	2000		C-S	42	24/18												↓							
Kajantie [37]	2004		C-S	151	0/151	0	0	0	0	0	0		↓											
Tull [21]	2005		C-S	53	0/53								↓											
Garcia-Prieto [38]	2006		C-S	41	0/48								↓											
Coutinho [27]	2006		C-S	47	0/47				0															
Kidambi [26]	2007		C-S	96	46/50		0		0															
Brydon [31]	2008		Exp	67	0/67			0						0	0									
Waist/hip ratio																								
Laederach-Hofmann [13]	2000		C-S	42	24/18												0							
Eller [15]	2001		C-S	121	37/84	0	0		0															
Kunz-Ebrecht[16]	2003		C-S	160	98/62									↓/0										
Steptoe [17]	2004		C-S	172	89/83	0			0	0	↑/0		↑/0											
Ward [18]	2004		C-S	678	678/0		↓																	
Ward [18]	2004		C-S	117	117/0		↓										0							
Power [22]	2006		C-S	6452	3176/ 3276		↓					↓						↓						
Steptoe [24]	2006		C-S	83	28/55					0	0		0				0							
Therrien [25]	2007		Pros	82	51/31	0/0					↑/0													
Coutinho [27]	2007		C-S	47	0/47				0															
Steptoe [29]	2007		C-S	2873	2126/747					↑														
Lasikiewicz [30]	2008		C-S	147	68/79					0			↓		0									

[a] Significant associations only in group with obese women (BMI>=30 kg/m^2).

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory stress test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory stress test increase/ground; d, DST, dexamethasone suppression test; C-C, Case-control; C-S, cross-sectional; Exp, experimental; Pros, prospective.

Cholesterol

Quantitative Analysis on the Evaluated Studies

In the 6 studies [14, 15, 17, 26, 30, 37], there were 47 analyses on the relationship with salivary cortisol (see Table 1b; 6 on total cholesterol, 6 on Low-Density Lipoprotein (LDL), 27 on High-Density Lipoprotein (HDL), and 8 on total cholesterol/HDL ratio). Of these, 7 associations were significant (15%); the other 40 (85%) showed non-significant findings. The only potential cluster was that a high total cholesterol/HDL ratio and a high LDL level were associated with a higher increase in cortisol under the laboratory stress test (2/4, 50%).

Consistency of the Material

Most studies did not reveal any association between different measurements of cholesterol and salivary cortisol. Of all 27 analyses on single time points, only 1 was significant. The significant association was based on a sub-population analysis [15]. Thus, given the low proportion of significant associations, this literature does not provide support for a strong relationship.

Methodological or Contextual Explanation on Divergent Findings

The findings on cholesterol and cortisol in a laboratory stress test were derived from the same study [14]. Although a high proportion was positively associated with an increase of cortisol under a laboratory stress test, the picture is not clear, as no associations could be found with total cholesterol and HDL in the same study.

Triglycerides

Quantitative Analysis on the Evaluated Studies

In the 5 studies [14, 18, 26, 30, 37], there were 15 analyses on the relationship with salivary cortisol (see Table **1b**). Of these, 9 were on single time points, 4 on deviation, and 2 on AUC.

Consistency of the Material

None of the studies evaluated showed a significant association with triglycerides.

Methodological or Contextual Explanation on Divergent Findings

The studies were performed under similar circumstances. Although the studies were cross-sectional, ruling out the possibility of discussing causality, the non-significant findings strongly suggest that there are no associations between circulatory levels of triglycerides and salivary cortisol.

Table 1b: Summary of the main findings of associations between measures of salivary cortisol and lipid status sorted by year of publication

References	Year	Exposure	Design	No. cortisol	m/w	Single time points (or sum/mean of two/more time points)					Deviation (difference/slope between two or more time points)				AUC				Dexamethasone suppression test
						a1	a2	a3	a4	a5	b1	b2	b3	b4	c1	c2	c3	c4	d
Total cholesterol																			
Roy [14]	2001		Exp	82	82/0									0					
Eller [15]	2001		C-S	121	37/84	0	0		0										
Kidambi [26]	2007		C-S	46/50	96		0		0										
Lasikiewicz [30]	2008		C-S	147	68/79					0			0		0				
Low-density lipoprotein (LDL)																			
Roy [14]	2001		Exp	82	82/0									↑					
Kidambi [26]	2007		C-S	46/50	96		0		0										
Lasikiewicz [30]	2008		C-S	147	68/79					0			0		0				
High-density lipoprotein (HDL)																			
Roy [14]	2001		Exp	82	82/0									0					
Eller [15]	2001		C-S	121	37/84	0	0		↑/0										
Steptoe [17]	2004		C-S	172	89/83	0			0	0	↓/0		↓/0						
Kajantie [37]	2004		C-S	151	0/151	0	0	0	0	0	0		0						
Kidambi	2007		C-S	46/50	96		0		0										

[26]																							
Lasikiewicz [30]	2008		C-S	147	68/79					0			0			0							
Total cholesterol/HDL																							
Roy [14]	2001		Exp	82	82/0										↑								
Steptoe [17]	2004		C-S	172	89/83	0			0	0	↑/0		↑/0										
Triglycerides																							
Roy [14]	2001		Exp	82	82/0										0								
Kajantie [37]	2004		C-S	151	0/151	0	0	0	0	0	0		0										
Ward [18]	2004		C-S	117	117/0		0										0						
Kidambi [26]	2007		C-S	46/50	96		0		0														
Lasikiewicz [30]	2008		C-S	147	68/79					0			0			0							

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory stress test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory stress test increase/ground; d, DST, dexamethasone suppression test; C-S, cross-sectional; Exp, experimental.

Plasma or Blood Glucose

Quantitative Analysis on the Studies Evaluated

In the 5 studies [18, 26, 30, 37, 39], there were 17 analyses on the relationship with salivary cortisol (see Table **1c**). Of these, 10 were on single time points, 4 on deviation, and 3 on AUC.

Table 1c: Summary of the main findings of associations between measures of salivary cortisol and blood glucose sorted by year of publication

References	Year	Exposure	Design	No. cortisol	m/w	Single time points (or sum/mean of two/more time points)					Deviation (difference/slope between two or more time points)				AUC				Dexamethasone suppression test
						a1	a2	a3	a4	a5	b1	b2	b3	b4	c1	c2	c3	c4	d
Plasma or blood glucose																			
Hucklebridge [39]	1999		C-S	27	14/13						0				0				
Ward [18]	2004		C-S	678	678/0		↑												
Ward [18]	2004		C-S	117	117/0		0									0			
Kajantie [37]	2004		C-S	151	0/151	0	0	0	0	0	0		0						
Kidambi [26]	2007		C-S	96	46/50		0		0										
Lasikiewicz [30]	2008		C-S	147	68/79					0			0		0				

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory stress test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory stress test increase/ground; d, DST, dexamethasone suppression test; C-S, cross-sectional.

Consistency of the Material

There was only one significant finding presented (6%), where a single time point in the morning were positively associated with fasting blood glucose. None of the other studies evaluated showed a significant association with blood sugar.

Methodological or Contextual Explanation on Divergent Findings

The studies were performed under similar circumstances. Although the studies were cross-sectional, ruling out the possibility of discussing causality, the amount of non-significant findings suggest that there are no strong associations between levels of fasting blood sugar and salivary cortisol.

Blood Pressure

Quantitative Analysis on the Studies Evaluated

Among the 14 studies [14-16, 18, 20, 26, 30, 37, 40-45], there were 53 analyses on the relationship with salivary cortisol (see Table **1d**; 18 on hypertension and 35 on systolic and diastolic blood pressure). Of these, 20 associations were significant (38%); the other 33 (62%) showed non-significant findings.

Consistency of the Material

There were no clear trends on blood pressure and cortisol measurements. The high percentage of significant findings is somewhat devalued by significant findings pointing in opposite directions. If anything, the results suggest that an increase in cortisol in a laboratory stress test is associated with increased blood pressure. This is shown in 4 out of 12 associations (33%). In addition 5 out of 15 reported findings on morning cortisol levels had a positive association (33%)

Methodological or Contextual Explanation on Divergent Findings

The literature does not provide any clear explanations on why the results vary. One possible explanation for divergent findings has been proposed by Weitz and colleagues [20]. The authors have reported that low birth weight may modify the association between blood pressure and salivary cortisol such that subjects with low birth weight show a clear negative correlation with salivary morning cortisol, whereas no correlation can be found in subjects with normal birth weight [20]. However, their hypothesis is hard to evaluate in the included studies in this overview, as most studies do not report data on birth weight.

Table 1d: Summary of the main findings of associations between measures of salivary cortisol and blood pressure sorted by year of publication

References	Year	Exposure	Design	No. cortisol	m/w	Single time points (or sum/mean of two/more time points)					Deviation (difference/slope between two or more time points)				AUC								Dexamethasone suppression test
						a1	a2	a3	a4	a5	b1	b2	b3	b4	c1		c2		c3		c4		d
Blood pressure																							
Kajantie [37]	2004		C-S	151	0/151	0	0	0	0	0	0		0										
Nyklicek [40]	2005		Exp	63	30/37									↑									
Wirtz [41]	2006		Exp	48	48/0	0		0													↑		
Kidambi [26]	2007		C-S	96	46/50		↑		↑														
Wirtz [42]	2007		C-S	42	42/0	0					↓		0			↓							↑

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory stress test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory stress test increase/ground; d, DST, dexamethasone suppression test; C-S, cross-sectional.

References	Year	Exposure	Design	No. cortisol	m/w	Single time points (or sum/mean of two/more time points)					Deviation (difference/slope between two or more time points)				AUC								Dexamethasone suppression test
						a1	a2	a3	a4	a5	b1	b2	b3	b4	c1		c2		c3		c4		d
Systolic blood pressure																							
Kunz-Ebrecht [16]	2003		C-S	160	98/62									0									
Gregg [43]	1999	Mental	Exp	100	50/50									0									
Gregg [43]	1999	Cold	Exp	100	50/50									↑									
Roy [14]	2001		Exp	82	82/0		0/↑ [a]								0								
Eller [15]	2001		C-S	121	37/84	0	0		0														

Ward [18]	2004		C-S	678	678/0		↑															
Ward [18]	2004		C-S	117	117/0		0									0						
Krantz [44]	2004		Exp	21	10/11								0									
Weitz [20]	2005		C-C	48	24/24		↓/0[b]															
Holt-Lunstad [45]	2007		C-S	301	146/155														↓			
Lasikiewicz [30]	2008		C-S	147	68/79					↑			0			↑						
Diastolic blood pressure																						
Kunz-Ebrecht [16]	2003		C-S	160	98/62								0									
Gregg [43]	1999	Mental	Exp	100	50/50								0									
Gregg [43]	1999	Cold	Exp	100	50/50								↑									
Roy [14]	2001		Exp	82	82/0		0/↑ [a]							0								
Ward [18]	2004		C-S	678	678/0		↑															
Ward [18]	2004		C-S	117	117/0		0									0						
Krantz [44]	2004		Exp	21	10/11								0									
Weitz [20]	2005		C-C	48	24/24		↓/0[b]															
Holt-Lunstad [45]	2007		C-S	301	146/155														↓			
Lasikiewicz [30]	2008		C-S	147	68/79					↑			0			0						

[a] Significant associations with blood pressure during stress task, but not with blood pressure at rest.
[b] Significant associations only in group with low birth weight.
Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory stress test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory stress test increase/ground; d, DST, dexamethasone suppression test; C-S, cross-sectional; Exp, experimental.

Heart Rate

All 3 studies evaluated [16, 43, 44] used a laboratory stress test (see Table **1e**). One out of 4 associations tested (25%) between an increase in cortisol level after the stress test and heart rate were significant [43]. However, this was true in only 1 of the 2 stressors tested in the study. Due to the low number, it is hard to further evaluate the consistency of the material.

Heart Rate Variability

Quantitative Analysis on the Evaluated Studies

In the 4 studies [16, 46-48], there were 7 analyses on the relationship with salivary cortisol (see Table **1e**). Of these, 2 were on single time points and 5 on deviations.

Consistency of the Material

Both studies investigating cortisol in a laboratory stress test found a negative association between increase in cortisol level and heart rate variability.

Methodological or Contextual Explanation on Divergent Findings

Heart rate variability can be divided into several components. These are not used uniformly in the literature, which makes it hard to compare different studies on heart rate. Even if there were more comparable studies, it may be relevant to investigate whether the associations are influenced by the stress level among subjects. A recent publication suggests that the associations between level of cortisol are associated with both heart rate and heart rate variability under stressful conditions, but that these associations are attenuated in periods of low stress [49].

Table 1e. Summary of the main findings of associations between measures of salivary cortisol and heart rate and heart rate variability sorted by year of publication

References	Year	Exposure	Design	No. cortisol	m/w	Single time points (or sum/mean of two/more time points)					Deviation (difference/slope between two or more time points)					AUC								Dexamethasone suppression test
						a1	a2	a3	a4	a5	b1	b2	b3	b4		c1		c2		c3		c4		d
Heart rate																								
Gregg [43]	1999	Mental	Exp	100	50/50									↑										
Gregg [43]	1999	Cold	Exp	100	50/50									0										
Kunz-Ebrecht [16]	2003		C-S	160	98/62									0										
Krantz [44]	2004		Exp	21	10/11									0										
Heart rate variability																								
Lucini [46]	2002		Exp	30	n.s.		↑																	
Kunz-Ebrecht [16]	2003		C-S	160	98/62			0						↓	↑									
Sgoifo [47]	2003		Exp	30	15/15									↓										
Eller [48]	2007		Pros	72	M/W						↑/0													

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory stress test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory stress test increase/ground; d, DST, dexamethasone suppression test; C-S, cross-sectional; Exp, experimental.

Interleukins and Other Markers Related with Inflammation

Quantitative Analysis on the Evaluated Studies

Combining all inflammatory markers, 30 analyses were presented in 10 studies [16, 29-31, 50-55] (see Table **1f**). Of these, were 12 on single time points, 11 on deviation, and 7 on AUC. Twelve of the analyses (40%) were significant.

Consistency of the Material

Two clusters without contradictory findings arise: High cortisol output throughout the day may be associated with higher average levels of inflammatory markers [29, 30]. 80% of the associations were significant, however derived from two studies only [29, 30]. There was also a possible cluster of significant negative findings in the laboratory stress tests (33 %), indicating that the ability to react with cortisol secretion on a stress test are associated with lower levels of inflammatory markers [16, 51]. However, this inference is derived from two studies only.

Methodological or Contextual Explanation on Divergent Findings

One question raised is the effect on cortisol and cytokine levels following acute stress. It is generally expected that an increase in cortisol levels will lower the level of cytokines. One explanatory factor for some of the non-significant findings may be that some commonly used acute stress tests may actually be too mild to affect the cortisol level to any major extent [56]. Thus, the possibility of finding any strong associations between cortisol and levels of cytokines following an acute stressor may be limited.

In addition, there is a large natural fluctuation of cytokines, depending on ongoing inflammations. Glaser *et al.* [50] have demonstrated that cortisol levels in the morning not is associated with cytokines a normal day but is inversely associated with cytokine levels measured 24 hours after an experimentally induced wound. Thus, occurrence of acute inflammation in some participants but not in others might add to the complexity when studying cortisol in relation to cytokines.

Table 1f: Summary of the main findings of associations between measures of salivary cortisol and inflammatory markers sorted by year of publication

References	Year	Exposure	Design	No. cortisol	m/w	Single time points (or sum/mean of two/more time points)					Deviation (difference/slope between two or more time points)					AUC								Dexamethasone suppression test
						a1	a2	a3	a4	a5	b1	b2	b3	b4		c1		c2		c3		c4		d
C-Reactive Protein (CRP)																								
Steptoe [29]	2007		C-S	2873	2126/747					0														
Lasikiewicz [30]	2008		C-S	147	68/79					↑			0			0								
IL-1																								
Glaser [50]	1999	IL-1 and IL-8	Exp	24	0/24		0/↓[a]	↓	↓															
Kunz-Ebrecht [16]	2003	IL-1ra	C-S	160	98/62									↓								↓		
Bower [51]	2007	IL-1beta	Exp	25	0/25									0										
Brydon [31]	2008	IL-1ra	Exp	67	0/67									0										
IL-6																								
Kunz-Ebrecht [16]	2003		C-S	160	98/62									0								↓		
Gaab [52]	2005		Exp	41	21/20																		0	
von Känel [53]	2005		Exp	21	21/0																	0		
Wirtz [54]	2007		Exp	44	44/0										0							0		
Bower [51]	2007		Exp	25	0/25									↓										
Steptoe [29]	2007		C-S	2873	2126/747					↑/↑														
Lasikiewicz [30]	2008		C-S	147	68/79					↑			0			0								
Brydon [31]	2008		Exp	67	0/67									0										
Tissue Necrosis Factor-alpha (TNF-α)																								
Luz [55]	2003		Exp	79	30/49		0	0	↑															
Gaab [52]	2005		Exp	41	21/20																		0	
Bower [51]	2007		Exp	25	0/25									0										
Wirtz [54]	2007		Exp	44	44/0										0							0		

[a] Morning values were not associated with cytokines prior to the experiment, but high cortisol level was associated with low cytokine levels 24 hours after an induced wound

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory stress test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory stress test increase/ground; d, DST, dexamethasone suppression test; C-S, cross-sectional; Exp, experimental.

Adrenaline and Noradrenaline

Only 2 studies were included in this literature study. Krantz *et al.* [44] reported that there were no associations between levels of salivary cortisol following a laboratory stress test and levels of urinary catecholamines. Cohen *et al.* [57] concluded that there is a non-association between an AUC throughout the day (with respect to ground) and urinary catecholamines in a population-based sample.

DISCUSSION

General Remarks

Before interpreting the results further, there are 4 aspects that should be considered.

First, we have focused our evaluation on biological correlates in healthy populations. This could be part of the explanation for the large number of non-significant findings. It is possible that studies on populations with diabetes would yield a different association between cortisol and glucose than the ones in this evaluation. The focus on healthy populations also contributes to the high loss of articles comparison with the number of hits in the first step of our search. A review on some of these articles can be found in the chapter on somatic outcome.

Second, we have studied only salivary cortisol. The aim was not to determine physiological correlates with cortisol, but rather to test the feasibility of using salivary cortisol in different contexts.

Third, a large proportion of the papers are based on relatively small study populations. This leads to the possibility of a high number of beta errors in our non-significant findings. On the other hand, the results may suffer from publication bias, where non-significant findings are not reported explicitly in some papers, even though analyses were done on cortisol and the biomarker under investigation.

Fourth, the search strategies used may be somewhat incomplete. It is likely that associations between any of the biomarkers and cortisol in saliva have been studied and presented in papers that could not be identified in our search.

Metabolic Abnormalities and Cortisol

The large number of non-significant findings may come as a surprise. This is in contrast to the widespread hypotheses that there is an association between cortisol and the metabolic syndrome. One of the more well-cited research groups [6, 11, 12] base their conclusions on a design that differs from the most commonly used design, as presented in Table **1**. Instead of any of the suggested measures, the authors base their calculations on "stress-induced cortisol secretion", combining 7 cortisol measurements throughout the day plus a dexamethasone test the following morning. The authors present non-significant findings with cortisol values and all of the metabolic parameters tested (including all criteria for metabolic syndrome). When the intra-individual variance (or the inverse intra-individual variance) between all time points is taken into account by weighting the correlations, the associations with the metabolic parameters become significant. This statistical approach has a considerable impact on the results and alters the conclusions in this study. This approach has not been used in any of the other papers examined in this chapter. Hence, the findings from this study that suggest an association between cortisol and metabolic abnormalities cannot easily be compared with the other studies.

One seemingly contradictory finding is that a high BMI and a high waist circumference are associated with a lower cortisol peak than normal. Based on experimental studies, several authors have proposed an opposite association, namely that an excess of cortisol would lead to an accumulation of abdominal fat. The feedback mechanisms to the HPA axis may be of relevance in observational studies. The high levels of cortisol potentially leading to an accumulation of fat will be counterbalanced by either lowering the sensitivity of receptors to circulating cortisol [58] or lowering the levels of cortisol.

An interesting design that occurs rarely in the literature but still may be of relevance is to consider the dynamics of a system. This is a central point of view in a study by Holt-Lunstad and colleagues on the association between a diurnal variation in cortisol and a nocturnal dip in blood pressure [45]. Going back to the theoretic assumptions in the concept of allostasis denoting stability through change [3, 4], studies linking dynamic capacity in 2 or more systems may be of particular importance. Approaches designed to study variability in other systems in relation to cortisol variability should be encouraged to increase knowledge about the role of cortisol in health and disease.

Table 2: Studies included on body mass index, waist circumference, waist/hip ratio, lipid status, glucose, blood pressure, heart rate, heart rate variability, inflammatory markers and other stress hormones sorted by first appearance in text in this chapter

References	Outcome	Study design/group characteristics	Sampling	Laboratory method and standardization in sampling	Statistical approach for cortisol measurement	Statistical analysis, cortisol in relation to outcome	Results	Discussion
Laederach-Hofman 2000 [13]	BMI Waist circumference WHR	Design: C-S No.: 42 m/w:24/18 Age: 42 (SD:9) Group: Inpatients at Psychosomatic Hospital aiming to induce weight reduction. Excl: medication (antihypertensives, antidepressants, tranquilizers) P rate: n.a.	Days: 1 Samples per day: 4 Times of sampling: wake up (usually 06.45 h), 20 min later,+ at 07:30 and 09:30 h Setting: Saliva collected at the hospital on a day following a mental stress test (the BonnDet reaction time)	HPLC	Measurement(s): c1: AUC with respect to ground, Samples used: All 4 morning values	Cortisol data: continous Stratified for age, sex and menopausal status. Correlative statistics (SAS)	c1: Waist circumference were negatively associated with AUC, but no relationship between cortisol and BMI or WHR	The results indicate a decrease of AUC in the morning with growing abdominal obesity
Roy 2001 [14]	BMI LDL Total cholesterol Total/HDL Triglycerides SBP DBP	Design: Exp No.: 82 m/w: 82/0 Age: 19-32 years Group: Healthy men, recently recruited as firefighters Excl: No exclusions for medications or diagnoses P rate:	Days: 1 Samples per day: 7 Times for sampling: Two at rest before stress test. Four during stress test, one after recovery trails. Setting: Laboratory stress test recovery	Biotin-Streptovidin immunoassay Morning sessions in a well-controlled laboratory setting.	Cortisol data: continuous Measurement(s): a2: Mean of resting levels b4: recovery (difference peak during stress test minus value after recovery trails)	Group comparisons of dichotomy on recovery (high vs low)	Group with high recovery (b4) had significantly higher LDL and Total/HDL ratio. a2 were positively assoc with SBP during stress task session. No other significant differences.	The results represent findings in comparatively young fit men, and this may be relavant for the interpretation.
Eller 2001 [15]	BMI WHR Total cholesterol HDL SBP	Design: C-S No.: 121 m/w: 37/84 Age: 44 (SD 8.5) Group: recruitment through public advertising Excl: hypertension P rate: n.a.	Days: 1 Samples per day: 4 Times of sampling: Awakening, +20 mins, +60 mins, 18.00h Setting: Ambulatory	RIA	Measurement(s): a1, awakening a2. +20 and +60 minutes a3: 18.00h	Cortisol data log-transformed Correlations with physiological parameters	No significant associations between cortisol and tested parameters, except for a4, being positively associated with HDL amongst men (but not women)	A correlation between WHR, lipids and cortisol could be expected. The lack of correlations in this material is probably caused by the small size of the study, as well as a small spread of physiological measures.
Kunz-Ebrecht 2003 [16]	BMI WHR SBP DBP Heart rate HRV IL-1ra IL-6	Design: C-S No.: 160 m/w: 98/62 Age: Group: subgroup from Whitehall II Excl: Heart disease, cancer, hypertension or psychiatric illness P rate: n.s	Days: 1 Samples per day: 3 Times for sampling: Just before stress test, immediately after and 45 mins after Setting: Laboratory	time resolved immunoassay with fluorescence detection	Cortisol data: continuous Measurement(s): b4: Reactivity in laboratory stress test (and recovery for HRV) c4: AUC during and after stress test (presented for cytokines)	Participants divided into two groups, upper 40% and lower 40% in response. Group comparisons with chi square tests and logistic regressions.	b4 was positively associated with a HRV inhibition during stress, a lower IL-1 and amongst women, a lower WHR c4 was negatively associated with IL1-ra and IL-6	The mental stress tests were not effective in inducing substantial increases in cortisol, so the responder and non-responder groups were not as distinct as would be desirable. However, lack of cortisol response was associated with heightened cytokine levels .

Steptoe 2004 [17]	BMI WHR HDL Total/HDL	Design: C-S n: 172 m/w: 89/83 age: 47-59 Group: Subgroup from Whitehall II, Excl: Heart disease, cancer, hypertension or psychiatric illness P rate: n.s.	Days: 1 Samples per day: 10 Times of sampling: wake up, 30 min later, and then within eight 30-min time window through the day and evening (8.00-8.30, 1000-1030,… 2200-22.30) Setting: Workday	time resolved immunoassay with fluorescence detection	Measurement(s): a1: Waking a4: Evening minimum (the lower of the values recorded at 2000 - 2030 and 2200-2230) a5: Average over the day (all values between 0800 and 22:30) b1: CAR (30 min - waking) b3: The slope= difference between 0+30 and evening minimum	Cortisol Data: continuous Partial product-moment correlations controlling for age, socioeconomic position, smoking, alcohol consumption and time of waking	a1: No associations a4: No associations a5: No associations b1: Positively with WHR and negatively with HDL and Total/HDL in men (but not in women) b3: Positively with WHR and negatively with HDL and Total/HDL in men (but not in women)	The relationship with b3 (cortisol change over day) was secondary to the association to b1 (cortisol awakening response). When the slope over the day was calculated as the differernce between waking and evening values, there were no relationships with WHR or lipids.
Ward 2004 [18]	BMI WHR SDP DBP Glucose Triglyceride	Design: No.: 678, 122 in indepth-study m/w: 678/0; 122/0 Age: 64 (SD 2.7) Group: Recuitment from population based study Excl: pituitary or adrenal disease, diabetes, glucocorticoid treatment P rate: n.a.	Days:1+1 Samples per day: 1+5 Times for sampling: day 1: 9.00 AM cortisol day 2: Awakening, +15 mins, +30 mins, +45 mins, +60 mins. Setting: Ambulatory	DELFIA	Cortisol data: continous Measurement(s): a2: In entire sample c1: AUC with respect to ground using all five samples day 2.		a1 negatively correlated with BMI and WHR, positively with SDP, DBP and fasting glucose in entire sample, but only associations with BMI and WHR remain in in-depth study c1: No associations	We have confirmed that fasting 9.00 AM cortisol is inversely related to adiposity. Our results did however not suggest that increased pituitary responsiveness was responsible for associations with the components of metabolic syndrome
Patel 2004 [19]	BMI	Design: C-S n: 248 m/w: 128/120 age: men: 41, 16-86 Women: 44, 16-98 Group: Patients attending an otorhinolaryngology clinic Excl: pregnancy, excessive physical exercise or alcohol consumption, night-shift workers, HPA axis dysfunction, hepatic, renal or psychiatric disorders, hormonal contraceptives, glucocorticoid therapy, P rate: n.a.	Days: 2 Samples per day: 1 Times of sampling: Immediately after waking on two consecutive days (06:00-08:00) Setting: Home.	Radioimmuno-assay Participants were instructed not to brush teeth, exercise, smoke eat or drink during the 60 minutes preceding saliva collection and to rince their mouth with water 10 min prior to sampling	Measurement(s): a1: Mean of samples from two days	Cortisol Data: continuous t-test, correlation and regression analyses were used to examine the relationship between cortisol and BMI	No correlation between BMI and cortisol at waking	Morning saliva cortisol levels were independent of age and gender, meaning that partitioning of reference intervals are unnecessary
Weitz 2005 [20]	BMI SBP DBP	Design: C-C No.: 48 m/w 24/24 Age: 26 (SD0.7) Group: Invitations to singletons with low bitrth weight. Participants with normal birth weight *via* public advetrisement Excl: Drug treatment or	Days: 1 Samples per day: 2 Times for sampling: 8.00 AM and 23.00 PM Setting: Ambulatory	RIA Instructed to be taken a leisure day. Subjects were asked to abstain from alcohol on the day of sampling and for one day beforehand, and not to smoke at least an hour prior to sampling.	Cortisol data: Continuous Measurement(s): a2:Sample at 8.00 AM a4: Sample at 23.00 PM	Correlations, split on birth weight	a2: negative association with BMI. Negative association with SBP and DBP amongst participants with low birth weight only. a4: No results presented	The influence of endocrine and metabolic signals on cardiovascular function may be changed specifically in subjects with low birth weight.

		any diagnose with direct impact on HPA-axis P rate: n.a.						
Tull 2005 [21]	BMI Waist circumference	Design: C-S No.: 53 m/w: 0/53 Age: 25-60 Group: Black women in population based study Excl: Diabetes P rate: 77%	Days: 1 Samples per day: 2 Times for sampling: 8.30 AM, 10.30 PM Setting: Ambulatory	Not stated	Cortisol data: continuous Measurement(s): b3: Difference between morning and evening sample	Spearman correlation	B3 was negatively associated with waist circumference. No association with BMI	These findings are consistent with results from other studies, in which higher level of psychological distress and waist circumference are associated with dysregulation of cortisol
Power 2006 [22]	BMI WHR	Design: Pros and C-S n: 6452 m/w: 3176/3276 age: birth, 7 years, 33 years, 45 years. Group: British birth cohort. Born in England, Scotland and Wales in 1 wk in March 1958 Excl: P rate: 54%	Days: 1 (at 45 yr) Samples per day: 2 Times of sampling: 45 minutes after awakening (t1) and 3 hours later on the same day (t2) Setting: Random day.	Commercial immunoassay kit with chemiluminescence detection Participants were instructed to avoid brushing or flossing their teeth, eating, or drinking for 15 minutes before taking each samples	Measurement(s): a2: Cortisol 45 mins after awakening (t1) b2: t1-t2 change in cortisol (decline vs abnormal pattern; *i.e.* flat or rise) c2: AUC with respect to ground	Cortisol Data: Continuous, truncated at <2 nmol/l and >100 nmol/l Data from earlier data collections at 7 years and 33 years were run against cortisol levels at 45 years as outcome. Adjusted for socioeconomic position.	a2 and c2: Negative association with BMI at 7years and 33 years. and with WHR at 45 years. b2: BMI at 33 years. and WHR at 45 years increases probability of abnormal pattern.	Our results demonstrate that adiposity across the life course are related to cortisol levels in midlife. There are however complexities in the associations. For instance, BMI at 33 yr has a significant non-linear association where AUC decreased from the lowest BMI to the 70th percentile, increasing thereafter among the most overweight adults.
Daniel 2006 [23]	BMI	Design: C-S No.: 129 m/w: 0/129 Age: 21-66 Group: workers at industrial sites in North Carolina Excl: P rate: 11%	Days:1 Samples per day: 2 Times for sampling: At awakening and midday (before lunch) Setting: Ambulatory, typical working day.	High sensitivity immunoassay. Instructions verbally and in writing: to avoid alcohol, eating and brushing teeth prior to sampling.	Cortisol data: Continuous Measurement(s): b2: midday minus awakening	Regression models adjusting for age, education, race and worksite	b2 were inversely associated to BMI	BMI may be inversely related because chronic stressors affect BMI directly through endocrine processes that mediate fat deposition and indirectly by behavioral or psychosocial responses that promote weight gain.
Steptoe 2006 [24]	BMI WHR	Design: C-S No.: 83 m/w: 28/55 Age: 18-25 years Group: students at unviersity Excl: medication, suffering from upper respiratory infection P rate: n.a.	Days: 1 Samples per day: 6 Times of sampling: Waking, 15 and 30 min later, and then within 3 30-min intervals over the day: 10:00-10:30 h, 16:00-16:30 h and 20:00-20:30 h Setting: Ambulatory	Participants were instructed not to drink coffee or tea, have breakfast, or brush their teeth before completing sample 3, and to avoid drinking or eating in the 15 min prior to samples 4-6 Participants attending a laboratory psychophysiologic stress testing session were asked after the session to collect saliva on a single day	Cortisol data: continuous Measurement(s): a5: average of samples 4-6. b1: Difference between sample +30 mins and awakening b3: Average slope (between the cortisol value on waking and sample 6 (20:00-2030 h)) c1: AUC with respect to increase using samples 1 to 3.	Participants with delayed samples were excluded. Partial correlation. Hierarchical regression analysis	No significant association for a5, b1, b3 or c1 with BMI or WHR, nor form men or for women.	Data were collected over a single day, and more stable estimates might emerge from repeated measurements.
Therrien 2007	BMI	Design: Pros	Days: 3	RIA	Cortisol Data:	ANOVA followed by	Men with visceral	From this observation, one can

[25]		n: 82 m/w: 51/31 age: 23-51 Group: Selected from public advertisement to match criteria for being lean, abdominal obese or reduced obese. Excl: depression, psychiatric or cardiovascular disorders, medication, smoking, alcohol consumption,	Samples per day: 2 Times of sampling: Awakening and 30 minutes after awakening Setting: Ambulatory. Three different occasions within a period of 2 months	Particiåpants were instructed to refrain from food and drink between the two morning samples. Allowed to drink water between the two samples but not during the 5 minutes before sampling. No alcohol, training or caffeine during a study day.	continuous Measurement(s): b1) CAR as the percentage of increase in cortisol levels between time of awakening and 30 minutes thereafter Cortisol values were adjusted for estradiol levels	post hoc paired Student's t-test to reveal differences between groups. Multivariate analysis of variance to analyse slope of morning cortisol. Tukey-Kramer post hoc test	obesity had a higher b1 (CAR) than lean and reduced obese state. Women in a reduced obese state had a higher b1 (CAR) than lean and obese women.	argue that a great part of gender difeerences between the obese and reduced obese groups came from a different pattern of body fat distribution.
Kidambi 2007 [26]	BMI Waist circumference Total cholesterol LDL HDL Triglycerides Blood glucose Blood pressure	Design: C-S n: 96 m/w: 46/50 age: 18-55 years Group: Subgroup from study on hypertension in a black population 40% hypertensives Excl: P rate: n.a.	Days: 1/2 Samples per day: 2 Times of sampling: 11:00 pm on the day of admission and at 7:00 am the following morning. Setting: Ambulatory	Enzyme immunoassay	Measurement(s): a2: 7.00 AM a4: 11.00 PM	Cortisol Data: continuous t-test or Wilcoxon rank sum test depending on the distribution of the variables.	a2 and a4 was positively associated with blood pressure (hypertension). There were no other associations to any of the tested metabolic parameters.	Despite the results, it seems reasonable based on the well-known actions of glucocorticoids, to hypothesize that cortisol plays a pathophysiological role in the metabolic syndrome.
Coutinho 2007 [27]	BMI Waist circumference WHR	Design: C-S No.: 47 M/W: 0/47 Age: 30-65 years Group: Obese women Excl: using any medication that could interfere metabolism, endocrine disease related to weight gain P rate: n.a.	Days: 1 Samples per day: 1 Times of sampling: 23:00 h Setting: Ambulatory, a random day		Measurement(s): a4. Single time point in the evening, 11.00 PM	Cortisol data: Continuous. Two tailed t-test and Fischer's exact test. Pearson's correlation coefficient	No statistically associations between a4 and BMI, waist circumference or WHR (BMI had a significant negative association in subgroup without binge eating disorder)	A trend toward a negative association between nocturnal salivary cortisol and BMI was found in the entire sample. In women with binge eating disorder, the severity of the binge eating was positively associated with nocturnal levels.
Roberts 2007 [28]	BMI	Design: Pros n: 71 m/w: 0/71 age: 43 (sd=7.1) Group: Healthy registered nurses Excl: Unemployment P rate: n.a.	Days: 2 (baseline and 12 weeks later) Samples per day: 12 Times of sampling: 2-hours intervals during a normal day (8AM to 8PM) Setting: Ambulatory	in-house radioimmunoassay. Participants were instructed to collect samples in the beginning of the academic semester and 12 weeks later during the participants' examination period	Measurement(s): c3: AUC over the day (with respect to ground ground), using all samples	Cortisol Data: Regression analysis	AUC (c3) was not related to BMI at baseline. An increase in AUC were however associated with an increase in BMI.	After inclusion of dietary restraint, there is no longer a relationship between cortisol and BMI, indicating that change of dietary restraint is a significant mediator in the relationship between stress and BMI.
Steptoe 2007 [29]	BMI WHR IL-6 CRP	Design: C-S No.: 2873 m/w: 2126/747 Age: 50-74 years Group: Phase 7 in the Whitehall II-study Excl: Steroids and cardiac medication. No history of CAD P rate: 90%	Days: Samples per day: 5 Times of sampling: After waking, 30 min, 2.5 h, 8 h, and 12 h after waking Setting: Ambulatory	Chemiluminescence immunoassay	Measurement(s): a5. cortisol over day Samples used: the last 4	Cortisol data: χ^2-test. Logistic regression using covariates of age, gender, ethnicity, income, smoking, employment and time of waking	Cortisol over day (a5) was positively associated with BMI, WHR, and IL-6. No association was found with CRP	The HPA axis is thought to downregulate proinflammatory cytokines, whereas IL-6 activates cortisol secretion

Lasikiewicz 2008 [30]	BMI WHR LDL HDL Triglycerides Plasma glucose SBP DBP CRP IL-6	Design: C-S n:147 m/w: 68/79 age: 46.2 (SD=7.18) Group: Healthy adults Excl: smokers and prescribed medication P rate: n.s.	Days: 3 (83 subjects); 1 (64 subjects) Samples per day: 8 Times of sampling: Awakening, + 15 mins, +30 min, +45 mins, +3hours, +6 hours, +9 hours, +12 hours post waking. Setting: Ambulatory	Non-commercial time-resolved fluorescenece (DELFIA) immunoassay All participants attended a pre-study briefing in which the procedure was explained. Participants were asked to re and refrain from consuming food or drink other than water prior to each sample collection time.	Measurement(s): a5: Diurnal mean= mean of 3, 6 ,9, 12h post waking. b3: Slope=regression of the line of decrease from 45 min post waking. c1: AUC with respect to increase (0, 15, 30 and 45 min post waking)	Cortisol Data: Log transformation A K-means cluster - analysis were performed. Two clusters were extracted. Cluster 1: Lower diurnal mean (a5) flatter slope (b3) and lower awakening response (c1), all significantly different from cluster 2.	No differences between cluster 1 and cluster 2 for any of the tested parameters, except for WHR, where cluster 1 had a significantly higher WHR. SBP was positively associated with a5 and c1 in regression models, and DBP was positively associated with a5.	Our results offer partial support to the theory that metabolic syndrome is a metabolic disorder, and that the morning cortisol profile may serve as an additional marker of metabolic vulnerability
Brydon 2008 [31]	BMI Waist circumference IL-1ra IL-6	Design: Exp No. 67 m/w: 0/67 Age: 18-25 Group: Recruited students Excl: BMI<16 & BMI >44 P rate: n.a.	Days: 1 Samples per day: 3 Times for sampling: Baseline, immediately after stress test and 45 minutes after stress test Setting: Laboratory	time-resolved fluorescenece immunoassay	Cortisol data: continuous Measurement(s): a3: Baseline before stresstest b4: reactivity and recovery after stress test	Partial correlations adjusted for age, ethnicity, smoking status and baseline levels.	There were no significant reported associations between a3 or b4 with BMI or waist circumference. There were no associations between cortisol response and cytokine response in stress test.	The tasks were only moderately stressful and cortisol responses were very small. More socially evaluative tasks eliciting more robust changes in cortisol may be required to observe correlations.
O'Donnell 2008 [32]	BMI	Design: C-S No.: 492 m/w: 350/192 Age: 60 (SD 5) Group: subgroup from Whitehall II Excl: Heart disease, cancer, hypertension or psychiatric illness P rate: n.s	Days: 1 Samples per day: 6 Times for sampling: Awakening, + 30 mins, +2.5 hours, +8 hours, +12 hours and just before going to bed Setting Ambulatory	time-resolved fluorescenece immunoassay	Cortisol data: continuous Measurement(s): b1: 30 mins after awakening minus awakening. b3: Slope as decrease per hour between awakening and bedtime values (not including peak) c3 AUC over day not including	Linear regressions adjusted for age, gender, smoking, depression, self-rated health, wake up time and household income.	High BMI was significantly associated with a higher AUC throughout the day, but there were no associations with b1 or b3.	Elevated cortisol is implicated is implicated in a range of health problems including adiposity and cardiovascular disease.
Wirtz 2008 [33]	BMI Glucocorticoid inhibition of inflammation	Design: Exp No.: 42 M/W: M Age:21-65 years Group: recruited from public advertising in Zurich, Switzerland Excl: any medication or chronic condition, current smoker P rate: n.a.	Days: 2 Samples per day: 8 the first day, 4 the second day Times of sampling: TSST starting between 14:00 h and 16:00 h the first day. Samples taken at rest, at TSST start, then 10, 20, 30, 40, 50 and 60 min after test. Day 2: 08:00 h, 11:00 h, 16:00 h and 20:00 h Setting: Laboratory day 1, ambulatory day 2	Chemiluminescence immunoassay Oral and written instructions. Subjects asked to refrain from eating and drinking 30 min prior to each sample. Electronic monitoring for sample times	Measurement(s): a3. Resting value before stress test b3. Diurnal slope b4. Reactivity in laboratory stress test Samples used: a3. Sample between 14:00 h and 16:00 h b3. All four samples day 2 b4. All eight samples day 1	Cortisol data: ANOVA and general linear models with repeated measurements, adjusted for age mean arterial blood pressure and cortisol level at rest	No relationship between cortisol and BMI for a3, b3 or b4 (however, BMI seems to have an impact, lowering the ability to inhibit inflammation with glucocorticoids following a stress reaction)	This study suggests that, while BMI does not seem to affect endogenous cortisol, inflammatory activity following stress is less effectively downregulated with glucocorticoids when BMI increases
Farag 2008	BMI	Design: C-S	Days: 1	Enhanced range enzyme	Cortisol data: log	Regression models	No significant	It is possible that metabolic

[34]		No.: 78 m/w: 0/78 Age: 24-72 Group: Employed in rural public school Excl: None P rate: 38%	Samples per day: 7 Times for sampling: Awakening, +40 mins, 11.00 AM, 2.00 PM, 6.00 PM, 9.00 PM and bedtime Setting:Ambulatory	immunoassay kits Participants received details instructions for producing samples. A typical workday following an overnight fast.	transformed Measurement(s): a1: Awakening a2: +40 mins a3: 11.00 AM and 2.00 PM a4: 6.00 PM, 9.00 PM, bedtime b3: Awake + 40 minutes minus mean of 9.00 PM and bedtime value	with loess fitting. Testing several model including socioeconomy and perceived stress and biological parameters to get best fitted model	associations for BMI with cortisol at any of the seven time points No association between BMI and diurnal slope in the entire sample, but a negative association in subgroup with BMI>=30.	consequences of obesity outweigh neuroendocrine changes due to stress, and are thus stronger predictors for HPA axis function in obese women.
Brummett 2009 [35]	BMI	Design: C-S No.: 328 m/w: not stated Age: 31 (SD:9) Group: Sibling pairs by public advertisements Excl: severe disease or pregnancy P rate: n.a.	Days: 1 Samples per day: 3 Times for sampling: Awakening, +30 mins, and bedtime Setting: Ambulatory	ELISA Samples collected day following laboratory stress test. Participants were instructed not to eat, drink or brush teeth 30 mins prior to each sample.	Cortisol data: continuous Measurement(s): b1: +30 mins minus awakening	Mixed models adjusting fo negative affect, age, ethnicity, sex, income and smoking statuts	None of the co-variates (except positive affect) were significantly associated with awakening response (b1)	
Boyne 2009 [36]	BMI	Design: BMI No.: 40 m/w: 0/40 Age: 37 (SD 5) Group: Cohort of mothers in Jamaica Excl: systemic illness P rate: n.s.	Days: 1 Samples per day: 4 Times for sampling: 8.00 AM, 12.00 AM, 4.00 PM, 8.00 PM Setting:Ambulatory	DELPHIA	Cortisol data: log-transformed Measurement(s): a2: 8.00 AM a3: 12.00 AM, 4.00 PM a4: 8.00 PM b3: a2 minus a4.	Multivariate logistic refgression	No associations between BMI and any of the cortisol measures	A more carefully measured cortisol awakening response could show more distinct associations.
Kajantie 2004 [37]	Waist circumference HDL Triglycerides Blood glucose Blood pressure	Design: C-S No.: 151 m/w: 0/151 Age: 76-77 Group: birth cohort study in Finland Excl: Glucocorticoid treatment P rate: n.a.	Days: 1 +1 Samples per day: Times for sampling: awakening, +15 mins, +30 mins, 12.00 AM, 5.00 PM, 8.00 PM: Day 2: Morning sample Setting: Ambulatory	DELFIA	Cortisol data: Converted to z-scores Measurement(s): a1: Awakening a2: +15 mins, +30 mins a3: 12.00 AM, 5.00 PM a4: 8.00 PM a5: Mean of all b1: Mean of first three samples b3 b1 minus mean of last three samples	Correlations and linear regression adjusted for postmenopausal hormone replacement therapy.	No cortisol measurement was associated with any of the components of metabolic syndrome, except waist circumference being negatively associated with diurnal variability (b3)	Our finding is surprising in the light of several studies showing an association to components of the metabolic syndrome.
Garcia-Prieto 2006 [38]	Waist circumference	Design: C-S No.: 41 m/w: 0/41 Age: 45 Group: Women born in a Spanish town during first six months 1960 Excl: None	Days: 1 Samples per day: 2 Times for sampling: 7.00 AM 10.00 PM Setting: Ambulatory	RIA	Cortisol data: continuous Measurement(s): b3: difference between morning and evening sample	Group comparisons of tertiles	Diurnal cortisol variability was inversely associated with waist circumference	Our results is suggesting that those with a pathological HPA-axis response develop an android pattern of body fat distribution.

		P rate: 80%						
Hucklebridge 1999 [39]	Blood glucose	Design: C-S No.: 27 m/w: 14/13 Age: 20-66 Group: Staff and students at a university Excl: ongoing medication P rate: n.a.	Days: 1 Samples per day: 4 Times for sampling: Awakening, + 10 mins, + 20 mins, +30 mins Setting: Ambulatory	Solid phase RIA	Cortisol data: continuous Measurement(s): b1: Difference between +30 mins and awakening c1: AUC on all values with respect to ground	Correlations with fasting blood glucose	There were no significant correlations with blood glucose for b1 or c1	The psyhiology of the awakening response differs to that from a acute stress response
Nyklicek 2005 [40]	Blood pressure	Design: C-C n: 63 m/w: 30/37 age: 44 (SD: 6) Group: 42 untreated hypertensives and 21 normotensives Excl: none (matched properties) P rate: n.a.	Days: 1+1 Samples per day: 5+1 Times of sampling: baseline sample, after each stressor and next morning at home at awakening. Setting: Laboratory stress test (music, pain, mental arithmetic, speech, films as stressors).	biotin-streptavidin immunoassay with TR-fluorometric detection Tampon kept in mouth 2 min, without stimulation by chewing. Seated in a comfortable chair. Participants were asked to refrain from using alcohol on the day of the experiment, form caffeine consumption for at least 3 hours, and from smoking for at least 2 h prior to the laboratory session.	Cortisol Data: log transformed Measurement(s): a1: sample at awakening a3: baseline (resting) before stress test) b4: response during stress test	Group comparisons of hypertensives vs normotesives MANOVAs Time of measurement was used as covariate for the salivary measures	Blood pressure reactivity correlated with cortisol responses (b4) but not with baseline cortisol (a3) or morning levels (a1).	Our results demonstrate a generalized pattern of physiological hyperactivity in hypertensives, which included the cardiovascular system, the HPA-axis and the immune system.
Wirtz 2006 [41]	Blood pressure	Design: Exp C-C n: 48 m/w: 48/0 age: 44 (SEM: 2) Group: 22 hypertensives and 26 normotensives Excl: Only inclusion of healthy non-smokers P rate: n.a.	Days: 1 Samples per day: 7 Times of sampling: baseline between 2.00 PM and 4.00 PM., and 10, 20, 30, 40, 50 and 60 min. after completion of test Setting: TSST, laboratory stress test	Competitive chemi-luminescence immunoassay with high sensitivity. Participants were instructed to abstain from food and drink (other than water) for 2h before experiment and from physical exercise, alcohol, and caffeinated beverages starting the evening before the test day.	Measurement(s): a3: baseline cortisol at rest before stress test c4: AUC with respect to increase, response during stress test	Cortisol Data: continuous Group comparisons between hypertensives and normotensives ANOVAs for repeated measures, regressions with BMI as covariate	Hypertensives showed higher cortisol reactivity (c4) compared with normotensive controls. Resting levels were not different (a3).	Hypertensive unmedicated and otherwise healthy men show higher cortisol, epinephrine, and norepinephrine secretions after stress.
Wirtz 2007 [42]	Blood pressure	Design: C-C n: 42 m/w: 42/0 age: 45 (SEM 3) Group: 20 unmedicated hypertensives and 22 controls Excl: Only inclusion of otherwise healthy non-smokers P rate: n.a.	Days: 2 Samples per day: day 1: 9/ day 2: 5 Times of sampling: min, at 8:00, 11:00, 15:00 and 20:00 h Setting: Ambulatory, on work days. Dexamethason test next evening - 0.5 mg dexam. at 23 h and new CAR next day.	Participants were instructed to wake up free (no preset time), remain in bed the first 15 min. and not have breakfast the first hour. Refrain from eating or drinking for 30 min before each sample.	Cortisol Data: Log-transformed Measurement(s): a1: Awakening b1: CAR (awakening, 15, 30, 45, 60) b3: Slope using 08.00, 11.00, 15.00 and 20.00 samples c1: AUC in the morning with respect to ground d: Dexamethasone administered at 23.00 h.	Group comparisons between hypertensives and normotensives Univariate analyses and ANOVAs	No difference in awakening levels (a1). Hypertensives had a lower CAR (b1) than did normotensives but circadian profiles were similar (b3). After dexamethasone hypertensives had higher levels of cortisol than normotensives (d).	Apparently healthy hypertensive and normotensive men had significantly different cortisol awakening response and feedback sensitivity suggestiong alterations in HPA functioning in systemic hypertension.
Gregg 1999 [43]	SBP DBP	Design: C-S n: 100	Days: 1 Samples per day: 5	RIA Sampling in connection with	Measurement(s): b4. Reactivity	Cortisol Data: continuous	For mental arithmetic, b4 was significantly	Cortisol measurements and hemodynamic variables

	Heart rate Correlations of salivary cortisol were a sub-result.	m/w: 50/50 age: 17-49 years, mean 20 years Group: healthy non-smoking Excl: P rate:	Times of sampling: 40 min before the first stressor, 25 and 45 min after each of two stressors. Setting: Tests: mental arimetic and cold pressor test.	stress-test in a sound-attenuated, temperature-controlled suite.	measured as change in levels before and after stress tests	Corrrelation analysis one-group t-test	correlated with change in heart rate, but uncorrelated with blood pressure. For cold pressor test b4 was significantly correlated with blood pressor and near significant with heart rate	generally correlated more strongly for the cold pressor test than mental arithmic, although coefficients were only moderate at best.
Krantz 2004 [44]	SBP DBP Heart rate Adrenaline Noradrenaline	Design: C-S n: 21 m/w: 10/11 age: 22.5 (19-29) Group: - Excl: - P rate: n.a.	Days: 1 Samples per day: 4 Times for sampling: Between 9 a.m. and 1 p.m.) Setting:. Laboratory test, mental and physical stress	RIA	Measurement(s): b4. Reactivity measured as change in levels before and after stress tests	Cortisol Data: continuous Correlation analyses	No significant correlation between b4 and any of the physiological parameters.	Cortisol was not significantly influenced by stress exposure tests whereas SBP DBP, Heart rate, adrenaline and noradrenaline increased significantly
Holt-Lunstad 2007 [45]	SBP DBP Measured as nocturnal blood pressure dip	Design: C-S No.: 301 m/w: 146/155 Age: 31.2 (9.8) years Group: Caucasians 82%, Hispanics 6.7%, Asian 4.3%) Excl: any medication, pregnancy P rate: n.a.	Days: 1 Samples per day: 5 Times of sampling: at 07:00 h, 12:00 h, 17:00 h, 22:00 h and on awakening before leaving the bed. Record the exact time of each sample collection Setting: Ambulatory at home	RIA with chemiluminescence Participants were instructed to avoid alcohol 24 h before, not to brush their teeth 3 h before, not to eat a major meal within 60 min before sample collection, to avoid dairy product 30 min before sampling and rinse mouth with water 10 min before sampling	Measurement(s): c3: AUC with respect to ground, using all samples Nocturnal blood pressure dipping was calculated by means of the change score (average day minus average night)	Cortisol data: continuous. Regression analyses, adjusted for age, gender, BMI, phase of menstrual cycle, sleeping quality, morning cortisol, and daytime blood pressure	Diurnal variation in cortisol was a significant predictor of BP dipping: the less cortisol changed the less BP dipping.	Cortisol variation was found to have a stronger relationship with blood pressure dipping than any of the other covariates measured
Lucini 2002 [46]	HRV (Correlations between HRV and salivary cortisol were sub-results.)	Design: C-S n: 30 m/w: not stated age: 22 (1) years Group: healthy, non-smokers of either gender Excl: n.s. P rate: n.a.	Days: 2 Samples per day: 1 Times of sampling: app. 10:30 a.m. two times, first time on a "stress-day" 30-60 minutes before examination. Control-day 3 months afterward. Setting: n. s.	n.s.	Measurement(s): a2: Samples at Stress day and regular day. Regular day and difference between levels at stress day and regular day were used.	Cortisol Data: 2-way ANOVA for repeated measures Paired t-test Correlation analysis	a2: Cortisol levels a regular day and stress-associated change of cortisol levels were significantly associated with indices of heart rate variability (RR, Lfnu, Hfnu, LF/HF).	A mild real life stressor is capable of significantly altering the HPA axis and the cytokine profile, raises arterial blood pressure and produces complex changes in major cardiovascular regulatory mechanisms, without significantly affecting the respiratory pattern.
Sgoifo 2003 [47]	HRV	Design: Exp No.: 30 m/w: 15/15 Age: 25 (SD:2) Group: university students Excl: P rate:	Days: 1 Samples per day: 2 Times for sampling: Before and after stress test Setting: Laboratory	RIA Tested in a laboratory with controlled temparture and light conditions. Two interviews were performed on every day life stressors	Measurement(s): b4: Reactivity during stress test (with continuous ECG recording)	Cortisol data: continuous ANOVa for repeated measures	b4 correlated negatively with HRV (RR variability), the higher cortisol reactivity, the lower variability	Noteworthy, associations were only significant during the first stress episode and not the second
Eller 2007 [48]	HRV (total power and high frequency variability)	Design: Pros. No.: 74 m/w: 24/50 Age: 46 (SD: 8) Group: Subgroup from larget study Excl: not stated P rate: n.a.	Days: 1 Samples per day: 2 Times for sampling: Awakening and +20 mins. Setting: Ambulatory	RIA	Cortisol data: Measurement(s): b1: +20 mins minus awakening	4 year follow-up GLM, repeated method with BMI, WHR, SBP, fibrinogen, cholesterol, HbA1c, catecholamines as covariates	CAR (b1) was negatively associated with HRV (total power and high frequency) for men but not women	The associations were different between the sexes. This can be due to conincidences related to the relatively large number of analyses in a small data set.

Glaser 1999 [50]	IL-1 IL-8 (combination of the two measures as outcome)	Design: Exp No.: 24 m/w: 0/24 Age: 57 (SD: 6) Group: Public advertisement Excl: None P rate: n.a.	Days: 1 Samples per day: 3 Times for sampling: 7.00 AM, 1:30 PM, 8.30 PM Setting: Laboratory	Chemiluminescence A blister in the forearm was induced by suction. All participants followed a detailed laboratory protocol	Cortisol data: continuous Measurement(s): a2: 7.00 AM a3: 1.30 PM a4: 8.30 PM	Group comparisons based on median splits on IL-1 and IL-8 24 hours after wound, participants with high vs low cytokine levels	a2 had no relation with cytokines before the induced wound, but a2, a3 and a4 was all inversely associated with cytokine level 24 hours after induced wound	Stress induced elevations in glucocorticoid levels can alter the carefully regulated system that controls development of the inflammatory response.
Bower 2007 [51]	IL-1beta IL-6 TNF-alpha	Design: Exp No.: 25 m/w: 0/25 Age: Group: Breast cancer survivors Excl: cancer recurrence, other severe condition or alcohol abuse P rate: n.a.	Days: 1 Samples per day: Times for sampling: Immediately before and at 15 minute-intervals Setting: Laboratory	Lab method not stated. Sessions started at 3.30 PM	Cortisol data: Measurement(s): b4: reactivity as baseline to changes 15 minutes after stress test	Spearman correlation to log transformed cytokine levels (LPS-stimulated)	There was a significant negative association between b4 and IL-6, but no associations with IL-1beta or TNF-alpha	Inadequate secretion of cortisol may set the stage for exaggerated inflammatory response to challenge, and possibly to a chronic inflammatory state.
Gaab 2005 [52]	IL-6 TNF-alpha	Design: Exp No.: 41 m/w: Age: Group: 21 participants with chronic fatigue syndrome, 20 matched controls Excl: Medical or psychiatric condition P rate: n.a.	Days: 1 Samples per day: 7 Times for sampling: Just before stress test and regularly until one hour after stressor Setting: Laboratory test (TSST)	Chemoluminiscence assays	Measurement(s): c4: Area under curve with respect to ground	Cortisol data: Continuous ANOVAs to compare groups. Correlations	c4 were not related to levels of IL-6 or TNF-alpha stress test responses	It is indicated that absolute individual salivary free cortisol responses were not related to the differences in pro-inflammatory cytokine responses in participants with or without chronic fatigue syndrome
von Känel 2005 [53]	IL-6	Design: Exp n: 21 m/w: 21/0 age: 47 (SD 7) Group: Employed at Institute of Technology CRP <10 mg/l and BMI <= 29 Excl: No major medical conditions, P rate: n.a.	Days: 1 Samples per day: 4 Times of sampling: Saliva sampled immediately before the preparation phase, immediately after, 45 min and 105 min after stress. All participants had morning sessions (starting between 7.30 AM and 8.30 AM) Setting: Laboratory ,TSST	ELISA All participants received a light standardized breakfast without caffeine and remained staed for additional 30 minutes before stress test	Measurement(s): a4: AUC with respect to increase were performed on all four samples	Cortisol Data: continuos Wilcoxon, Sperman's rho against AUC for IL-6. repeated measures ANOVA.	There were no correlation between c4 and IL-6 stress test response, although both levels of IL-6 and cortisol changed significantly following the stress test.	Unlike previous studies, we did not find a relationship with IL-6. TSST provoke a higher cortisol activity than stressors used in previous studies that could suppress other pro-inflammtory cytokines (IL-1, TNF-alpha) even before IL-6 stimulation occurs in the inflammation cascade.
Wirtz 2007 [54]	IL-6 TNF-alpha	Design: Exp n: 44 m/w: 44/0 age: 21-65 Group: Subpopulation in a larger cohort Excl: Only inclusion of otherwise healthy non-smokers. No atopic allergies P rate: n.a.	Days: 1 Samples per day: day 1: 8 Times of sampling: Baseline, immediately after stress test, then every 10^{th} minute up to 60 minutes after completion of the stress test Setting: Laboratory	Chemiluminiscence immuno assay Laboratory session started between 2.00 PM and 4.00 for all subjects. Participants were asked to abstain from alcohol, physical exercise, and caffeinated beverages since the previous evening.	Measurement(s): c4: AUC with respect to increase following stress test	Cortisol Data: continuous Regressions on AUC with respect to increase of LPS-stimulated cytokine levels, adjusted for BMI and mean arterial pressure.	There were a borderline significance (p=0.057) for an association betwwen a high c4 and a low TNF-alpha. No relation between c4 and IL-6	We did not find that the lowering of cytokine levels throughout the test was significantly affected by the release of stress hormones, as could have been expected from the literature

Luz 2003 [55]	TNF-alpha	Design: Exp. No.: 79 m/w: 30/49 Age: 20-40 + 60-91 Group: Recruited to the SENIEUR study Excl: chronic medical condition and use of glucocorticoids or drugs P rate: n.a.	Days: 1 Samples per day: 3 Times for sampling: 9.00 AM, 12.00 AM, 10.00 PM Setting: laboratory	RIA All samples were taken prior to venepuncture or food intake	Cortisol data: Measurement(s): a2:9.00 AM a3: 12.00 AM a4: 10.00 PM	Pearsons moment product correlations with cytokines stimulated with endotoxin	TNF-alpha levels were positively associated with evening cortisol (a4), but had no relation to the other time points tested (a2 and a3).	Levels of pro-inflammatory cytokines determined in serum may not necessarily reflect those found *in vitro*
Cohen 2006 [57]	Adrenaline Noradrenaline	Design: C-S No.: 193 m/w: 95/98 Age: 21-55 Group: responders to advertisements in news papers Excl: pregnant or chronic disease P rate: n.a.	Days: 3 Samples per day: 7 Times for sampling:Awakening and 1, 2, 4, 7, 9, 11 h after wake up Setting: Ambulatory at home	ELISA	Cortisol data: log transformed c3. Mean over day as the log of AUC for the 3 days	Correlations with adrenaline and noradrenaline as assessed by averaging two 24-hour urine samples.	Neither average adrenaline nor noradrenaline was correlated with cortisol.	The lack of a correlation between average cortisol and catecholamine levels suggests that these systems are not closely associated. It may also have to do with different rates of degradation and uptake of cortisol and catecholamines.

Abbreviations: AUC, Area under curve; BMI, body mass index; CAR, cortisol awakening response; CRP, C-reactive protein; C-C, case-control; C-S, cross-sectional; DBP, diastolic blood pressure; ELISA, enzyme linked immonusorbent assay; Excl, exclusions; Exp, experimental; HDL, high-density lipoprotein; HPA, hypothalamic-pituitary-adrenal axis;; HPLC, high-performance liquid chromatography; HRV, heart rate variability; IL, interleukin; LDL, low-density lipoprotein; m/w, men/women; n.a., not applicable; n.s. not stated; P rate, participation rate; Pros, prospective; RIA, radioimmunoassay; SBP, sysstolic blood pressure; SD, standard deviation; SEM, standard error of mean; TNF, tissue necrosis factor; TSST, Trier social stress test; WHR, Waist-hip ratio.

Inflammation and Cortisol

It is well known that cortisol exerts anti-inflammatory effects. The discovery in the late 1940s that a synthesized cortisol derivate could reverse inflammation in rheumatoid arthritis [59] led to a Nobel Prize. The anti-inflammatory properties have resulted in widespread clinical use of exogenous cortisol (or hydrocortisone as the synthetic form is named).

Thus, the associations in this overview may have been expected to be stronger than shown. In addition to the general problem with possible beta errors due to low statistical power in some analyses, it should be remembered that cytokines are measured in very low concentrations (pg/ml). Thus, statistical analyses on cortisol and cytokines are much more vulnerable to possible insensitivity of laboratory methods than statistical analyses on the other markers covered in this chapter. Moreover, there might also be a considerable variation of cytokine levels within subjects. For instance, von Känel and colleagues compared levels of interleukin-6 (IL-6) measured at a laboratory visit and then a visit 2 weeks later, and found no correlations between IL-6 concentrations at the two time points [60].

However, a couple of studies reported that a higher capability to react with cortisol secretion on a stress test and good capability to recover after a stress test are associated with lower levels of inflammatory markers [16, 50, 51]. Thus, the reported positive association between a higher cortisol output throughout the day and IL-6 [29, 30] is in line with earlier research on the role of cortisol in immunoregulation in studies on patient populations [7, 61]. Fanatidis and colleagues proposed that "inappropriately normal" cortisol levels due to limited capability to respond with increased cortisol levels may not be sufficient to limit an ongoing inflammation [61]. Raison and Miller describe a situation "when not enough is too much", with increased levels of cortisol due to downregulation of receptors on target cells, making glucocorticoid signaling in immunoregulation insufficient [7].

The total numbers on cytokines and other inflammatory markers are few, especially compared with the number of studies focusing on some or several aspects of metabolic abnormalities. This implies that the main paradigm in stress research on cortisol and its physiologic effects has focused on energy supply rather than regulation of inflammation. More research on the association between cortisol in saliva and inflammatory markers is needed to elucidate whether salivary cortisol may be a marker of relevance in clinical settings regarding diseases related to inflammation such as autoimmune diseases and coronary events.

Adrenaline, Noradrenaline and Cortisol

The number of studies reviewed is low perhaps because it is unusual to draw blood and collect saliva samples in the same study design (in comparison, there were 2090 hits on PubMed for a search on "cortisol and "adrenaline" but omitting "saliva*"). It is therefore not easy to draw any general conclusions on associations between salivary cortisol and adrenaline or noradrenaline. The few studies included in this overview do not provide support for a strong relationship.

In recent research, salivary alpha-amylase has been suggested as a good proxy for activity in the sympathetic adrenal medullary axis [62, 63]. This opens up the possibility to explore the association between the two main stress hormone systems in observational studies, using non-invasive procedures. A few studies have investigated the association between salivary alpha-amylase and salivary cortisol [64, 65]. None have reported any significant correlations between the two stress hormone systems, for single time points or change over the day. This is in line with the studies presented in this chapter evaluating salivary cortisol in relation to urinary catecholamines.

Limitations

A number of problems can be listed when studying the association between salivary cortisol and other physiological measures. Most of the other physiological measures were measured in other matrices than saliva, typically blood or urine. Using different matrices may pose problems *e.g.* due to half-life and timing of samples. When looking at well-designed studies of cortisol, measurement of salivary cortisol has been found to be an

excellent indicator of unbound concentrations of cortisol in serum [66-69]. The studies show that the correlation between mean saliva cortisol and mean serum cortisol were approx. r = 0.6 and with a mean cortisol concentration in serum 10-20 times higher than the concentration measured in saliva. Furthermore, even though there is a close correspondence regarding circadian fluctuations of cortisol in saliva and plasma [70], the half lives of various biological measures are not easily comparable to cortisol and per se dependent on the biological fluid. In most of the studies included in the present chapter the sampling of saliva and blood or urine was most often carried out at different time points. A profound diurnal variation will affect the results, particularly if there is a large variation in the time difference between samples or when during the day samples are taken. *E.g.* the difference in concentrations of cortisol between two saliva samples taken on hour apart is much larger in the morning compared to the evening due to the diurnal variation in cortisol.

Comparison between concentrations of cortisol in saliva and other physiological measures in urine poses yet another problem. Whereas concentrations in saliva is affected only by the past few minutes concentrations measured in urine represent the mean excretion since the past urine void.

The sampling design is very closely related to whether the topic of concern is acute or long term stress or a mixture. If the biological measure has a low diurnal and monthly variation and there is an effect of a stressor, it is relevant to talk about a stable measure over time. In contrast, a biological measure with a large diurnal and monthly variation will be influenced by a number of daily hassles. However, the general level of which the measure is fluctuating around may be influenced by a long term perception of stress [71].

CONCLUSIONS

The number of non-significant findings was considerable. This is true for metabolic abnormalities, markers related to inflammation as well as other stress hormones. This overview may suffer from a large number of studies with relatively small study populations. With regard to metabolic abnormalities, the data point to an association between a lower cortisol peak than normal and high BMI or high waist circumference. Further studies on inflammatory markers are needed to elucidate the association with cortisol in saliva.

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CHAPTER 6

Sleep and Salivary Cortisol

Anne Helene Garde[1,*], Berndt Karlson[2], Åse Marie Hansen[3], Roger Persson[4] and Torbjörn Åkerstedt[5]

[1]Senior reseacher at the National Research Centre for the Working Environment, Lersø Parkallé 105, 2100 København Ø, Copenhagen, Denmark; [2]Doctor in Occupational and Environmental Medicine,at the Department of Public Health and Clinical Medicine, Umeå University, Sweden; [3]Senior reseacher at the National Research Centre for the Working Environment, Copenhagen, Denmark; [4]Senior reseacher at the National Research Centre for the Working Environment, Copenhagen, Denmark and [5]Professor at the Stress Research Institute, Stockholm University, Stockholm, Sweden

Abstract: The aim of the present chapter was to analyze whether measures of cortisol in saliva were associated with measures of sleep and to explore if divergent results were related to underlying differences in theoretic assumptions and methods. Measures of sleep quality included sleep duration, overall sleep quality, difficulty falling asleep, disturbed sleep, and sleep deprivation. Twenty-three papers were found to fulfil the inclusion criteria. Cortisol measures were grouped into single time points at different times during the day, deviations at different time periods during the day, reactivity and recovery after a standardized laboratory test, area under the curve and response to dexamethasone test. A large proportion of the studies included showed nonsignificant findings, which, in several cases, may be a result of low power. The most consistent results were a positive association between sleep duration and single measures of salivary cortisol at awakening, which was observed in 3 studies. In these studies, sleep duration was also associated with low evening cortisol levels, steep diurnal deviation of cortisol and/or high area under the curve. Together these findings suggest that longer sleep duration is related to a more dynamic cortisol secretion. Two of the 6 studies on disturbed or restless sleep showed relations to flat diurnal deviation and low laboratory stress test reactivity. This to some extent corroborates the findings on sleep duration. However, the many nonsignificant findings as well as the theoretical and methodological differences (*e.g.*, heterogeneity in measures) complicate comparisons. Conflicting results may be at least partially due to differences in methods and underlying assumptions.

Keywords: Salivary cortisol, sleep, sleep quality, sleep duration, sleep deprivation, difficulty falling asleep, single time point measures, deviations measures, area under the curve, dexamethasone.

INTRODUCTION

The stress response can be described as an increase in arousal in response to a real or anticipated perturbation of homeostasis [1]. The Hypothalamus-Pituitary-Adrenal Cortex (HPA) axis is one of the main stress systems with cortisol as a main actor [2, 3]. The underlying anatomy of the stress response is closely interconnected with the anatomy that regulates sleep and wakefulness [4, 5]. Emotional and cognitive arousal may therefore provide inputs that override the normal circadian and homeostatic processes that otherwise govern sleep and wakefulness in normally healthy humans [4, 6]. The interconnectedness also makes sleep a potent factor that may modulate most components of the endocrine system [6]. To summarize, there is a possible bidirectionality between stress and sleep.

Cortisol levels have a circadian peak early in the morning, show a decline throughout the day and are near the limits of detection in the late evening [6]. The secretion of cortisol is inhibited at sleep onset, and during the early part of the sleep period, and cortisol concentrations continue to decrease until a few hours before normal waking time when they start to rise again [6-8].

In experimental studies, induced sleep deprivation lead to higher cortisol concentrations the subsequent

***Address correspondence to Anne Helene Garde:** Senior reseacher at the National Research Centre for the Working Environment, Lersø Parkallé 105, 2100 København Ø, Copenhagen, Denmark; Tel: +45 39 16 52 00; Fax: +45 39 16 52 01; E-mail: ahg@nrcwe.dk

Margareta Kristenson, Peter Garvin and Ulf Lundberg (Eds)

evening [8] and HPA axis hormones such as cortisol-releasing hormone had a negative effect on sleep quality with increased episodes of rapid eye movement sleep and inhibited Slow-Wave Sleep (SWS). In contrast, cortisol has been shown to promote SWS [9].

Although the theoretic and empirical evidence of a close interconnectedness between sleep and HPA axis hormones is strong, there are still several unknowns with regard to understanding the interplay between stress reactions and sleep. As in other areas of stress research, findings have been disparate on these interactions.

AIM

The aim of the present chapter was to analyze whether measures of cortisol in saliva were associated with measures of sleep and to see if possible divergent results were functions of differences in assumptions made and methods used.

METHOD

In a first step, an online search of the NCBI PubMed database (National Library of Medicine, National Institutes of Health, Bethesda, MD, USA-http://www.ncbi.nlm.nih.gov/PubMed) was conducted. The search covered the time period up to October 1, 2009. The search terms were "sleep AND (saliva OR salivary) AND cortisol". One hundred and eight-eight papers were found after limiting the search to papers written in English and studies on humans. Of these, 69 were selected for further scrutiny based on the titles and abstracts. They were supplemented with hand searches. In this step, studies were only included in this review if the study group comprised healthy adults and the study included specific statistical analyses of the association between sleep and cortisol.

Measures of sleep quality included (1) sleep duration, (2) overall sleep quality, (3) difficulty falling asleep, (4) disturbed sleep, (5) premature awakening, and (6) sleep deprivation. Sleep duration is a well-defined measure of the number of hours a person sleeps. It may be assessed from self-reports, actigraphy, or polysomnography (PSG). Reports on sleep quality such as ease of awakening, sleep efficiency, and sufficient sleep by use of questionnaire, logbook or actigraphy were all considered as indicators of overall sleep quality. Sleep quality may be related to sleep problems and divided into categories related to different parts of the sleep: difficulty falling asleep, disturbed sleep (difficulties maintaining sleep), and premature awakening. Difficulty falling asleep covered ease of sleep (inverted), speed of sleep onset (inverted), sleep latency, but not sleep onset, and time of falling asleep. Disturbed sleep covered restless sleep, nocturnal awakenings, time awake after sleep onset, number of microarousals during the night, and number of wake periods after sleep onset. In studies of sleep deprivation participants are actively kept awake.

In the following analyses findings were considered significant if p-values were <0.05. As most of the studies had small numbers and seemingly low statistical power, we also included marginally significant results ($0.05<p<0.10$) denoted by arrows in parentheses in Table **1**.

RESULTS

In total 23 papers fulfilled the inclusion criteria. A brief summary of the results (indicated as arrows denoting positive associations, or negative association and zero for a nonsignificant finding) are presented in Table **1**. More detailed information on study design, statistical approach, main results, and discussion for each of the 23 papers is presented in Table **2**.

Results are presented for each sleep measure. Cortisol measures were grouped as follows. Single time points at: a1, awakening; a2, morning; a3, midday; a4, evening; a5, all day. Deviations during: b1, morning; b2, midday; b3, morning to evening; b4, laboratory test. Area Under the Curve (AUC): c1, morning (increase/ground). Suppression test: d, response to dexamethasone (DST). No studies were found for premature awakening.

Sleep Duration

Thirteen papers were found to test the association between salivary cortisol and sleep duration [10-22]. In the 13 papers there were 37 analyses on relationships between measures of salivary cortisol and sleep

duration. The proportion of significant relationships were 4/16 (25%) for single time points, 6/12 (50%) for deviations, 2/8 (25%) for AUC and 0/1 (0%) for dexamethasone test.

The most consistent results were a positive association between sleep duration and a single measure of salivary cortisol at awakening found in 3 studies [19-21]. In these studies, sleep duration was also associated with low evening cortisol levels [19], steep diurnal deviation of cortisol [19, 20], and with high AUC [21].

In 7 studies the authors failed to find any statistically significant associations between single measures of cortisol and sleep duration [11, 12, 15-17, 22]. The size of these studies was, in general, very small.

The association between sleep duration measures and deviations in cortisol measures was investigated in 7 studies. Morning deviations in cortisol concentrations were found to be positively associated with sleep length in an experimental study of 16 young people (8 morningness and 8 eveningness) using PSG [16]. In 2 ambulatory studies with more than 200 participants [10, 14] and a study of 2761 civil servants using self-reports negative associations to morning deviation in cortisol concentrations [20] were found.

Two studies showed a positive association between self-reported sleep duration and diurnal deviation of cortisol [19, 20]. In 4 other studies, no significant associations were found [11, 12, 15, 22], although tendencies were observed in 1 [22].

Morning AUC was the only AUC investigated in relation to sleep duration [12, 13, 18, 21, 22]. One study, a case study with 50 days of sampling, showed a positive relationship. In contrast, 1 study, which used an insomnia scale and defined sleep duration as “more than six hours sleep”, showed a negative relationship. Two out of 4 studies had only nonsignificant findings.

Overall Sleep Quality

Associations between sleep quality and measures of salivary cortisol were assessed in 8 studies [11, 12, 15, 17, 19, 21, 23, 24]. In the 8 papers there were 28 analyses on relationships between measures of salivary cortisol and overall sleep quality. The proportion of significant relationships was 5/21 (24%) for single time points, 1/5 (20%) for deviations, and 0/2 (0%) for AUC. Sleep quality was measured mainly by use of self-reports, but also PSG [13].

The most consistent pattern, a positive association to a single measure at awakening [11] or in the morning [17, 23], was observed in 3 studies. However, 5 other studies found no associations with a single morning or awakening cortisol measure [12, 15, 19, 21, 24]. In 4 studies, sleep quality was examined in relation to single measures in the afternoon or an evening measure; no associations were found [11, 15, 19, 24]. No significant associations were seen for sleep quality and deviations in cortisol concentrations [12, 19, 24].

One study found a positive relationship between stress reactivity and sleep quality measured as sleep efficiency by actigraphy, but not by self-reports [15]. One study examined associations between sleep quality and morning AUC, and found no significant relationship [21].

Difficulty Falling Asleep

Three studies assessed a total of 10 associations between salivary cortisol and difficulty falling asleep [15, 23, 25]. The proportion of significant relationships was 0/5 (0%) for single time points, 2/3 (67%) for deviations, and 1/2 (50%) for AUC. Difficulty falling asleep was assessed by use of actigraphy and self-reports (ease of sleep (inverted), speed of sleep onset (inverted), sleep latency, and time to fall asleep). The studies all used different types of cortisol measures.

Only 1 of the 3 studies reported significant associations, and the results were mixed [25]. In the same study the association between self-reported difficulty falling asleep in terms of ease of sleep was positively related to slope, whereas speed of sleep onset was negatively related [25]. High self-reported difficulty falling asleep was related to high AUC morning [25]. No other significant associations were observed between self-reported ease of sleep and measures of cortisol [15, 23, 25].

Disturbed Sleep/Restless Sleep

Disturbed or restless sleep was examined in 6 studies [11, 12, 15, 20, 21, 26] analyzing a total of 22 relationships. The proportion of significant relationships was 3/13 (23%) for single time points, 4/7 (57%) for deviations, and 0/2 (0%) for AUC. Disturbed sleep was assessed as the number of microarousals during the night using PSG, forced awakening, actigraphy, and self-reports (restless sleep, nocturnal awakenings, time awake after sleep onset, and number of wake periods after sleep onset).

Four studies included associations with a single cortisol measure at awakening or in the morning: 1 found a positive association with the number of microarousals [12], 1 found a negative association with self-reported frequency of nightly awakenings, but no association with self-reported wake time after sleep onset [11], and 2 found no associations [20, 21]. No associations were observed for single measures of cortisol later in the day [11, 15].

One study investigated the relation between disturbed sleep and diurnal deviation and found a negative association [20]. No significant findings were seen in the 3 studies that investigated the relationship between morning deviations of cortisol and disturbed sleep in terms of nightly microarousals[12], forced awakenings [26], and sleep disturbance [20]. One study investigated the effect of disturbed sleep the night before a laboratory stress test, and found negative associations with reactivity [15].

AUC in the morning was tested in relation to disturbed sleep on a day to day basis in a case study with 50 days of sampling; and no significant associations were found [21].

Sleep Deprivation

Six studies investigated a total of 8 associations between sleep deprivation and measures of salivary cortisol with mixed results [27-32]. The proportion of significant relationships was 2/5 (40%) for single time points, 1/3 (33%) for deviations, and 0/0 (0%) for AUC. The studies used either 1 night of sleep deprivation [28-30, 32] or 5-6 nights of only 4 h sleep [27, 31].

In 1 study sleep restriction was associated with increased concentrations of cortisol in the evening and smaller decline in cortisol during the afternoon [27]. In another study it was found that cortisol concentrations were higher in the afternoon after sleep deprivation [29]. In 4 studies using cortisol concentrations in the morning, evening, and during the day following sleep deprivation, no associations were observed [28, 30-32].

Table 1: Summary of main findings of associations between measures salivary cortisol and studied domains sorted by year of publication

References	Year	Exposure	Awakening time	Design	*n*	M/W	Single time points (or sum/mean of two or more time points)					Deviation Difference/slope for two or more time points				AUC		Dexamethasone suppression test
							a1	a2	a3	a4	a5	b1	b2	b3	b4	c1		d
Sleep duration																		
Wüst [10]	2000	SR		C-S	509	190/319						↓						
Backhaus [11]	2004	SR		C-C	29	21/8	(↓)	0		0								
Ekstedt [12]	2004	PSG	07:00 h ±1 h	C-S	24	10/14	0	0				0						
Federenko [13]	2004	SR	04:00 h	Exp	49	0/49						0					0	
Schlotz [14]	2004	SR		C-S	219	102/117	0					↓						
Wright [15]	2007	SR/AG			53	0/53			0						00			
Griefahn	2008	PSG		Exp	16	16/0		0				↑[b]						

[16]																		
Gustafsson [17]	2008	SR		C-S	25	13/12		00										
Liberzon [18][a]	2008	SR		Pros	31	13/18										0	0	
Hsiao [19]	2009	SR	06.65 h, SD 1.3	C-S	106	35/71	↑			↓				↑				
Kumari [20]	2009	SR	06:13-07:44 h	C-S	2751	?	↑					↓		↑				
Stalder [21]	2009	SR		C	1	1/0	↑									0	↑	
Vreeburg [22]	2009	SR	07:20, SD 1.1	C-S	491	199/292		0		0		(↓)		(↓)		↓	0	0
Overall sleep quality																		
Bailey [23]	1991	SR		C-S	20	16/4		↑*										
Backhaus [11]	2004	SR		C-C	29	21/8	↑↑	00		00								
Ekstedt [12]	2004	PSG	07:00 h ±1 h	C-S	24	10/14	0	0				0						
Wright [15]	2007	SR			53	0/53			00						↑0			
Gustafsson [17]	2008	SR		C-S	25	13/12		↑↑00										
Dahlgren [24]	2009	SR		Pros	14	8/6	0	0		0		0						
Hsiao [19]	2009	SR	06.65 h, SD 1.29	C-S	106	35/71	0			0				0				
Stalder [21]	2009	SR		C	1	1/0	0									0	0	
Difficulty falling asleep																		
Bailey [23]	1991	SR		C-S	20	16/4		00										
Wright [15]	2007	AG			53	0/53			0						0			
Lasikiewicz [25]	2008	SR		C-S	147	68/79					00			↓↑		↓0		
Disturbed sleep																		
Backhaus [11]	2004	SR		C-C	29	21/8	↓0	00		00								
Ekstedt [12]	2004	PSG	7 AM ± 1 h	C-S	24	10/14	↑	↑				0						
Dettenborn [26]	2007	Forced awake		Exp	13	0/13						0						
Wright [15]	2007	AG			53	0/53			000						↓↓↓			
Kumari [20]	2009	SR	6:13-7:44	C-S	2751		0					0		↓				
Stalder [21]	2009	SR		C	1	1/0	0									0	0	
Sleep deprivation																		
Spiegel [27]	1999	Sleep restricted		Exp	11	11/0				↑			↓					
Heiser [28]	2000	Forced		Exp	10	10/0								0				
Goh [29]	2001	One night		Exp	14	0/14			↑									
Pagani [30]	2009	One night		Exp	24	12/12		0		0								
Van Leeuwen [31]	2009	Restricted		Exp	19	19/0					0							
Birchler-Pedross [32]	2009	40 h		Exp	32	16/16								0				

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory test reactivity/recovery; c1, morning increase/ground; AG, actigraphy; AUC, Area under the curve (increase vs ground) ; C, case; C-C, case-control; C-S, cross-sectional; Exp, experimental; M, men; Pros, prospective; PSG, polysomnography; SR, self-reported; W, women. ↑ indicates that the slope is steeper.

[a]Sleep length >6 h.

[b]Significant finding only in evening types.

Table 2: Descriptives of the articles on salivary cortisol and sleep parameters sorted by domain of sleep parameter and year of publication

References	Outcome	Study design/group characteristics	Sampling	Laboratory method and standardization in sampling	Statistical approach for cortisol measurement in relation to sleep	Statistical analysis, cortisol in relation to outcome	Results on cortisol and sleep
Wüst 2000 [10]	*Sleep length:* TST *Method:* Self-report	Design: C-S No.: 509 M/W: 190/319 Age: 37.3 (18-71) years Group: Healthy	Days: 2 Samples per day: 4 Times for sampling: Awakening, +15, 30, and 60 min, Setting: Ambulatory (at home)	RIA	Measurement(s): a1. Cortisol on awakening b1. Mean increase from awakening c1. AUC	Cortisol data: Continuous Statistics: Pearson correlation and ANOVA with repeated measures	Positive correlation between sleep duration and mean cortisol increase from awakening (b1)
Backhaus 2004 [11]	*Sleep length:* TST *Sleep quality:* PSQI, feeling of recovery *Disturbed sleep:* Frequency of nightly awakenings, wake time after sleep onset *Method:* Questionnaire (PSQI), feeling of recovery	Design: C-C No.: 29 M/W: 21/8 Age: 32-62 years Group: insomniacs (*n*=14) and healthy controls (n=15)	Days: 7 Samples per day: 3 Times for sampling: Awakening, +15 min and before going to bed Setting: Ambulatory (at home)	RIA Not to use food, alcoholic beverages, caffeine, fruit juice, or brush teeth 1 h before sampling	Measurement(s): All by means of same time point over the 3 consecutive days a1. Cortisol on awakening a2. Cortisol 15 min after awakening a4. Cortisol at bedtime	Cortisol data: Continuous Statistics: Pearson correlation and ANOVAs	Trend for negative correlation between TST and cortisol at awakening (a1) Positive correlation between sleep quality, and feeling of recovery, and cortisol at awakening (a1) Negative correlation between frequency of nightly awakenings and cortisol at awakening (a1). No correlation between wake time after sleep onset and awakening cortisol (a1) No correlation between sleep parameters and cortisol 15 min after awakening (a2) or cortisol at bedtime (a4)
Ekstedt 2004 [12]	*Sleep length:* *TST* *Sleep quality:* Sleep efficiency *Disturbed sleep:* Number of arousals *Method:* 2 PSG recordings carried out in the subject's home (before workday/ day off)	Design: C-S No.: 24 M/W: 10/14 Age: 30.5 ± 0.5 years Group: High (n=12) and low (n=12) burnout, recruited from a Swedish IT company	Days: 2 Samples per day: 9 Times for sampling: Awakening, +15, 30, and 60 min, 11:00, 15.00, 19:00, 21:00 h and bedtime Setting: Ambulatory. Saliva collected at day after the PSG	RIA No current smokers, non-sedentary lifestyle and moderate alcohol intake	Measurement(s): a1. Single awakening sample a2. Awakening cortisol as a mean morning value of 4 samples, at awakening, 15, 30, 60 min post awakening b1. Deviation morning value (CAR (difference 0-60 min)	Cortisol data: Log transformed Statistics: Stepwise multiple regression analyses. Pearson correlation coefficient Confounders:	No association between total sleep time and awakening cortisol or mean cortisol within 60 min after awakening No association between sleep efficiency and cortisol More nightly arousals were associated with higher awakening cortisol and mean cortisol within 60 min after awakening No association between any sleep measure and morning deviation
Federenko 2004 [13]	*Sleep duration:* TST *Method:* Self-report	Design: Exp No.: 49 M/W: 0/49 Age: nurses: 40.3 years, students: 25 years Group: Nurses working shifts (n=18) and students with regular sleep cycle (n=31)	Days: 2 Samples per day: 4 Times for sampling: Awakening, +30, 45 and 60 min Setting: Nurses: collected 1st and 2nd day of 3 different shifts. Students: after early evening nap on 2 days	RIA Not to smoke, eat and drink just water in the first hour after awakening, not to brush teeth, avoid microinjuries in oral cavity	Measurement(s): b1. Mean increase from awakening c1. AUC_{ground}	Cortisol data: Statistics: Person's correlations Confounders: Oral contraceptives	No correlation between sleep duration and mean increase from awening or AUC_{ground}

Schlotz 2004 [14]	*Sleep length:* ? *Method:* ?	Design: C-S No.: 219 M/W: 102/117 Age: 48.6 (24-83) years Group: Healthy	Days: 7 consecutive Samples per day: 4 Times for sampling: Awakening, +15, 30, and 60 min, Setting: Ambulatory (at home)	RIA	Measurement(s): a1. Cortisol on awakening b1. Mean increase from awakening	Cortisol data: Continuous Statistics: ANOVA with repeated measures	No association between sleep duration and cortisol on awakening (a1) Positive association between sleep duration and mean cortisol increase from awakening (b1)
Wright 2007 [15]	*Sleep duration:* TST (actigraphy and self-reports) *Sleep quality:* Sleep quality and sleep efficiency *Difficulty falling asleep:* Sleep latency *Disturbed sleep:* Wake up %, minutes awake, number of wake periods *Method:* Actigraph and sleep log (Pittsburgh sleep diary) over 7 days	Design: C-S No.: 53 M/W: 0/53 Age: 37.3 (± 9.9) years Group: Healthy	Days: 1 Samples per day: 4 Times for sampling: Base line cortisol before stress test (14:00 h), post test, +30 min and 45 min post test Setting: Laboratory with stress test	Immunoassay After stress test the participants were asked to relax and read general interest magazines	Measurement(s): a3. Single measure at baseline (14:00 h) b4. Reactivity to test	Cortisol data: Logarithmic (base 10) Statistics: Pearson´s correlations, univariate analysis and partial correlations adjusting for baseline cortisol	Positive association between sleep efficiency (actigraph) and cortisol reactivity to test Negative association between all 3 disturbed sleep and cortisol reactivity to test No association between TST, sleep latency, self-reports of sleep quality and length and cortisol reactivity to test No association between actigraph measures and baseline cortisol (14:00 h)
Greifahn 2008 [16]	*Sleep duration:* TST *Method:* PSG	Design: Exp No.: 16 M/W: 16/0 Age: 19-27 years Group: morningness (n=8), eveningness (n=8)	Days: 6 days (?) Samples per day: 2 Times for sampling: 7:00 h, +30 min. Only those when wakeup is after 06:50 h Setting: Laboratory	LIA (IBL) No smoking, no teeth brushing prior sampling	Measurement(s): a2. Single measures at 07:00 h b1. Deviation morning concentration, at 07:00 h and 30 min later	Cortisol data: Continous? Statistics: ANCOVA with repeated measures correlation Confounders:	TST had positive association with b1 after night sleep TST not associated with cortisol at awakening (a2)
Gustafsson 2008 [17]	*Sleep duration:* Sleep length *Sleep quality:* Sufficient sleep, generally difficulties sleeping because of work *Method:* Questionnaire	Design: C-S No.: 25 M/W: 13/12 Age: 24-62 years Group: White collar workers	Days: 2 Samples per day: 6 Times for sampling: 15-30 min after awakening and every 2 h until 20:00 h Setting: Ambulatory	RIA All participants were asked to rise and go to bed at the same times during days of measurement	Measurement(s): a2. Two measures, approx. 07:00 h and 09:00 h	Cortisol data: ? Statistics: Linear regression. Repeated measures ANOVA Confounders:	Association between less sufficient sleep (better sleep quality) and higher morning cortisol. (both measures) No association between sleep duration or difficulties sleeping because of work and morning cortisol
Liberzon 2008 [18]	*Sleep duration:* *Method:* Not mentioned in methods section	Design: Follow-up No.: 31 M/W: 13/18 Age: 18-38 years Group: Students (n=23) and science staff (n=4) and ships crew member (n=4)	Days: 6 Samples per day: 4 Times for sampling: Awakening, +15, 30, 45 min Setting: Ambulatory	RIA Not to eat, drink, smoke, brush teeth or rinse mouth until after 45 min sample	Measurement(s): c1g. AUC with respect to ground c1i. AUC for increase (awakening response)	Cortisol data: Statistics: Confounders: Perceived stress and control (Likert scale)	No other correlation between total sleep time and cortisol measures in total sample
Hsiao 2009 [19]	*Sleep duration:* Total time slept *Sleep quality:* Sleep quality last night *Method:* Questionnaire	Design: C-S No.: 106 M/W: 35/71 Age: 38.5 years (SD 9.7) Group: 106 healthy subjects (and 126 patients	Days: Samples per day: 5 Times for sampling: Awakening, + 45 min, 12:00 h, 17:00 h, 21:00 h Setting: Ambulatory	RIA Not to brush teeth, avoid oral blood contamination before sampling, Not to eat 45 min after awakening and 30 min before collecting samples	Measurement(s): single measures at awakening and over a day a1. Awakening cortisol a4. Evening cortisol b3. Deviation (diurnal	Cortisol data: Natural logarithm Statistics: Two-level individual growth curve model. (multiple regression model for nested, repeated data) Confounders: Several	Association between longer sleep and steeper slope (b3), higher awakening cortisol (a1), lower cortisol in the evening (a4) No association between sleep quality and cortisol

		with major depression - not used in present review)			profile)	confounders are adjusted for in two different models	
Kumari 2009 [20]	*Sleep duration:* TST divided into 1-h categories *Disturbed sleep:* Sleep disturbance *Method:* Logbook and questionnaire	Design: C-S No.: 2751 M/W: ? Age: ? Group: Whitehall	Days: Samples per day: 5 Times for sampling: Awakening, +30 min, +2.5, 8, 12 h Setting: Ambulatory	Immunoassay method Provide 6 samples on a normal weekday	Measurement(s): a1. Single time point, awake b1. Deviation morning profile b3. Deviation morning to evening (profile)	Cortisol data: Log cortisol data Statistics: Multilevel, interaction term Confounders: Age, sex, employment grade, awakening time, smoking status, waist circumference	Association between long sleep duration and higher cortisol. at awakening (a1) and steeper diurnal slope (b3). Association between long sleep duration and flatter morning slope (b1) Association between less disturbed sleep and steeper diurnal slope (b3)
Stalder 2009 [21]	*Sleep duration:* TST *Sleep quality:* *Disturbed sleep:* Nocturnal awakenings *Method:* Sleep log	Design: Case study No.: 1 M/W: 1/0 Age: 27 years	Days: 50 with 3 days interval Samples per day: 4 Times for sampling: Awakening, +15, 30 and 45 min Setting: Ambulatory	ELISA method Sat for 15-30 min when sampling, otherwise moved freely in relation to sampling times	Measurement(s): a1. Awakening concentration c1. AUC_{ground} c1. $AUC_{increase}$	Cortisol data: Log transform Statistics: Repeated measures ANOVA Confounders: Alcoholic drinks consumed the evening before measurement day	Positive association between sleep duration and awakening cortisol and AUC_{ground} No association between disturbed sleep and sleep quality and cortisol No association between any sleep parameters and $AUC_{increase}$
Vreeburg 2009 [22]	*Sleep duration*: Sleep length (more or less than 6 h) *Method:* Insomnia rating scale	Design: C-S No.: 491 M/W: 199/292 Age: 43.0 years Group: volunteers without psychopathology Excl: Taking antidepressants, pregnant or breastfeeding, on medication with corticosteroids P rate: 78.3%, with at least one usable cortisol measurement	Days: 1 Samples per day: 7 Times for sampling: Awakening, +30, 45, 60 min, 22:00 h, 23:00 h. Samples taken more than 5 min from protocol time were discarded Setting: Ambulatory. Day after dexamethasone 0.5 mg directly after sampling time at 23:00 h	Immunoassay method When sampling no eating, smoking, drinking tea or coffee, or brushing teeth 15 min before	Measurement(s): a2. a4. b1. Deviation morning b3. Deviation 23:00 h, awakening time divided by numbers of hours in between (diurnal slope) c1i. $AUC_{increase}$ morning c1g. AUC_{ground} morning d. Post dexamethasone (DST)	Cortisol data: AUC, evening cortisol and DST were log transformed Statistics: Linear mixed models or linear regression analysis Confounders: Sociodemographic factors, health indicators	Less than 6 h sleep is associated with increase in CAR ($AUC_{increase}$) Tendency for less than 6 h sleep is associated with steeper morning deviation (b1) and a steeper diurnal slope (b3) No association with morning cortisol (a2), evening cortisol (a4), AUC_{ground} morning, or post dexamethasone
Bailey 1991 [23]	*Sleep quality:* Sleep quality *Difficulty falling asleep:* Sleep onset *Method:* Sleep log	Design: C-S No.: 20 M/W: 16/4 Age: 23-39 years Group: morning types (n=10) and evening types (n=10). Recruited from the general population	Days: 1 Samples per day: 7 Times for sampling: Arising, +20, 40, 60, 80, 100, and 120 min Setting: Ambulatory	RIA	Measurement(s): a2. Single cortisol levels	Cortisol data: Continuous Statistics: *t*-test, Pearson product-moment correlation coefficients, Spearman rho correlation coefficients	Positive association between sleep quality and total cortisol in evening type group, but not in morning type group No association between sleep onset and total cortisol in evening or morning type group
Dahlgren 2009 [24]	*Sleep quality:* *Method:* Karolinska sleep diary for 4 weeks	Design: C-S No.: 14 M/W: 8/6 Age: 44 years Group: Office workers	Days: 28 Samples per day: 3 Times for sampling: Awakening, +15 min, bedtime Setting: Ambulatory	RIA No food, no teeth brushing 30 min before saliva sampling	Measurement(s): Single time points: a1. Awake a2. Morning a4. Evening b1. Deviation morning	Cortisol data: Log data Statistics: Multiple regression analyses by time. ANOVA of repeated measurements Confounders: Work day, work load, awakening time, stress at bedtime, sleep quality, stress, sleepiness exhaustion, self related health	No associations between sleep quality and measures of cortisol

Lasikiewicz 2008 [25]	*Difficulty falling asleep:* Ease of sleep and speed of sleep onset *Method:* Questionnaire (Leeds Sleep Evaluation Questionnaire)	Design: C-S No.: 147 M/W::68/79 Age: :mean age 46.2 years (±7,2) Group: volunteers	Days: 1 (*n*=64) or 3 (*n*=83) Samples per day: 8 Times for sampling: Awakening, +15, 30, 45 min, +3, 6, 9, 12 h Setting: Ambulatory	Immunoassay method Not to consume food or drink other than water in relation to sample collection. Avoid teeth brushing and vascular leakage	Measurement(s): Mean of same time point on consecutive days a5. b4. Deviation evening from 45 min post awakening (slope) c1. AUC not specified	Cortisol data: Log transformed Statistics: Pearson's correlation. Cluster analysis (M)ANOVA Confounders: Age, gender	Association between higher ease of sleep (less difficulty falling asleep) and low AUC Association between high ease of sleep (less difficulty falling asleep) and less steep slope (b3) Association between high speed of sleep onset (less difficulty falling asleep) and more steep slope (b3) No association between ease of sleep, speed of sleep onset and diurnal mean (a5)
Dettenborn 2007 [26]	*Disturbed sleep:* *Method:* Three experimentally induced awakenings (phone call). The wake up in the morning was optional or set up by alarm clock. No other sleep registration	Design: Exp No.: 13 M/W: 0/13 Age: 24 years	Days: 3 intervention nights + 3 reference nights Samples per day: 8 on intervention nights and 2 on recovery nights Times for sampling: Awakening and +15 min in the morning Setting: Ambulatory	CLIA	Measurement(s): b1. Repeated measures	Cortisol data: continous Statistics: ANOVA and ANCOVA Confounders: Oral contraceptives, thyroid hormone	The morning CAR after disturbed nights was not different from CAR on undisturbed nights There was a lack of HPA axis activation by forced nightly awakenings
Spiegel 1999 [27]	*Sleep deprivation:* Sleep restriction	Design: Exp No.: 11 M/W: 11/0 Age: 18-27 years Group: Young healthy volunteers	Days: 3 Samples per day: 12-20 Times for sampling: Every 30 min between 15.00 and bedtime Setting: Laboratory. 3 nights with 8 h in bed, 6 nights with 4 h in bed, and 7 nights with 12 h in bed	RIA	a4. Single evening concentration b2. Deviation between 16:00 h and 21:00 h	Cortisol data: Continuous Statistics: ANOVA for repeated measures	Higher evening cortisol concentration after sleep restriction Lower rate of decrease in the afternoon after sleep restriction
Heiser 2000 [28]	*Sleep deprivation:* 3 days covered, after ordinary sleep, 1 night of total sleep deprivation and 1 night of recovery	Design: Exp No.: 10 M/W: 10/0 Age: 27.4 ± 2.8 years Group: healthy volunteers	Days: 3 Samples per day: 3 Times for sampling: 07:00, 13:00, 19:00 h Setting: Laboratory	RIA All intake of pineapples, bananas, almonds, nuts, tomatoes, vanilla, or alcohol forbidden, no smokers	Measurement(s): b3. Diurnal profile with 3 measures per day over 3 days	Cortisol data: ? Statistics: ANOVA with repeated measures	No effect on salivary cortisol rhythm of sleep deprivation
Goh 2001 [29]	*Sleep deprivation:* 24 h sleep deprivation or 8 h sleep (control)	Design: Exp No.: 14 M/W: 14/0 Age: 20-30 years Group: healthy subjects, military service members	Days: 2 Samples per day: 3-5 Times for sampling: 08:00, 13:30, 18:00, 21:00, 24:00 (day 1), 08:00, 13:30, 18:00 (day 2) Setting: Laboratory	Immunoassay	b3. Deviation, all day	Cortisol data: Continuous Statistics: Two-way ANOVA with repeated measures and interaction terms	Significant interaction between sleep status and time. Cortisol levels at 13:30 h were increased after sleep deprivation
Pagani 2009 [30]	*Sleep deprivation:* 7 normal nights + 24 h sleep deprivation) or normal living conditions (strict sleep-wake schedule 23:00-07:00 h)	Design: Exp No.: 24 M/W: 12/12 Age: 27-45 years Group: healthy subjects	Days: 8? Samples per day: 2 Times for sampling: 10.30 and 18.00 h Setting: Laboratory	RIA	Measurement(s): a2. a4.	Cortisol data: Continuous Statistics: Mixed model or GLM analysis. Intraclass correlations Confounders:	No effect of sleep deprivation on cortisol

Van Leeuwen 2009 [31]	*Sleep deprivation:* 2 baseline (8 h sleep), 5 nights of 4 h sleep, and 2 recovery nights of 8 h. Controls (8 h sleep) all nights	Design: Exp No.: 19 M/W:19/0 Age: 19-29 years Group: 13 young healthy men + 6 controls	Days: Samples per day: 10 Times for sampling: Not specified Setting: Laboratory	Competitive CLIA Napping during day time was not allowed, meals standardized (calories and time), controlled illumination and room temperature	Measurement(s): a5. Averaged throughout the day	Cortisol data: Statistics: *t*-tests and Wilcoxon signed ranks test for not normally distributed differences Confounders:	No change in salivary cortisol after sleep restriction
Birchler-Pedross 2009 [32]	*Sleep deprivation:* 40 h sleep deprivation or nap protocol	Design: Exp No.: 32 M/W: 16/16 Age: 25.0±3.3 and 65.0±5.5 years Group: Young and older healthy volunteers	Days: 2 Samples per day: 5-6. Times for sampling: every 30 min collapsed into 08:00, 12:00, 16:00, 20:00, 24:00 h (day 1), 04:00, 08:00, 12:00, 16:00, 20:00, 24:00 h (day 2) Setting: Laboratory	RIA	b3. Deviation, all day	Cortisol data: Continuous Statistics: Repeated measures ANOVA with interaction terms	Significant four-way interaction term (time of day, age, gender, sleep pressure) in model for cortisol, most likely driven by time of day None of the other variables were significant
Vreeburg 2009 [22]	*Sleep duration:* Sleep length (more or less than 6 h) *Method:* Insomnia rating scale	Design: C-S No.: 491 M/W: 199/292 Age: 43.0 years Group: volunteers without psychopatology Excluded: Taking antidepressants, pregnant or breastfeeding, on medication with corticosteroids P rate: 78.3%, with at least one usable cortisol measurement	Days: 1 Samples per day: 7 Times for sampling: Awakening, +30, 45, 60 min, 22:00, 23:00 h. Samples taken more than 5 minutes from protocol time were discarded Setting: Ambulatory. Day after dexamethasone 0.5 mg directly after sampling time at 23:00 h	Immunoassay method When sampling no eating ,smoking, drinking tea or coffee, or brushing teeth 15 min before	Measurement(s): a2. a4. b1. Deviation morning b3. Deviation 23:00 h to awakening time divided by number of hours in between (diurnal slope) c1i. $AUC_{increase}$ morning c1g. AUC_{ground} morning d. Post DST	Cortisol data: AUC, evening cortisol and DST were log transformed Statistics: Linear mixed models or linear regression analysis Confounders: Sociodemographic factors, health indicators	Less than 6 h sleep is associated with increase in CAR ($AUC_{increase}$) Tendency for less than 6 h sleep is associated with steeper morning deviation (b1) and a steeper diurnal slope (b3) No association with morning cortisol (a2), evening cortisol (a4), AUC_{ground} morning, or post dexamethasone

Abbreviations: AUC, Area under the curve; C-C, case-control; C-S, cross-sectional; CAR, cortisol awakening response; CLIA, chemiluminescence-assay; DST, Dexamethasone test; ELISA, Enzyme Linked Immuno-Sorbant Assay; Exp, experimental; GLM, generalized linear model; LIA, luminescence immunoassay; M, Male; PSG, polysomnography; PSQI, Pittsburgh Sleep Quality Index; P rate, Response rate; RIA, radioimmunoassay; TST, total sleep time, W, women.

DISCUSSION

The aim of the present chapter was to analyze whether measures of cortisol in saliva were associated with measures of sleep to see if divergent results were functions of differences in theoretic assumptions made and methods used. Relatively few papers were first identified (n=188), and only 23 papers met the final inclusion criteria.

The most consistent results were a positive association between sleep duration and a single measure of salivary cortisol at awakening, which was observed in 3 studies [19-21]. In these studies, sleep duration was also associated with low evening cortisol levels [19], steep diurnal deviation of cortisol [19, 20], and with high AUC [21]. Together these findings suggest that longer sleep duration is related to a more dynamic cortisol secretion.

However, long sleep duration is also associated with a lower CAR [10, 14, 20]. Since a lower CAR implies a less dynamic response these observations seems to contradict the suggestion that longer sleep duration is related to a more dynamic cortisol secretion. However, a lower CAR needs not to be mutually exclusive with a dynamic cortisol secretion as assessed by the decline in concentrations during the entire day. Indeed, a lower CAR might just reflect the diurnal rhythmicity of cortisol secretion. For example, to the extent that participants, who sleep long wake up later than usual, they are likely to take their first sample at a time when the concentrations of cortisol already have risen due to the normal diurnal rhythm. Hence, there is simply less room to obtain a high CAR as the morning value has been inflated by the underlying diurnal rhythm. However, there might also be several other explanations and this question deserves more attention in future studies.

Two of the 6 studies on disturbed sleep showed that it was associated with less diurnal deviation [20], and lower reactivity to a laboratory stress test [15]. This to some extent corroborates the findings on sleep duration. However, the many nonsignificant findings as well as the theoretic and methodological differences (*e.g.* heterogeneity in measures) complicate comparisons.

As expected, statistically significant associations were more often reported in studies with a large number of participants or a high sampling frequency. Among the papers included, statistically significant findings were generally seen in studies with more than 100 participants [10, 14, 19, 20], or samples (case study) [21]. In the large studies, it was more common to focus on general sleep patterns, whereas the case study registered day to day variations within the same person. The results suggest that a relationship between sleep and salivary cortisol is observable both within and between subjects, but requires many observations due to high variability in both cortisol and sleep measures. The use of small study samples and non-optimal measurement procedures might lead to studies with low power and failure to detect statistically significant results.

Even if the inclusion criteria drastically reduced the number of papers, the papers included covered several different types of measures and indicators for cortisol and sleep. This heterogeneity in measures and methods appears to generate a mixed pattern of results that are also conflicting at times. For example, in the same study a positive relationship was observed between stress reactivity and sleep quality measured as sleep efficiency by actigraphy, but not by self-reports [15]. As differences between objectively measured and self-reports of sleep have been observed previously, this conflict may be partially related to the selected instrument for measurement of sleep quality [33].

There are several methodological problems in studies of sleep and saliva cortisol. Because saliva sampling requires that the person is awake, it is virtually impossible to sample saliva during sleep and to establish the relationship with different sleep stages or other processes that may occur during sleep. It is often not practically feasible in field studies to obtain direct measurements during the sleep period with, for example, PSG or endocrine measures. Even if it is technologically possible to use such measures, the procedures and commitments necessary for successful implementation have often been considered to be cumbersome and too expensive to be considered as a realistic option. Stress researchers often have to rely on less sophisticated and simpler approaches such as motion logging (*e.g.*, actigraphy) and subjective reports of various aspects of sleep (*e.g.*, bedtime, awakening time, and perceived quality of sleep).

Many of the studies were not conducted primarily to evaluate the relationship between sleep and salivary cortisol. Even so, all studies included in the present chapter have used statistical tests to address the overall question about the relationship between sleep and saliva cortisol. Considering that research have been driven by many points of departures, it is not surprising that most of the result do not act in concert.

CONCLUSIONS

In total 23 studies examining sleep quality in relation to salivary cortisol measures were identified. There was a large proportion of nonsignificant findings and many operational definitions of sleep quality and cortisol secretion. Because many of the studies included were small and entailed few measurements, there is reason to believe that the nonsignificant findings partly reflect low statistical power.

The most consistent results were our observation of a positive association between sleep duration and a single measure of salivary cortisol at awakening, which was observed in 3 studies. In these studies, sleep duration was also associated with low evening cortisol levels, steep diurnal deviation of cortisol, and/or with high AUC. Together these findings suggest that longer sleep duration is related to a more dynamic cortisol secretion. Two of the 6 studies on disturbed or restless sleep showed a relationship with flat diurnal deviation and low laboratory stress test reactivity. This to some extent corroborates the findings regarding sleep duration. However, the many nonsignificant findings as well as the theoretic and methodological differences (*e.g.*, heterogeneity in measures) complicate comparisons. Conflicting results may be at least partially due to differences in methods and underlying assumptions.

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CHAPTER 7

Mental Health and Salivary Cortisol

Ingibjörg H. Jonsdottir[1,*] Christina Halford[2] and Frida Eek[3]

[1]Associate professor at the Institute of Stress Medicine, Carl Skottsbergs gata 22 B, 413 19 Gothenburg. Sweden; [2]Researcher at the Department of Public Health and Caring Sciences, Uppsala University, Sweden and [3]Associate professor at the Division of Occupational and Environmental Medicine, Lund University, Sweden.

Abstract: The aim of this chapter was to analyze associations between measures of cortisol in saliva and mental health and to see if divergent results were functions of the methods used. Measures of mental health outcome included Major Depressive Disorder (MDD), symptoms of depression, and symptoms of anxiety, Burnout (BO), and Vital Exhaustion (VE). Only studies on otherwise healthy individuals were included. Cortisol measures were grouped into single time point measures, measures of deviations, laboratory test responses, Area Under the Curve (AUC), and response to dexamethasone. Some consistency is seen for MDD, mainly higher mean levels. The results regarding single measures and depressive mood are less consistent, but the overall picture for depression shows poorer diurnal deviation and response to stress. Inconsistency among papers studying depression seems to be related mainly to the study population. Very few significant findings were found for anxiety, therefore cortisol does not seem to be strongly related to anxiety. Most of the statistical analysis does not show a significant relationship between BO and cortisol, and when these are present, the results are inconsistent. One explanation seems to be the measures of BO used, probably due to the different conceptual basis for BO. VE measured using the Maastricht Questionnaire seems to be related to a poorer cortisol response to stress and poorer diurnal deviation. The coexistence of BO and VE in many studies does make it difficult to conclude how the different concepts are related to cortisol. However, an interesting difference appeared between MDD and VE in response to dexamethasone administration, showing lower suppression in MDD patients and higher suppression in VE patients. A general conclusion for all mental health measures is that a large proportion of non-significant findings are due to low power and few sampling days combined with low contrasts between study groups and within study populations. Generally, deviation measures such as diurnal deviation seem to be more valid measures compared with single measures to capture possible changes in the hypothalamus-pituitary-adrenal axis, measured using salivary cortisol.

Keywords: Salivary cortisol, depression, anxiety, major depression disorder, burnout, vital exhaustion, single time point measures, deviations measures, area under the curve, laboratory test, dexamethasone.

INTRODUCTION

Mental health consequences of long-term stress exposure can vary greatly between individuals, and symptoms of depression, anxiety, Burnout (BO), and Vital Exhaustion (VE) are often, but not always, related to psychosocial stress exposure. There is a great discrepancy in the literature regarding the relationship between the Hypothalamus-Pituitary-Adrenal (HPA) axis function and mental health outcomes. This review only deals with salivary cortisol and the aim is to analyze if the existing literature, studying the above-mentioned mental health symptoms and salivary cortisol, show consistent findings and if not, can the discrepancy be explained. Previous literature on depression shows inconsistent findings and the finding on hypercortisolemia in patients with depression is not always confirmed in the literature [1, 2]. In this chapter, the studies are separated depending on whether patients with Major Depressive Disorder (MDD) were studied or if the study deals with the relationship between salivary cortisol and depressive mood.

Anxiety has been shown to be characterized by hypocortisolemia and supersuppression after

***Address correspondence to Ingibjörg Jonsdottir:** Associate professor at the Institute of Stress Medicine, Carl Skottsbergs gata 22 B, 413 19 Gothenburg. Sweden; Tel: +46 31 342 0716; Fax: +46 31 41 42 73; E-mail: Ingibjorg.jonsdottir@vgregion.se

Margareta Kristenson, Peter Garvin and Ulf Lundberg (Eds)

dexamethasone [3] but there is a discrepancy in the literature regarding the relationship between HPA axis function and anxiety, one possible explanation being that anxiety is a multifacetted phenomenon. Cortisol Awakening Response (CAR) does not seem to be significantly affected by anxiety according to the meta-analysis by Chida and Steptoe [2] and HPA axis reactivity in response to a stressor does not seem to be significantly affected by anxiety [4].

The constructs of BO and VE are suggested to reflect responses, in terms of exhaustion, due to long-term stressor exposure. BO is a mental condition defined as a result of continuous and long-term stress exposure, particularly related to psychosocial factors at work [5, 6]. However, the theoretic basis for the term burnout differs between the available self-report instruments constructed to assess BO, and different instruments do seem to measure quite different aspects depending on the theoretic base for the instrument. However, they share the fact that they were mainly developed for research on work-related stress. The most widely used instrument is the Maslach Burnout Inventory (MBI) and the conceptual basis for MBI is thus often considered as synonymous with the construct BO [7]. Maslach and colleagues originally defined BO as a psychological syndrome of emotional exhaustion, depersonalization (later replaced with the construct cynicism) and reduced effectiveness or personal accomplishment [6]. Another conceptual approach was presented by Melamed and coworkers, viewing BO as a multidimensional construct consisting of emotional exhaustion, physical fatigue, and cognitive weariness, which together represents the core component of BO [5, 8]. The concept of VE is characterized by unusual fatigue, loss of mental and physical energy, increased irritability, and feeling of demoralization. The concept was developed in the search for premorbid psychological characteristics of people who developed myocardial infarction, and has thereafter been used to define the psychological state viewed as chronic stress [9, 10]. The concepts of BO and VE do share several features and many studies do not distinguish between these two concepts. Furthermore, within the same concept, *e.g.*, BO, different measures can differ considerably and the dissimilarities could thus be larger within the concept BO compared with dissimilarities between the BO and VE concepts. Based on the definitions used, the concepts of MDD, depressive state, BO and VE, all share several conceptual similarities; *e.g.*, high correlation between the scales used to measure BO and VE is seen [11], and between scale scores of depressive state and VE [12]. In this review, BO and VE are treated partly as the same construct when the general interpretations are done, but both constructs are also initially divided into respective measures as these can differ considerably as previously mentioned. Kudielka and coworkers have recently reviewed the literature on cortisol measurements in BO and VE, concluding that there seems to be a considerable divergence on data regarding HPA axis functioning in chronically distressed individuals [11]. As both hypoactivity and hyperactivity of the HPA axis has been reported in studies on BO or VE, the direction of the supposed dysregulation of the HPA axis remains inconsistent.

Another related concept is chronic fatigue which is not considered in this review, mainly because it is difficult to discriminate chronic fatigue and chronic fatigue syndrome, which is sometimes, but not always, due to postviral infection or other somatic conditions. Also, several symptoms related to chronic fatigue, *e.g.,* muscle pain, joint pain, sore throat, and tender cervical nodes, are of different character compared with BO, even though fatigue is the core feature as for both BO and VE. Furthermore, chronic fatigue syndrome is not as distinctively related to psychosocial stress as the concepts of BO and VE.

AIM

The aim of this chapter is to analyze if measures of cortisol in saliva are associated with depression, anxiety, BO, and VE, all of which are measures of mental health frequently related to chronic psychosocial stress. Furthermore, we aim to see if the divergent results in studies involving cortisol assessments and mental health are functions of differences in the theoretic assumptions made and methods used.

METHOD

Search Strategies

For all outcome measures, an electronic search was performed in the NCBI PubMed database (National Library of Medicine, National Institutes of Health, Bethesda, MD, USA-http://www.ncbi.nlm.nih.gov/PubMed). The

database PsycINFO was also searched for relevant papers not found in the PubMed database. The search covered the time period up to October 1, 2009. Only full-length articles published in English in peer-reviewed journals, based on adult study populations, including direct statistical analyses of associations between cortisol and the actual outcome measure, were included. Studies were selected in two steps, with the first step based on titles and abstracts and, when relevant, by reading the full-length article.

Depression

Search terms "depression" and truncated "salivary cortisol" generated 324 abstracts. The exploration and results were divided into two sections: one included papers studying MDD according to DSM-IV and salivary cortisol and the second part included papers studying depression measured with different self-rated scales and salivary cortisol in otherwise healthy individuals. Thus, studies on subjects with apparent somatic diseases (*e.g.*, cancer and traumatic brain injury) or psychiatric illness (*e.g.*, posttraumatic stress disorder (PTSD) and bulimia) other than anxiety were excluded. Studies on pregnancy and postnatal depression were also excluded. Intervention studies (*e.g.*, medications) were included if comparison between groups was available before the intervention. However, the effects on salivary cortisol in response to the intervention are not included in this review. Papers studying a possible relationship between salivary cortisol and vulnerability to developing future depression were not included. In some cases, the same research group published different data sets using the same patient material and in the cases where the salivary cortisol data were repeated, only one of the papers is included in this review. Studies using instruments measuring mood state (*e.g.*, Profile of Mood States Questionnaire) were not included. The final number of papers included was 21 studying MDD and salivary cortisol and 14 studying the relationship between scores measuring symptoms of depression and cortisol. Reasons for exclusions were: 116 papers because the patients had comorbid conditions (such as cancer and cardiovascular diseases), in 103 papers statistical analyses between depression and cortisol were not presented; 24 papers studied pregnancy or postnatal depression; in 13 papers, cortisol was measured in children. Twenty-seven papers studied MDD and cortisol in saliva, but did not include a control group and 6 papers studied previous or future depression.

Anxiety

The search terms were "anxiety" and truncated "salivary cortisol". The search on PubMed generated 281 abstracts. In order to limit variation in the anxiety measures, we decided to limit the inclusion to three scales used to measure symptoms of general anxiety levels: the Spielberger State-Trait Anxiety Inventory (STAI), the Beck Anxiety Inventory (BAI) and Hospital Anxiety and Depression (HAD) scale. Only papers on otherwise healthy individuals were included. Thus, studies on subjects with apparent somatic diseases (*e.g.*, cancer and traumatic brain injury) or psychiatric illness (*e.g.*, PTSD and bulimia) other than comorbid depression were excluded. Studies reporting cortisol responses to psychosocial stress were included if anxiety levels were related to salivary cortisol in the statistical analysis. Studies of salivary cortisol and its relation to mood changes (state anxiety) were not included. Results from intervention studies (*e.g.*, medication) were included if in the statistical analysis the relation between trait anxiety and salivary cortisol was measured before the intervention (basal levels). Studies of acute anxiety scores and salivary cortisol levels due to, for example, parachute jumping or other similar physical stress were not included. The final number of papers included was 17 and all except 1 used STAI to measure anxiety levels.

Burnout and Vital Exhaustion

The following search terms were used: "cortisol", "saliva*", "burnout" and "exhaustion". The first step identified 31 papers investigating associations between cortisol and BO and 28 studies investigating associations between cortisol and exhaustion. Studies based on patient populations (other than BO), pregnant women, and studies investigating effects of intervention on cortisol were excluded, as were studies in which analyses of direct associations between BO or exhaustion and cortisol were not presented. Concerning exhaustion, studies primarily investigating the effects of physical exhaustion on cortisol were excluded. Finally, 21 papers were included, 12 of which investigated associations between BO measures and cortisol in saliva, and 7 with associations with exhaustion measured using the Maastricht Questionnaire (MQ) [9] or the exhaustion subscale of MBI.

RESULTS

Results are presented for each mental health measure. Cortisol measures are grouped into: single time points at: a1, awakening; a2, morning; a3, midday; a4, evening; a5, all day. Deviations during: b1, morning; b2, midday; b3, morning to evening; b4, laboratory test. AUC: c1, morning; c2, midday; c3, morning to evening; c4. laboratory test. Dexamethasone suppression test: d, response to dexamethasone (DST).

MDD and Depressive Mood

Twenty-one papers were included covering MDD, and diagnosis based on DSM-IV was used in these studies to confirm depression. In the 14 papers studying depressive mood, 6 different instrument were used: Becks Depression Inventory (BDI) (5 papers), Center for Epidemiologic Studies Depression Scale (CES-D) (4 papers), the Zung Self-Rating Depression Scale (2 papers), Hamilton Depression Inventory (HDI) (1 paper), HAD scale (1 paper) and the Patient Health Questionnaire (PHQ) (1 paper). A brief summary of the results (indicated as a positive association, a negative association, or a non-significant finding) is presented in Tables **1a** and **1b**. The study design, statistical approach, main results, and discussion for each of the 35 articles are briefly presented in Table **2a** (MDD) and Table **2b** (depressive mood).

As seen in Tables **1a** and **1b**, there is a large variation in the salivary cortisol measures used in different studies. Among the 21 studies on MDD, most of the measures (39 measures) were from single time points, and 7, 10 and 6 measures were on deviations, AUC, and after DST, respectively. The proportion of significant relationships (in any direction) among the 21 papers studying MDD was 20/39 (51%) for single measure, 3/7 (43%) for deviation measures, 6/10 (60%) for AUC measures, and 4/6 (67%) for the DST studies.

Among the 14 studies on depressive mood, the proportions of significant relationship were 8/20 (40%) for single measures, 4/10 (40%) for deviation measures, and 1/2 (50%) for AUC measures. No DST suppression test study was found for depressive mood.

The only consistency among studies on MDD was reactivity on laboratory stress (one study) or daily event measured in ambulatory settings (one study), as both studies showed poorer ability to respond with cortisol to acute stress. The only study included in this review, studying response to stressor in relation to depressive mood, also showed that response to naturalistic stressors was poorer in women with higher scores of depressive mood.

No single measure of salivary cortisol showed full consistency regarding either significant or non-significant results for either of the groups (MDD or depressive mood). The most consistent significant findings among the MDD papers were single evening measures and single morning measures; 5 out of 10 measures of evening cortisol (50%) showed increased cortisol levels and 10 out of 20 morning measures (50%) showed increased levels. Among the papers studying single measures in relation to depressive mood, no clear consistency can be found, and there are too few measures for each time point to draw any firm conclusions regarding depressive mood and single measures of salivary cortisol.

For deviation measures, 1 study examining CAR in MDD patients showed non-significant findings, 1 study of 4 on diurnal deviation was significant and showed an inverse relation for MDD and cortisol. Both studies on stress reactivity were significant (one on men only), showing poorer cortisol response among MDD patients. The same pattern is seen for the studies on depressive mood, as the 3 studies examining CAR did show non-significant findings; 3 out of 6 studies on diurnal deviation were significant, all showing an inverse relation between depressive mood and cortisol. Three out of 5 studies measuring salivary cortisol after dexamethasone administration reported that MDD patients showed less suppression, usually calculated as more non-suppressors among the patients.

Comparing results from case-control studies on MDD patients and population studies on depressive mood, two measures showed full consistency. Thus, as all three studies on cortisol reactivity in response to stress

showed that depression or depressive mood was related to poorer response to stress and the four studies including CAR deviation measures reported non-significant findings. Full consistency across MDD and depressive mood studies was also found among significant findings for the deviation between morning and evening, as all studies reporting significant relation, showed that flatter diurnal curve was related to depression or depressive mood.

Table 1a: Summary of main findings of associations between measures of salivary cortisol and MDD sorted by year of publication

References	Year	Exposure	Design	No. cortisol	m/w	Single time points (or sum/mean of two/more time points)					Deviation (difference/slope between two or more time points)					AUC								Dexamethasone suppression test
						a1	a2	a3	a4	a5	b1	b2	b3	b4		c1		c2		c3		c4		d
Copolov [13]	1989		C-C	88	0/88				↑															0
Galard [14]	1991		C-C	32	5/15[a]		↑																	
Michael [15]	2000		C-C	85	26/59		↑		↑															
Stanton [16]	2001		C-C	27	12/15					0														
Weber-Hamann [17]	2002		C-C	45	0/45		↑																	
Galard [18]	2002		C-C	26	4/10[a]		↑																	↑[b]
Young [19]	2002		C-C	89	31/58		0		0															
Watson [20]	2002		C-C	57	30/27																0			
Bauer [21]	2002		C-C	67	27/40		0																	↑
den Hartog [22]	2003		C-C	63	32/31		0		↑	0			↓											
Porter [23]	2003		C-C	40	19/21																0			
Peeters [24]	2003		C-C	86	35/49								0	↓[c]										
Assies [25]	2004		C-C	26	6/20		0		0				0											
O'Brien [26]	2004		C-C	101	23/78		0	↑	↑												↑			
Bhagwagar [27]	2005		C-C	60	29/31	0	↑↑↑0				0						↑							
Stetler [28]	2005		C-S	73	0/73	0	↓↓										↓							
Juruena [29]	2006		C-C	32	7/25					↑											↑			↑
Alhaj [30]	2007		C-C	56	27/29		0		0				0											
Treadway [31]	2009		C-C	38	18/20		↑	0	0	↑														
Chopra [32]	2009		C-C	54	28/26									↓[d]									↑[e]	
Vreeburg [33]	2009		C-S	1009	376/633	↑	↑↑0		↑0							0	↑							0↓[f]

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory test increase/ground; C-C, Case-Control; C-S, Cross-sectional.

[a]Information regarding males and females only reported for the patient group.

[b]Measured after DST 16:00 h . No suppressors among the patients (compared with none among controls) but statistical analyses not presented.

[c]Lack of response to negative daily event in patients with MDD compared with controls.

[d]Significant in males only.

[e]Significant in women only.

[f]Level of cortisol not significant (0), cortisol suppression ratio significantly different. More suppression in the patient.

Anxiety

In total, 17 papers studying the relation between salivary cortisol and general anxiety trait were included. A brief summary of the results (indicated as a positive association, a negative association or a non-significant

finding) is presented in Table **1c**. Study design, statistical approach, main results and discussion for each of the 17 papers are briefly presented in Table **2c**.

Table 1b: Summary of main findings of associations between measures salivary cortisol and depressive mood sorted by year of publication

References	Year	Exposure	Design	No. cortisol	M/W	Single time points (or sum/mean of two/more time points)					Deviation (difference/slope between two or more time points)					AUC								Dexamethasone suppression test
						a1	a2	a3	a4	a5	b1	b2	b3	b4		c1		c2		c3		c4		d
Van Eck a [34]	1996	Zung	C-S	87	87/0					↑														
Van Eck b [35]	1996	Zung	C-S	87	87/0																		0	
Da Rosa [36]	2001	BDI	C-S	30	12/18			0																
Pruessner [37]	2003	HDI	C-S	40	40/0	0	↑↑									↑								
Vedhara [38]	2003	HAD	C-S	54	0/54					0			0											
Tse [39]	2004	BDI	C-S	60	26/34			↑																
Burke [40]	2005	CES-D	EXP	1109	0/1109					0				↓										
Ryff [41]	2006	CES-D	C-S	135	0/135								0											
Gallagher-Thompson [42]	2006	CES-D	C-S	45	0/45		0	0	0				↓											
McCallum [43]	2006	CES-D	C-S	54	0/54								0											
Sjögren [44]	2006	BDI	C-S	257	129/128	↓	↓		0		0		↓											
Therrien [45]	2008	BDI	C-S	78	50/28						0													
Schulze [46]	2009	BDI	C-S	21	12/9						0		↓/											
Muhtz [47]	2009	PHQ	C-S	215	107/108		0	0/↑	0/↑	0														

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory test increase/ground; C-C, Case-Control; C-S, Cross-sectional.; Exp, Experimental.

The proportion of significant relationships among the 33 measures presented in the papers studying anxiety and salivary cortisol was as follows: 2/12 (17%) for single measure (both measures in the same study), 8/18 (44%) for deviation measures, and 1/3 (33%) for AUC in response to a laboratory stress test. No study included dexamethasone administration.

As for depression, the cortisol measures varied among the studies and there were relatively few studies on each measure making it difficult to draw firm conclusions. Full consistency was seen for single measures in the afternoon, single measures in the evening and the means of all day measurements of cortisol, as all studies reported non-significant findings. Significant findings among single time point measures were seen only for morning measures and in one study only (showed higher cortisol among military men). For deviation measures, only 1 study examined CAR, showing lower CAR among more anxious men but not among women. Diurnal deviation measures showed significant findings in 3 of 4 studies: a negative relationship between anxiety and cortisol among women attending a breast cancer clinic (receiving a benign diagnosis) and positive relationships in two studies among white-collar middle-aged men and among military men; the latter were the same group as those with high morning levels.

Stress reactivity was found to be higher in anxious women (only in the follicular phase); 2 studies found blunted reactivity in anxious individuals compared with controls. One study showed that pre-test values were positively correlated to anxiety, but no relation was found for stress reactivity.

Table 1c: Summary of main findings of associations between measures salivary cortisol and anxiety sorted by year of publication

References	Year	Exposure	Design	No. cortisol	M/W	Single time points (or sum/mean of two/more time points)					Deviation (difference/slope between two or more time points)				AUC								Dexamethasone suppression test
						a1	a2	a3	a4	a5	b1	b2	b3	b4	c1		c2		c3		c4		d
Hubert [48]	1989	STAI	EXP	17	17/0									0									
Bohnen [49]	1991	STAI	EXP	24	0/24									0									
Hubert [50]	1992	STAI	EXP	64	64/0									0/								↓	
Van Eck [34]	2006	STAI	C-S	87	87/0								↑										
Van Eck [35]	2006	STAI	EXP	87	87/0																	0	
Filarie [51]	1999	STAI	C-S	20	0/20				0														
Vedhara [38]	2003	HAD	C-S	54	0/54					0			↓										
Takai [52]	2004	STAI	EXP	83	53/30									0/0									
Jezova [53]	2004	STAI	EXP	27	27/0									0/↓									
Takahashi [54]	2005	STAI	EXP	20	20/0									↑/0									
Schlotz [55]	2006	STAI	C-S	71	31/20					0													
Ryff [41]	2006	STAI	C-S	135	0/135								0										
Ellison [56]	2007	STAI	C-S	95	0/95	0																	
Therrien [45]	2008	STAI	C-S	78	50/28						0/↓												
Taylor [57]	2008	STAI	C-S	28	28/0		0↑↑	0	0	0			↑										
Hlavacova [58]	2008	STAI	EXP	40	0/40									0/↑ FP 0 LP									
Preville [59]	2008	STAI	EXP	315	131/184		0							0/↓									

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory test increase/ground; C-C, Case-Control; C-S, Cross-sectional.; Exp, Experimental, STAI, State-trait Anxiety Inventory; FP, Folicular phase; LP, Luteal phase.

Burnout and Vital Exhaustion

The final analysis includes 13 articles on BO. Four studies used the Shirom Melamed Burnout Questionnaire (SMBQ), 8 studies used the MBI and one combined MBI and Teachers Burnout Scale (TBI). The final analysis on VE included 9 articles, 6 of which used the MQ on VE and 3 used the exhaustion subscale of MBI. One study is presented twice as two different instruments were used to measure vital exhaustion [60]. A brief summary of the results (indicated as a positive association, a negative association,

or a non-significant finding) are presented in Table **1d**. Study design, statistical approach, main results, and discussion for each of the articles are briefly presented in Table **2d**.

With regard to BO (Tables **1d** and **1e**), there is a large variation of the salivary cortisol measures used in different studies. Among the studies measuring BO using SMBQ, the proportion of significant relationships among the 19 statistical analyses performed was 5/19 (26%), all in the same direction; *i.e.*, BO measured with SMBQ was positively related to salivary cortisol. The proportion of significant relationships (in any direction) among the 9 articles studying BO using MBI was 10/20 (50%) for single measure showing both positive and negative relationship with BO. All deviation measures showed non-significant results except for the 1 studying acute stress responses to laboratory stress, showing higher reactivity in BO. Two studies using AUC measures, and 3 studies on DST response showed non-significant results.

The proportion of significant relationships (in any direction) among the 6 articles studying VE using MQ was 2/14 (14%) for single measures, 3/7 (42%) for deviation measures, 0/1 study using AUC measures; the only DST study showed a significant relationship between VE and lower cortisol level, showing higher suppression. Among the studies measuring VE with the MBI-EE subscale or an electronic diary using an exhaustion item from MBI-EE, the proportion of significant relationships (in any direction) among the 3 articles was 0 for single measures (only 1 measure presented), 2/5 for deviation measures, and 2/6 for DST.

Thus, with regard to BO, most of the significant findings for both SMBQ and MBI were seen for single measures. However, there was no clear tendency for any time point to be more relevant, and within each time point the direction of the relationship varied. The full consistencies found in the data set were all related to non-significant findings. Regarding VE, among single measures (MQ and MBI-EE), significant findings between cortisol and exhaustion were all in the negative direction. For deviation measures, the CAR measure findings were divergent, 1 positive and 1 negative; for diurnal measures all showed negative relationships, *i.e.*, flatter diurnal curve among people with VE. All significant DST results (3/7) showed higher suppression related to VE.

Table 1d: Summary of main findings of associations between measures of salivary cortisol and burnout sorted by instruments

References	Year	Exposure	Design	No. cortisol	M/W	Single time points (or sum/mean of two/more time points)					Deviation (difference/slope between two or more time points)				AUC				Dexamethasone suppression test
						a1	a2	a3	a4	a5	b1	b2	b3	b4	c1	c2	c3	c4	d
Burnout (SMBQ)																			
Melamed [8]	1999	SMBQ	C-S	111	M/W					↑			0						
Ekstedt [61]	2004	SMBQ	C-S	24	M/W	↑	0				0								
Grossi [62]	2005	SMBQ	C-S	64	M/W	0/↑	0/↑				0/0					0/↑			
Söderström [63]	2006	SMBQ	C-S	20	M/W	0	0	0	0	0			0						
Burnout (MBI)																			
Pruessner [64]	1999	MBI+TBS MBI TBS	C-S	66	M/W		↓ ↓ ↓				0								↓
Morgan [65]	2002	MBI	C-S	41	-		↓		↑										
De Vente [66]	2003	MBI	C-S	45	M/W		↑	0			0			↑					
Langelaan [67]	2006	MBI	C-S	88	M		0				0								0
Mommersteeg	2006	MBI	C-S	43	M/		↓			0	0		0		0	0			

[68]					W																		
Mommersteeg [69]	2006	MBI	C-S	94	M/W		0				0												0
Mommersteeg [70]	2006	MBI	C-S	109	M/W		0			0	0		0			0	0						0
Österberg [71]	2009	MBI	C-C	220	M/W	0	0		↓		0		0										
Wingenfeld [72]	2009	MBI	C-S	279	M/W		↑[a]	0	0	↑			0										

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory test increase/ground; C-C, Case-Control; C-S, Cross-sectional.; Exp, Experimental

[a]Slight positive association between cortisol at 07:00 h and exhaustion subscale, but no association between cortisol and DP, or PA subscales.

Table 1e: Summary of main findings of associations between measures of salivary cortisol and burnout and exhaustion sorted by instruments

Referencea	Year	Exposure	Design	No. cortisol	M/W	Single time points (or sum/mean of two/more time points)					Deviation (difference/slope between two or more time points)				AUC				Dexamethasone suppression test
						a1	a2	a3	a4	a5	b1	b2	b3	b4	c1	c2	c3	c4	d
Exhaustion (MQ)																			
Kristenson [73]	1998	MQ	C-S	183	M									0					
Nicolson [74]	2000	MQ	C-S	59	M	0	0	0	0, ↓	0	0		↓	↓					
Sjögren [75]	2006	MQ	C-S	257	M/W	0	↓		0		0		↓						
Wirtz [76]	2007	MQ	C-S	50	M													0	
Bellingrath [60]	2008	MQ	C-S	135	M/W					0									↓
Wingenfeld [72]	2009	MQ	C-S	279	M/W		0	0	0	0			0						
Exhaustion (MBI-Exhaustion subscale)																			
Mommersteeg [77]	2006	MBI-GS[e]	C-S [L]	74	M/W						↑		0						0
Sonnenschein [78]	2007	MBI-GS[e] ESM[a] ESM[b]	C-S	42	M/W						0 0 ↓								0 0, ↓ 0
Bellingrath [60]	2008	MBI[e]	C-S	135	M/W					0									↓

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory test increase/ground; C-C, Case-Control; C-S, Cross-sectional.;

[a]ESM: aggregated 2-week score.

[b]ESM: same-moment assessment.

Table 2a: Studies on MDD sorted by year of publication

References	Diagnostic and scales	Study design/group characteristics	Sampling	Laboratory method and standardization in cortisol sampling	Statistical approach for cortisol measure	Statistical analysis, cortisol in relation to outcome	Results	Discussion
Copolov 1989 [13]	DSM-III Hamilton depression scale	Design: C-C, C-S No.: 71 P, 17 C M/W: 28%/ 53% Age: 42/31 years Group: MDD (melancholic and non-melancholic) Excl: Organic brain syndromes, eating disorders and drug and alcohol abuse P rate: Part of a larger study with more groups with other psychiatric diagnoses	Days: 1 + 1 DST Samples per day: 1 + 3 Times for sampling: 23:00 h before DST and 07:00 h, 15:00 h and 23:00 h post DST Setting: Mostly inpatients included. Laboratory setting with DST at 23:00 h (1 mg)	Radioimmunoassay Prior to collection of each sample, the subject brushed their teeth and rinsed their mouth thoroughly with water	Measurement(s): a4. Single measure evening before DST d. Deviation 3 measures after DST Cortisol data: log transformation	Log transformed, ANOVA Confounders: Age added as a covariate. Possible effect on medication tested in the patient group. Non-melancholic and melancholic patients were compared	Higher evening cortisol pre-DST in MDD compared with controls. No difference in the level of DST suppression compared with controls, although the controls started from a considerably lower pre-dexamethasone baseline. DST discriminated between hospitalized psychiatric patients and controls	Higher evening cortisol levels in MDD but MDD patients could not be discriminated from other psychiatric diagnoses included in the study. Higher cortisol in MDD inpatients compared with outpatients
Galard 1991 [14]	DSM-III Newcastle depression scale used to discriminate between endogenous and exogenous depression	Design: C-C, C-S No.: 20 P, 12 C M/W: 5/15 P Age: 20-65 years Group: 11 patients with MDD and 9 with dysthymic disorder (non-endogenous) Excl: medications P rate:	Days: 2 Samples per day: 1 Times for sampling: Between 08:00-09:00 h before DST (1 mg at 23:00 h) and + 16:00 h the day after DST Setting: Laboratory	Radioimmunoassay	Measurement[s]: a2. Single measure the morning before DST Data regarding post DST not reported as this was not performed in the control group Cortisol data:	Mann-Whitney U-test	Before receiving DST the endogenous depressed patients (MDD) had significantly higher cortisol concentration than the comparison subject. Trend to higher than the non-endogenous group	Our data show that morning pre-DST cortisol levels are significantly higher in endogenously depressed patients than in normal comparison subjects
Michael 2000 [15]	DSM-IV Clinical interview for depression [CID] HAM-D	Design: C-C, C-S No.: 44 P, 41 C M/W: 12/32 P 14/27 C Age: 43 years (11)/42 years (9) Group: MDD patients , mostly inpatients and controls Excl: Drug and alcohol abuse Remitted patients also included. Data not reported here	Days: 4 (usually consecutive) Samples per day: 2 Times for sampling: 08:00 h and 20:00 h Setting: Ambulatory	ELISA Not to clean teeth 2 h before. Wash mouth with clean water immediately before	Measurement(s): a2. Mean of 4 morning samples a4. 4 evening samples	ANOVA Spearman correlation Cortisol not correlated with age	Salivary cortisol in the depressed subject significantly higher at both time points Within the depressed group no correlation between cortisol at either time point to HAM-D or CID scores	Increased cortisol is a well-known association in this condition
Stanton 2001 [16]	DSM-IV BDI	Design: C-C, C-S No.: 14 P and 13 C M/W: 6/8 vs 6/7 Age: 36 years (21-55) Group: MDD (outpatients) and	Days: 1 Samples per day: 4 Times for sampling: 08:00, 12:00, 16:00 and 20:00 h	Radioimmunoassay	Measurement(s): a5. Mean of several measures c3. AUC to measure overall cortisol output and correlate with BDI	Repeated measure ANOVA Pearson correlation	No difference between groups regarding cortisol No significant correlation between overall cortisol output (AUC) and BDI score within the MDD group	We failed to show a significant difference between healthy controls and MDD patients, possibly due to the relatively small numbers studied and the limited time frame of our measures, but also because the measure of basal HPA function may be relatively

		controls Excl: PTSD and substance abuse. Patients medication free for 4 weeks Patient with depersonalization disorder also studied. Data not presented here	Setting: Ambulatory		scores Cortisol data: Log transformation			insensitive
Weber-Hamann 2002 [17]	DSM-IV HAM-D	Design: C-C, C-S No.: 22 P / 23 C M/W: 0/45 Age: 65 years (9.2)/64 years (7.2) Group: Postmenopausal female inpatients with MDD Excl: History of substance abuse, neurological or relevant medical disorder, BMI <30 kg/m^2	Days: 7 Samples per day: 1 Times for sampling: 08:00 h Setting: Inpatients. 16 controls collected saliva	Time-resolved immunoassay with fluorescence detection	Measurement(s): a2. Mean of 7 days collection at 08:00 h	ANOVA Age, BMI and years of menopause were similar for the groups (not controlled for)	On average, cortisol concentrations were significantly higher in patients than controls	
Galard 2002 [18]	ICD-10 MADRS HAM-D	Design: C-C, C-S, Exp No.: 14 P/12 C M/W: 4/10, /? Age: 48 vs 49 years (mean) Group: MDD outpatients Excl: chronic infections, neoplasm, other chronic disease, history of drug and alcohol abuse	Days: 2 Samples per day: 1 Times for sampling: 00:80-09:00 h the day before DST and 16:00 h the day after DST taken at 23:00 h (1 mg) Setting: Laboratory	Radioimmunoassay Overnight fast before the first sample (morning). Pre-sampling rest	Measurement(s): a2. Single measure morning (before DST) d. Single measure afternoon after DST Cortisol data:	Mann-Whitney U test Paired Wilcoxon test used to compare patients and controls before and after DST	Higher cortisol before DST test (basal) in the MDD patients. Cortisol significantly decreased in both patients and controls after DST 4/14 (33%) of the depressed patients were non-suppressors., none among controls (proportions not statistically tested)	As expected we obtained higher salivary cortisol in depressive disorder. The percentage of non-suppressors (33%) was within the range accepted in several laboratories for this neuroendocrinological psychiatric test and reinforces the clinical utility of salivary cortisol in psychiatry
Young 2002 [19]	DSM-IV MADRS HAM-D	Design: C-C, C-S No.: 44 P and 45 C M/W: 15/29 and 16/29 Age: 33 years (SD 11) and 32 years (SD 11) Group: Unipolar outpatients MDD Excl: No current diagnoses of substance abuse or dependence	Days: 2 Samples per day: 2 Times for sampling: 08:00 h and 20:00 h Setting: Ambulatory Data available from 39 patients and 41 controls	Corti-cote radioimmunoassay	Measurement(s): Mean of two morning samples (a2) and of two evening samples (a4)	Repeated measure ANOVA Age and sex matched. The 26 patients were entirely medication free and the others were drug-free for at least 6 weeks	No difference in cortisol levels either morning or evening between groups	The lack of hypercortisolemia in the depression group is not surprising because low rates of hypercortisolemia are typically reported in non-melancholic outpatients
Watson 2002 [20]	DSM-IV	Design: C-C, C-S No.: 29 P and 28 C M/W: 15/14 and 15/13 Age: 49 years (SD 9.6) and 49 years (SD 9.4) Group: MDD Excl: History of head	Days: 1 Samples per day: 4 Times for sampling: 08:00, 12:00, 16:00 and 20:00 h before DST	Radioimmunoassay Permitted to smoke as usual to reduce the influence of nicotine withdrawal	Measurement(s): c3. AUC (baseline corrected) Cortisol data: Obtained for 19 patients and 22 controls	One-way ANOVA	No difference in AUC between patients and controls	The response shown here differs from previous papers. One explanation may be that the HPA axis might be normalized by medication

		injury, neurological disorder, problem use of alcohol or drugs	Setting: Ambulatory. DST was performed but cortisol was measured in plasma post DST (1.5 mg)					
Bauer 2002 [21]	DSM-IV HAM-D BDI used for controls	Design: C-S, C-C, Exp No.: 36 P and 31 C M/W: 15/21 and 12/19 Age: 49 years (SD 12) and 49 years (SD 13) Group: MDD inpatients Excl: Steroid use, heavy smoking, infection, somatic disease, other psychiatric diseases, pregnancy, lactation	Days: 2 Samples per day: 1 + 15 post DST (only 1 in controls) Times for sampling: 09.00 h before DST. 08:00 to 22:00 h after DST (1 mg) Setting: Laboratory	Time-resolved fluorescence immunoassay	Measurement(s): a2. Single morning measure before DST and single measure after DST Cortisol data:	Student t-test χ^2 test 35 patients on stable medication for at least a week	No difference in a single morning measure before DST Significantly more non-suppressors in the patient group after DST. Other DST results only done in patients and therefore not reported here	We did not observe increased cortisol in treatment-resistant depressed patients. This is in contrast with an extensive literature that indicated increased cortisol levels in major depression. Medication speculated as one explanation
den Hartog 2003 [22]	DSM-IV BDI	Design: C-C, C-S No.: 27 P and 36 C M/W: 12/15 and 20/16 Age: 42 years (SD 13) and 45 years (SD 12) Group: MDD outpatients Excl: Any psychotropic medication, other current axis 1 disorder, neurological disorder, somatic disorder affecting cognitive function, drug and alcohol abuse	Days: 2 Samples per day: 3 Times for sampling: 08:00, 16:00 and 21:00 h Setting: Ambulatory (allergy patients also included, data not presented)	Radioimmunoassay Instructed not to eat, drink, smoke or practice tooth care 1 h prior to saliva sampling Awakening time not recorded	Measurement(s): a2. a4. Mean value for the two samples used a5. DAC = mean of all three measures b3. Deviation from morning to evening. Higher values reflecting steeper curve Cortisol data: log transformed cortisol values	MANCOVA Post hoc (Scheffés) Adjusted for age and sex. BDI sleep item added to the model	Main effect of group, post hoc showing MDD patient having increased evening cortisol DAC not different between the groups MDD group showed lower delta cortisol (a flatter curve) compared with healthy controls (related to significantly higher evening value)	MDD group was not characterized by an overall increase or decrease in cortisol level, but instead by a flattening of the cortisol curve over the day, mainly appearing to be due to higher evening cortisol values. One possible explanation could be early morning awakening
Porter 2003 [23]	DSM-IV MADRS	Design: C-C, C-S No.: 20 P and 20 C M/W: 9/11 and 10/10 Age: 34 years(SD 11) and 33 years (SD 12) Group: MDD outpatients Excl: Current substance abuse, medication (last 8 weeks) P rate:	Days: 3 Samples per day: 4 Times for sampling: 08:00, 12:00, 16:00 and 20:00 h Setting: Ambulatory for 3 days before exp [tryptophan infusion]. The EXP part not reported further	Radioimmunoassay	Measurement(s): c3. AUC, average of the 3 measures, log transformed	One-way ANOVA	No difference between patients and controls in baseline levels of cortisol before the intervention. Results from the intervention not relevant for this paper	Only a few of the patients met criteria for melancholic depression as this subtype appears to give rise to a higher incidence of biological abnormalities including HPA dysfunction
Peeters 2003 [24]	DSM-IV HAM-D BDI	Design: C-C, C-S No.: 45 P and 39 C M/W: 19/26 and 16/23 Age: 40 years (11) and	Days: 6 Samples per day: 10 Times for	Radioimmunoassay Physical exertion, food, alcohol, coffee, smoking and medication recorded	Measurement(s): b3. Diurnal pattern of cortisol secretion. Mean level for each time of the day was	Multiple regression Many factors such as age, food intake and awakening time recorded and	Intercept showed that depressed participants had no increase in basal cortisol levels (day cortisol curve) compared with healthy	In contrast to healthy subjects, depressed subjects showed no increase in cortisol after a negative event. Positive events had no significant effect on cortisol levels in either group

		42 years (12) Group: MDD outpatients Excl: Substance abuse, psychotic symptoms, biopolar disorder, pregnancy, weight loss (>15%) in the last 6 months, endocrine and RA disorder, medication (inclusive antidepressants)	sampling: Experience sampling, recording moment of daily activity 10 times/day starting 07:30 h at 90 min interval until 22:30 h. Salivary cortisol taken at the same times Setting: Ambulatory		first calculated from the 6-day sampling period Cortisol data: Log transformation	adjusted for when relevant	participants b4. Negative events associated with significant cortisol increase in healthy subjects, but no evidence of cortisol response in the depressed subjects	
Assies 2004 [25]	DSM-IV HAM-D MADRS	Design: C-C, C-S No.: 13 P and 13 C M/W: 3/10 and 3/10 Age: 40 years (11) and 41 years (10) Group: MDD mostly inpatients (10/13) Excl: Organic brain syndrome, alcohol or drug abuse, medical illness, recent electroconvulsive therapy and psychotic feature	Days: 1 Samples per day: 2 Times for sampling: 08:00 and 22:00 h Setting: In the inpatients, sampling was supervised by nursing staff. Others were ambulatory	ELISA Instructed to rinse their mouth with water and not to brush their teeth before sampling. Morning samples were collected after overnight fast	Measurement(s): a2. Morning sample a4. Evening sample b3. Diurnal slope change from morning to evening Cortisol data:	MANOVA with repeated measures Pearson correlation	No difference in either cortisol levels or diurnal slope between patients and controls No correlation between any of the cortisol measures and MADRS score	In the current sample of unipolar, non-psychotic medicated depressed patients the levels of DHEA-S were increased but not cortisol. Might be due to the type of depression or that treatment has normalized the HPA dysfunction
O'Brien 2004 [26]	DSM-IV MADRS	Design: C-C (only baseline considered here) No.: 61 P and 40 C M/W: 13/48 and 10/30 Age: 74 years (SD 6.7) and 73 years (SD 6.7) Group: Older MDD, both in- and outpatients Excl: History of cognitive impairment, stroke or TIA, severe or unstable medical illness. Substance abuse, >2 months steroid use, ECT (<3 months). Medication (SSRI allowed)	Days: 3 at baseline Samples per day: 3 Times for sampling: 08:00, 12:00, 16:00, 20:00 h Setting: Inpatients samples were not taken within a week of admission. Outpatient samples taken at home	^{125}I disequilibrium assay, the radioactive cortisol	Measurement(s): Single measure (mean of 3 days) a2. Morning a3. Noon, afternoon and a4. Evening c3. AUC for all samples during the day (mean of 3 days)	Student t-test	Significantly higher cortisol level at all time points except for 08:00 h in depressed subject. AUC also significantly higher	Depressed subjects exhibited hypercortisolemia during depression, as demonstrated by increased salivary cortisol levels
Bhagwagar 2005 [27]	DSM-IV BDI HAM-D	Design: C-C, C-S No.: 20 P and 40 C M/W: 10/10 and 19/21 Age: 44 years (SD 11) and 41 years (SD 14) Group: MDD outpatients	Days: 1 Samples per day:5 Times for sampling: awakening +15, +30, +45, +60 min Setting:	Radioimmunoassay Self reports for wakening time. Premenstrual week was avoided	Measurement(s): b1. Interaction effect of the morning curve a1, a2. Post hoc for 5 time points c1. AUC measured by the trapezoid method	ANOVA Pearson correlation Age and gender included as covariates	Main effect group, main effect time but no time × group interaction effect. Post-hoc showed that cortisol levels at +15, +30 and +45 min were higher but not at awakening AUC significantly greater No correlation between	Medication-free subjects with a diagnosis of major depression secrete significantly greater amounts of salivary cortisol in relation to wakening (post). First hour after wakening the cortisol level is the same as in healthy controls. Does not seem to be related to severity of depression

		Excl: Current past serious medical or neurological illness, alcohol or illicit substance dependence or other axis 1 disorder. Patients medication free for >4 weeks	Ambulatory		Cortisol data:		cortisol and BDI or HAM-D scores	
Stetler 2005 [28]	DSM-IV HAM-D PRIME-MD	Design: C-C, C-S No.: 37 P and 36 C M/W: 0/37 and 0/36 Age: 27 years (6.6)/26 years (6.8) Group: MDD (33) or mild depression (4) Excl: Chronic medical illness, acute infection, pregnancy, use of antidepressants or any medication except OC during previous month	Days: 3 Samples per day: 3 Times for sampling: Awakening, + 30 min and + 60 min Setting: Ambulatory	Chemiluminescence assay Palm Pilot computer used to program the time of sampling. Instructed not to eat or brush their teeth immediately prior to collection	Measurement(s): a1. Awakening a2. Single measure 2 time points post-awakening c1. AUC ground Cortisol data: 3 patients and 1 control excluded due to missing samples	Hierarchal linear modelling OC, tobacco use, education. 32% of MDD also met criteria for GAD. No difference between these groups	Similar cortisol levels at awakening, lower levels in depressed patients at +30 and + 60 min (blunted response in depression). AUC greater in control subjects Cortisol negatively associated with HAM-D but not with BDI	Blunted morning cortisol response among depressed women compared with non-depressed controls. Depressed patients failed to increase cortisol after awakening. Community-based samples with milder symptoms are studied
Juruena 2006 [29]	DSM-IV HAM-D BDI	Design: C-C, C-S, EXP No.: 18 P and 14 C M/W: 4/14 and 3/11 Age: 50 years (SD 3) and 49 years (SD 3) Group: MDD inpatients Excl: All subject physical healthy, no OC and no history of hypersensitivity to corticosteroids	Days: 3 (1 placebo, 2 exp) Samples per day: 10 Times for sampling: 09:00-17:00 h (hourly) + 22:00 h Setting: One capsule taken at 22:00 h the day before, containing placebo, DST (0.5 mg) or prednisolone	Time-resolved immunofluorescent assay DELIFA No alcohol, coffee, tea or meals allowed after 22:00 h the day before sampling. During sampling, snacks, meals, drinks standardized. Sedentary activities. No coffee allowed	Measurement(s): a5. Mean of day measures c3. AUC d. DST Cortisol data	General linear model for repeated measure Pearson correlation	Patients with MDD had higher mean level of cortisol after placebo and after DST (d) Patients had higher c3 AUC compared with controls after placebo and after DST. Less suppression after DST in MDD than controls	MDD higher level of cortisol and more non-suppressors among MDD. Prednisolone suppression also studied. Data not reported here
Alhaj 2007 [30]	DSM-IV HAM-D BDI	Design: C-C, C-S No.: 27 P and 29 C M/W: 11/16 and 16/13 Age: 47 years (SD 10.8) and 46 years (SD 11) Group: MDD, outpatients (recruited through advertisement) Excl: Psychotropic medication (<6 weeks), patients with other psychiatric diagnosis	Days: 2 Samples per day: 2 Times for sampling: 08:00 and 20:00 h Setting: Ambulatory	Radioimmunoassay	Measurement(s): a2, a4. Cortisol level (morning and evening) b3. Group × time interaction (morning/evening) Cortisol data: Available form 23 patients and 29 controls	ANOVA Effect of gender tested	No significant difference in cortisol level between the groups. No time × group interaction indicating that diurnal variation of cortisol occurred in both patients and controls	
Treadway	DSM-IV	Design: C-C, C-S	Days: 3	Enzyme immunoassay	Measurement(s): a5. Mean of all seven	t-test	Average cortisol levels (a5: the mean of all seven samples)	Cortisol was primarily measured to be related to volumetric measures of

2009 [31]	HAM-D	No.: 19 P and 19 C M/W: 9/10 and 9/10 Age: 35 years (SD 11) and 30 years (SD 9) Group: MDD outpatients Excl: No history of neurological disease or brain injury, substance abuse <6 months, comorbid axis-A disorder.Patients were antidepressant free.	Samples per day: 3 sample per day on two consecutive days and a morning sample on the third day Times for sampling: within 0.5 h after awakening, 15:00 h and 21:00 h Setting: Ambulatory		sample s= average cortisol levels, single measure a2. Morning a4. Evening a3. Afternoon also statistically analyzed		were increased in the patients group. However the difference in the cortisol secretion between the patient and control groups was greatest for average morning cortisol. No difference for other time points	different brain areas and the cortisol data alone was not discussed
Chopra 2009 [32]	DSM-IV HAM-D	Design: C-C, EXP No.: 26 P and 28 C M/W: 14/12 and 14/14 Age: 39 years (SD 6) and 43 years (SD 6) Group: MDD Excl: Major medical illness (affecting cortisol), heart disease, current steroids use, substance abuse, pregnancy, bipolar disorder, acute suicide ideation, history of psychotic symptoms	Days: 1 Samples per day: 7 Times for sampling: -35 min, -20 min, 0 (before TSST), +20, +40, +60 and +80 min post TSST Setting: TSST	Radioimmunoassay	Measurement(s): c4. AUC total cortisol secretion b4. Peak percentage change (max post stressor cortisol - T0 cortisol/T0 × 100) Cortisol data:	RANOVA A series of two-way RANOVAs and Pearson correlations were performed to see if age, BMI, psychotropic medication PTSD or anxiety diagnosis, menstrual phase and smoking were associated with cortisol	AUC during the whole test period showed greater levels in females with MDD, not males Mean peak percentage change was lower in MDD males. No difference in females	Data suggest that females with MDD do in fact secrete more cortisol in response to the TSST than healthy females. In males, MDD was associated with significantly lower peak percentage change in cortisol in response to the TSST
Vreeburg 2009 [33]	DSM-IV	Design: C-C, C-S, EXP No.: 701 P and 308 C M/W: 243/458 and 133/175 Age: 42 years (SD 12.0) and 47 years (11.7) Group: MDD (remitted MDD also included, not accounted for here) Excl: pregnancy, breastfeeding, taking corticosteroids P rate: Cohort study	Days: 2 (1 post DST) Samples per day: 6 +1 (post DST) Times for sampling: Awakening, +30, +45, +60 min (CAR), 22:00 h and 23:00 h. On day 2 after DST at awakening Setting: Ambulatory, DST test on the evening day 1 at 23:00 h (0.5 mg)	Electrochemiluminescence immunoassay No eating, smoking, drinking or brushing teeth within 15 min	Measurement(s): a1, a2. Single measure morning and CAR measure with c1. AUC_{ground} and c2. $AUC_{increase}$ a4. Single measure evening d. Single measure morning after DST b3. Deviation between the two evening samples d. Cortisol suppression ratio (awakening day 1/awakening day2 after DST) Cortisol data: At least 3 samples available to be able to measure AUC. Missing values imputed. Log transformed (DST data)	LMM analyses (morning response) ANOVA (c1 and c2) Full adjustments, sex, age, education, northern Europe ancestry, working, weekday, time of awakening, sleep, months with more daylight, smoking, physical activity	After adjustments, cortisol levels were found to be significantly higher in MDD at awakening (a1) and +30 and +45 min (a2) after awakening. The evening cortisol at 22:00 h significantly higher in subject with current MDD but no difference in cortisol levels was found at 23:00 h or post DST. The suppression rate however differs as MDD had higher suppression rate (lower cortisol) AUC higher overall cortisol levels, but no interaction effect group × time. Higher AUC_{ground}, ns $AUC_{increase}$	Higher cortisol values in the hour after awakening were found for MDD. Although not confirmed with at 11:00 h, significantly higher evening cortisol was found in MDD at 22:00 h. MDD did not show more cortisol non-suppression after DST and current MDD was even associated with more suppression. The use of psychoactive medication was generally associated with decreased cortisol levels and less cortisol suppression after DST

Table 2b: Studies on depressive mood sorted by year of publication

Reference	Depression scale	Study design/group characteristics	Sampling	Laboratory method	Statistical approach for cortisol measure	Statistical analysis, cortisol measure in relation to outcome	Results	Discussion
Van Eck 1996 [34]	Zung self-rating scale for depression	Design: C-S No.: 87 M/W: 87/0 Age: 42 (27-57) years Group: White-collar workers Excl: Chronic illness, endocrine disorder, medications known to affect cortisol, mental health problems P rate: 316 screened. Only high and low stress included according to the lowest and upper tertiles of PSS	Days: 5 Samples per day: 10 Times for sampling: Between 08:00 h and 22:00 h Setting: Ambulatory, 10 watch beeps per day with approx. 90-min interval. Cortisol sampling done at the same time point	Radioimmunoassay 5 Extreme values were excluded (>1200 ng/dL)	Measurement(s): a5. Day curve of all cortisol measures by using average from the 5 days sampling Cortisol data: Log transformation	Main aim to relate cortisol to daily activity. Trait of depression was entered into Hierarchical Linear Model. Possible confounders including exercise, smoking, coffee and food intake were included in the model as explanatory variables	Trait depression was associated with significant increase in cortisol levels (estimate 0.003, p=0.05)	Trait depression showed small but significant positive association with cortisol in healthy subjects
Van Eck 1996 [35]	Zung self-rating scale for depression	Design: Exp No.: 87 M/W: 87/0 Age: 42 (27-57) years Group: White-collar workers Excl: see above P rate: see above	Stress-inducing laboratory test (speech task) taking place between 11:00 and 13:00 h. Groups compared were high and low stress. 4 time points before, during and after stress task	Radioimmunoassay	Measurement(s): c4.. AUC Cortisol data:	The possible effect of trait depression was tested by using stepwise multiple regression	Trait depression did not predict cortisol response during stress task	Trait depression failed to predict cortisol response
Da Roza Davis 2001 [36]	BDI	Design: C-S No.: 30 M/W: 12/18 Age: 68.8 (30-75) years Group: Caregivers (controls also studied, but data not relevant) Excl: Fulfilling criteria of MDD. Psychotic drugs	Days: 1 Samples per day: 4 Times for sampling: 08L00, 12:00, 16:00 and 22:00 h Setting: Ambulatory	Radioimmunoassay	Measurement(s): a3. Only 12:00 h sample used to relate to depression. Correlation only carried out in caregivers	Log transformation. Pearson correlation between depression score and cortisol at 12:00 h	Depression scores (BDI) was not significantly correlated to 12:00 h cortisol levels	Caregivers had higher cortisol levels compared with controls but cortisol was not related to depression score
Pruessner 2003[37]	HDI	Design: C-S No.: 40 M/W:40/0 Age: 24.3 years (SD 4.33) Group: Male university students Excl: History of psychiatric disorder, CVD problems and	Days: 4 days (1 day/week for 4 weeks) Samples per day: 3 Times for sampling: Awakening, +30 and +60 min Setting: Ambulatory	Time-resolved immunoassay with fluorescence detection. Asked to refrain from smoking, caffeinated drinks. Not to brush teeth and not to eat and drink 10 min before and to rinse mouth with water	Measurement(s): c1. AUC other a1, a2. Post hoc analysis of single measures Correlation; cortisol levels were transformed into a single value by calculation of AUC for each day and then computing the median. Two-way repeated measures	Pearson correlation computed between HDI scores and AUC (median value). First for the whole group and then repeated excluding the subject scoring above the non-clinical range of depression. ANOVA: two groups compared (median split of HDI)	i. Positive correlation between HDI score and AUC. Significant only in the non-clinical group (p=0.05) f. Significantly higher Aw response in subjects with high HDI scores. Group × time also significant, best reflecting in +30 and +60 min values	Positive association between increased cortisol levels after awakening and self-reported severity of depressive symptoms in a normal population

		alcohol abuse. Medication free at time of testing. Six smokers			ANOVA (group × time)			
Vedhara 2003 [38]	HAD	Design: C-S No.: 54 M/W:0/55 Age: 44 years Group: Women attending one-stop breast clinic (receiving benign diagnosis) Excl: Medication affecting cortisol P rate: 55/158	Days: 1 Samples per day: 5 Times for sampling: 07:00-08:00 h, 60 min after the first (08:00-9:00 h), 12:00-13:00 h, 16:00-17:00 h and 23:00-24:00 h Setting: ambulatory one of the days before appointment but not on the appointment day	ELISA Asked to avoid meals within 60 min of each sample and to avoid caffeine-containing products during the day. 18 of 55 reported current medication use	Measurement(s): a5. Mean of all measurements = absolute log cortisol levels b3. Deviation several measures. The rate of change of cortisol over time Cortisol data:	d. Pearson's product correlation coefficients (with Bonferroni correction) f. GLM. Interaction effects of depression × time (linear change and non-linear change)	d. No correlation between depression and absolute level of cortisol f. Depression significantly predicted non-linear time interaction, but not the linear interaction. (the change in cortisol better described as a non-linear slope)	When the non-linear interaction is tested we find a probability value approaching significance for depression
Tse 2004 [39]	BDI	Design: C-S No.: 60 M/W: 26/34 Age: 28.35 years (SD 9.09) Group: Healthy volunteers Excl: Current or previous history of psychiatric illness, current physical illness and current use of medication	Days: 1 Samples per day: 1 Times for sampling: After 12:00 h Setting: Laboratory. 30 min resting before sampling	Chemiluminescence immunoassay Asked to refrain from any consumption of food or drinks (except water) from morning	Measurement(s): a3. Single measure afternoon Cortisol data: Log transformed. Three subjects were excluded from the analysis (medication affecting the cortisol and 2 due to high BDI scores)	General linear modelling, BDI as dependent variable and age, sex and cortisol as independent. Other analyses are not accounted for here	Cortisol concentration was positively associated with BDI scores	Depression scores were significantly associated with basal cortisol level and social functioning
Burke 2005 [40]	CES-D (the Spanish version)	Design: EXP No.: 1109 M/W: 0/1109 Age: 28.81 years (6.07) Group: Low-income women from families across Mexico. At least one child between age 2 and 6 years P rate: 95% of baseline survey agreed to participate	Days: 1 Samples per day: 3 (between 08:00 and 16:00 h) Times for sampling: At baseline (arrival of the team) and 2 samples during naturalistic stressor Setting: An unexpected visit of health professionals who conducted an interview lasting 1 h	Time-resolved fluorescence immunoassay. The samples were schedule 25 min apart starting at different time points sometimes between 08:00 and 1600 h. All women remained seated during the visit and sampling	Measurement(s): a3. Baseline measure before stress (somewhere between 08:00 and 16:00 h). Control for the time point done in the analysis b4. Deviation several measurements Cortisol data: Log transformation	Hierarchical linear modelling procedures. Depression scores (3 groups, high cut-off (35), high risk (16) and low risk of depression (10) according to CES scores. This was entered as predictors at level 2. Adjusted for age, time since waking, time of sample	No difference in baseline levels between depression groups (trend). Women with scores above 35 failed to mount cortisol response to naturalistic stressor	Women with very high depressive symptoms exhibit blunted cortisol response to naturalistic psychological stressor. No difference between the two other groups
Gallagher-Thompson 2006 [42]	CES-D	Design: C-S No.: 45 M/W:0/45 Age: 53 (40-70) years Group: Total 4 groups. Caregivers and noncaregivers divided into Hispanic and non-Hispanic white.	Days: 3 Samples per day: 3 Times for sampling: 08:00 h, 17:00 h and 21:00 h Setting: Ambulatory	Radioimmunoassay and enzyme immunoassay . 30 samples were assayed using both to ascertain the comparability between assays	Measurement(s): a2, a3, a4. Single measure morning, evening, and afternoon b3. Daily average slope from 9 measurements (3 per day for 3 days) Cortisol data: The slope of diurnal change in log	a2, a3, a4. ANOVA b3. Linear regression analysis to analyse the slope. Regression analysis entering depression as predictor. Confounders (included in the model); ethnicity, caregiving status, perceived stress	Depression scores did not predict cortisol levels at any time point. Greater depression symptoms were associated with flatter daytime cortisol slope	Absence of relationship between depressive symptoms and morning cortisol but association between higher depressive symptoms and flatter daytime curve

		Subsample from 83 used after matching for age and education within each group			transformed cortisol level			
McCallum 2006 [43]	CES-D	Design: C-S No.: 54 M/W: 0/54 Age: 58 years (8)/67 years (11) Group: Caregivers, divided in two groups (ethnicity) Excl: < 0 years P rate:	Days: 2 Samples per day: 5 Times for sampling: Awakening. 09:00, 12:00, 17:00 h and 21:00 h Setting: Ambulatory	Immunoassay Participants were asked to register sleep and exercise deviation from normal days	Measurement(s): b3. Deviation other Cortisol data: Log transformation. Cortisol slope during the day Samples used: All	Correlation. Hierarchical linear modelling. Depression scores included in the model as a predictor. Confounders (included in the model); age, ethnicity and caregiving status	No significant correlation between cortisol slope and CES-D score. Depression did not predict the cortisol day slope	Mental health outcomes showed no association with physiological stress responses as measured by cortisol
Sjögren 2006 [44]	Major Depressive Disorder Scale (Becks)	Design: C-S No.: 257 M/W: 129/128 Age: 30-64 years Group: Random sample from a health survey (n=10, 000) Excl: P rate: 400 invited. 64.5% participation rate	Days: 3 (mid-week) Samples per day: 3 Times for sampling: awakening, +30 min and before bedtime Setting: Ambulatory	Time-resolved fluorescence Registration of time of awakening. For +30 sample less than 20 min and more than 40 were excluded. + 30 taken before breakfast	Measurement(s): a1, a2, a4, b1, b3 Cortisol data: Log transformation. Diurnal deviation = difference between morning values and evening. Awakening response +30 min minus awakening Samples used: All	Partial correlation analysis (a1, a2, b1, b4). Adjusted for age, gender, awakening time, taking medicine regularly, smoking, and alcohol use	Negative correlation between depression score and awakening and +30 min but not evening. Negative correlation between wakening and evening and 30 min and evening. No correlation between 30 min and awakening	Depression related to lower morning cortisol and flatter diurnal rhythm
Therrien 2008 [45]	BDI	Design: C-S No.: 78 M/W: 50/28 Age: 38 years (1.0)/37 years (1.4) Group: Healthy subjects recruited through advertisements Excl: No history of depression or psychiatric disorder, CVD or smoking, menopause	Days: 3 randomly assigned within a 2-month period Samples per day: 2 Times for sampling: awakening and +30 min Setting: ambulatory	Radioimmunoassay Abstain from alcohol and physical activity 24 h before, caffeine during the same day. No brushing and no food or drink except water between sampling. Fasting since the evening before (20:00 h)	Measurement(s): b1. Deviation between awakening sample and +30 min after awakening Cortisol data: Average from the 3 days (except for 20 individuals for 2 days due to missing samples) Samples used:	Correlation	No significant correlation between depression scores and cortisol response in either men or women	
Schulze 2009 [46]	BDI	Design: C-S No.: 21 M/W: 12/9 Age: 53.4 (24-81) years Group: Colorado ranchers Excl: P rate: Invitation sent to 105 ranchers	Days: 3 × 3 (3 typical days during each of 3 periods of 2 weeks representing high, medium, and low stress) Samples per day: 3 Times for sampling: Awakening, +30 min and before bedtime Setting: Ambulatory	High sensitive commercial EIA kit SPIT books were used	Measurement(s): b1. Deviation between +30 min and awakening b3. Daytime decline = deviation between peak value (+30 min) to evening Cortisol data: Samples used:	Hierarchical multiple linear regression. Depression was entered as dependent variable. Age, perceived stress and pre-study survey also included in the model as predictors b1. Morning cortisol was not significantly related to depression	Depression score were predicted by cortisol day slope but this was only significant when males were separately analyzed). Not significant in the total group	High depression scores reflected in flattened cortisol decline (only in males)
Muhtz 2009 [47]	PHQ	Design: C-S No.: 215	Days: 1 Samples per day: 4	Radioimmunoassay Advised not to drink, eat, smoke, brush teeth or use	Measurement(s): a2, a3, a4, a5. Single measures cortisol profile	MANCOVA. Effects of depressive symptoms on cortisol profile. Gender ×	No significant difference between participant with and without depressive symptoms	Women with depressive symptoms exhibit greater 16:00 and 22:00 h

		M/W: 107/108 Age: 30-70 years Group: Healthy recruited from the general population Excl: Chronic disease, particularly CVD and psychiatric disease, medication (except from HRT and OC) P rate: 149 provided cortisol samples	Times for sampling: 08:00, 12:00, 16:00 and 22:00 h Setting: Ambulatory	mouthwash 30 min prior to sampling. Not to drink alcohol during the day of collection	was analyzed Cortisol data: Including 149 participants using linear interpolation for missing values Samples used: All	depression interaction for variables of cortisol also analyzed. Differences were adjusted for age, education, level of physical activity and smoking	for the total group. In women, significant effects of depressive symptoms were seen on cortisol levels. Post hoc revealed that 16:00 and 22:00 h cortisol levels where higher in the group with depressive symptoms. Remained after adjustments	salivary cortisol values which partially mediated the association between depressive symptoms and variables of the metabolic syndrome

Table 2c: Studies on anxiety sorted by year of publication

References	Outcome	Study design/group characteristics	Sampling	Laboratory method and standardization in cortisol sampling	Statistical approach for cortisol measure	Statistical analysis, cortisol in relation to outcome	Results	Discussion
Hubert 1989 [48]	STAI	Design: C-S, EXP No.: 17 M/W:17/0 Age: 27 years (SD 1.0) Group: Healthy volunteers Excl: No medication or drugs P rate:	Days: 1 Samples per day: 3 Times for sampling: 0 (before), +25 and +45 min in a response to stressor (LHRH stimuli) Setting: Stress reaction related to procedures of LHRH test performed between 14:00 and 16:00 h. Data not considered here	Radioimmunoassay	Measurement(s): b4. Cortisol levels before stressor Cortisol data:	b4. Pearson correlation (one-tailed) between trait anxiety scores and cortisol secretion	No significant correlation between trait anxiety scores and cortisol secretion	Like other investigators we could not find correlation between personality traits and cortisol release
Bohnen 1991[49]	STAI	Design: C-S; EXP No.: 24 M/W: 0/24 Age: 41-49 years (n=12), 61-69 years (n=12) Group: Female volunteers in good physical and mental condition Excl:	Days: 2 Samples per day: 6 Times for sampling: 10:20, 12:40, 14:10. 14:45: 15:00, 15:30 h Setting: Continuous mental task for 4 h compared with non-stressful control session (on a separate day)	Radioimmunoassay During the experimental procedure, the participants abstained from smoking and coffee. At lunch time they received 150 mL of lemonade. 10 min interruption for lunch	Measurement(s): b4. Mean of several measure in a response to stress Cortisol data: The mean of the 3 latest afternoon samples were highly intercorrelated and the mean was calculated as an index of cortisol response to stress	Spearman rank correlation	There was no significant relationship between trait anxiety and saliva cortisol response	The present finding failed to demonstrate a significant relationship between levels of trait anxiety and individual glucocorticoid susceptibility
Hubert 1992 [50]	STAI Based on the ratings on STAI, subjects were divided by median split into high	Design: C-S, EXP No.: 64 M/W: 64/0 Age: 18-40 years Group: Healthy volunteers, forming two	Days: 1 Samples per day: 10 Times for sampling: From the onset of film (0 min) and then 20 min intervals until 180 min	Radioimmunoassay Conducted between 14:00 and 18:00 h. Subjects asked to refrain from exercise, drinking coffee, tea, and alcohol 1 h before the session	Measurement(s): a3. Single measure = baseline before the session performed between 14:00 and 18.00 h c4. Cortisol concentration	a3. ANOVA c4. MANOVA interaction effect with repeated measures was calculated	a3. No significant difference in cortisol baseline level between the groups (HA and LA) c4. The saliva cortisol response increased significantly in the LA	Lack of cortisol reaction in the high anxiety group during unpleasant film stimuli

	anxiety (HA) and low anxiety (LA)	groups (HA and LA) Excl: medication P rate:	Setting: Half the group exposed to unpleased film and half the group exposed to a control film. 120 min		time curve (AUC) during stressor was calculated Cortisol data:		group only. HA group did not differ between the two sessions	
Van Eck 1996 [34]	STAI	Design: C-S No.: 87 M/W: 87/0 Age:42 (27-57) years Group: White-collar workers Excl: Chronic illness, endocrine disorder, medications known to affect cortisol, mental health problems P rate: 316 screened. Only high and low stress included	Days: 5 Samples per day: 10 Times for sampling: Between 08:00 h and 22:00 h Setting: Ambulatory, 10 watch beeps per day with approx. 90-min interval. Cortisol sampling done at the same time point	Radioimmunoassay 5 extreme values were excluded (>1200 ng/dL)	Measurement(s): Day curve (slope) of all cortisol measures. Average from the 5 days sampling	Main aim to relate cortisol to daily activity. Trait of anxiety was entered into the Hierarchical Linear Model. Alcohol, coffee, food intake, smoking, and physical exertion were entered into the model	Positive association between cortisol levels (the day slope) and anxiety	Trait anxiety showed small but significant positive association with cortisol in healthy subjects
Van Eck 1996 [35]	STAI	Design C-S , EXP No.: 87 M/W: 87/0 Age: 42 (27-57) years Group: White-collar workers Excl: See above P rate: See above	Stress-inducing laboratory test (speech task) taking place between 11:00 and 13:00. Groups compared were high and low stress. 4 cortisol samples	Radioimmunoassay	Measurement(s): c4. AUC calculated. 4 time points before, during and after stress task	The possible effect of trait anxiety was tested by using stepwise multiple regression	Trait anxiety did not predict cortisol response during stress task	Trait anxiety failed to predict cortisol response to stressor
Filarie 1999 [51]	STAI	Design: C-S No.: 20 M/W: 0/20 Age:24 years (4.8)/25 years (2.6) Group: Handball (n = 13) and volleyball (n = 7) players Excl: Drugs, medication, OC, no history of endocrine disease	Days: 1 Samples per day: 2 (1 after competition not further accounted for here) Times for sampling: 5 min before and after competition lasting 1.5-2 h Setting: Handball or volleyball competition, played between 20:00 h and 22:00 h	Radioimmunoassay At least 3 h since last ingested food. Only water allowed during the games. None had trained the day before	Measurement(s): a4. According to the data presented correlation was done between two single measures (evening) and trait anxiety Cortisol data:	Data presented for trait anxiety is a correlation analysis between single measures (before the competition) for the two different groups	No pre-competition level of cortisol was significantly correlated to trait anxiety. Trait anxiety was significantly correlated with post-competition cortisol values. As this involves intense physical activity, the results are not relevant for this review	Both pre- and post-competition level of cortisol was significantly higher in the handball players. No correlation between pre-competition level and trait anxiety
Vedhara 2003 [38]	HAD	Design: C-S No.: 54 M/W:0/54 Age: 44 years Group: Women attending one-stop breast clinic (receiving benign diagnosis)	Days: 1 Samples per day: 5 Times for sampling: 07:00-08:00 h, 60 min after the first (08:00-9:00 h), 12:00-13:00, h 16:00-17:00 h, 23:00-24:00 h	ELISA Asked to avoid meals within 60 min of each sample and to avoid caffeine-containing products during the day 18 of 55 reported current medication use	Measurement(s): a5. Mean of all measurements = absolute log cortisol levels b3. Deviation several measures. The rate of change of cortisol over time Cortisol data: log	a5. Pearson's product correlation coefficients (with Bonferroni correction) b3. GLM. Interaction effects of anxiety × time (linear change and non-linear change)	a5. No correlation between anxiety and absolute level of cortisol b3. Anxiety significantly predicted non-linear time interaction, but not the linear interaction. (the change in cortisol better described as a non-linear	When the non-linear interaction is tested we find a significant, but small, effect of anxiety (when the lines of best fit are presented, high anxiety showed somewhat lower cortisol (at midday)

		Excl: Medication affecting cortisol P rate: 55/158	Setting: Ambulatory one of the days before appointment but not on the appointment day		transformation		slope)	
Takai 2004 [52]	STAI	Design: C-S, EXP No.: 83 M/W: 53/30 Age: 23.3/23.8 years Group: Healthy volunteers Excl: Physical or mental illness, pregnancy, taking corticosteroids or OC P rate:	Days: 1 Samples per day: Accumulated saliva was collected every 3rd minute during the stress exposure. In total 11 samples are presented Times for sampling: Setting: Stressful video viewing for 15 min performed between 09:00 and 11:00 h	ELISA Instructed to abstain from eating, smoking, drinking any beverage except water and exercising 2 h before the experiment	Measurement(s): a2. Single measure = resting level b4. Maximum level of cortisol during stress (as a percentage of basal) Cortisol data:	Pearson's correlation coefficient	Cortisol concentration in the resting saliva did not correlate with STAI score. In contrast with amylase the cortisol level did not statistically correlate with the STAI score	No significant correlation between cortisol and STAI score
Jezova 2004 [53]	STAI	Design: C-S, EXP No.: 27 M/W: 27/0 Age: 20-40 years Group: Healthy, divided into high and low anxiety Excl: Somatic or mental diseases, family history of psychiatric disease, BMI >28 kk/m^2, BP >140/90 mmHg, medication	Days: 1 Samples per day: 6 samples during and post-stress Times for sampling: Tests were performed during early afternoon Setting: Modified version of TSST	RIA Asked to abstain from eating at least 3 h prior to the experiment	Measurement(s): a3. Basal levels before the stress test b4. Mean of several measurements during the stress test Cortisol data:	Two-way ANOVA with time and group as factor	a3. No significant difference between basal parameters measured b4. In anxious subjects the salivary cortisol was significantly lower in a response to stress compared with non-anxious group	The present data indicate reduced responses during psychosocial stress in subjects with high anxiety
Takahashi 2005 [54]	STAI	Design: C-S, EXP No.: 20 M/W: 20/0 Age: 20 (19-23) years Group: Healthy male students Excl: Smokers, drinkers, medication, acute or chronic hormonal regulation, atopic, psychosomatic psychiatric disease	Days: 1 Samples per day: 2 samples Times for sampling: pre-stress and 10 min post stress Setting: TSST conducted between 14:30 and 17:30 h	ELISA Not to drink anything containing coffee or alcohol the day before. Not eat/drink anything other than water or exercise 1 h before the trial	Measurement(s): a3. Single measure = pre-stress and post-stress afternoon b4, Deviation = cortisol increase post-stress minus pre-stress Cortisol data:	Pearson's correlation analysis	a3. Trait anxiety was positively correlated to basal (pre-stress) and post-stress cortisol level b4. Trait anxiety was not significantly correlated with cortisol increase during stress	The results indicate that a high level of anxiousness as a trait personality is associated with chronic high levels of cortisol, irrespective of psychosocial stress exposure
Schlotz 2006 [55]	STAI	Design: C-S No.: 71 M/W: 31/40 Age: 52.6 years (SD 16) Group: Healthy Excl: Corticosteriods, pregnancy, diabetes P rate: Subsample of	Days: 2 weekdays approx. 3 months apart Samples per day: 3 Times for sampling: 11:00, 15:00, 18:00 h Setting: Ambulatory	Luminescence immunoassay Handheld computer device to monitor compliance. Questions regarding exercise, smoking, meals, coffee were asked (computer) before sampling	Measurement(s): Model with 3-level structure (person, days, time/day) a5. The correlation with STAI score was performed with cortisol secretion Cortisol data: log	Mixed models testing the impact of control variables, stressors and affect on cortisol. Controlling for sex, age, exercise, smoking as other did not contribute (OC, food intake, coffee)	a5. No significant association of trait anxiety with cortisol secretion. The association of performance pressure with cortisol not relevant for this review	Trait anxiety did not have significant main effect on cortisol. However HPA activation to performance pressure is stronger in subjects with high anxiety (beyond the scope of this review)

		broader community-based sample			transformed			
Ryff 2006 [41]	STAI	Design: C-S No.: 135 M/W: 0/135 Age: 74 years (SD 7.08) Group: Women recruited from previous longitudinal study P rate: Half of the original sample. Responders younger and more educated	Days: 4 Samples per day: 3 Times for sampling: 30 min after awakening, midday (before lunch) and evening before bedtime Setting:	Cortisol enzyme immunoassay kit No brushing, eating or drinking coffee before the morning sample. No lunch before and no brushing before evening sample	Measurement(s): b3. Deviation other Cortisol data: Normalizing transformation done when appropriate. Daily average slope from the 4 days was calculated Samples used: All	Correlation	Daily slope of salivary cortisol not significantly correlated to trait anxiety	The trait anxiety and cortisol results were not discussed separately
Ellison 2007 [56]	STAI	Design: C-S No.: 95 M/W: 0/95 Age: 21-40 years Group: Healthy women Excl: OC, pregnancy or lactation for at least 6 months. Two parts, only part 2 reported	Days: every day during one menstrual cycle Samples per day: 1. Morning samples soon after wakening Setting: Ambulatory. In part two, participants were divided into 4 groups based on STAI scores	Radioimmunoassay previously described by the authors	Measurement(s): a1. Mean of several collected during every third day over a month Cortisol data: Around 1/3 of the samples used, representing every third day unless sample was taken after 09:00 h. Samples were averaged	ANOVA	There was no significant difference between the different anxiety groups in cortisol levels	Differences in anxiety levels that were established by the study design are not reflected in differences in HPA axis activity
Therrien 2008 [45]	STAI	Design: C-S No.: 78 M/W: 50/28 Age: 38 years (1.0)/37 years (1.4) Group: Healthy free of medication Excl: History of depression or psychiatric disorders, CVD problems, smoking	Days: 3 randomly assigned within a 2-month period Samples per day: 2 Times for sampling: Awakening and +30 min Setting: ambulatory	Radioimmunoassay Abstain from alcohol and physical activity 24 h before, caffeine during the same day. No brushing and no food or drink except water between sampling Fasting since the evening before (20:00 h)	Measurement(s): b1. Deviation between morning sample +30 min minus awakening (ACR) Cortisol data: Average from the 3 days (except for 20 individuals for 2 days due to missing samples)	Correlation	Awakening cortisol response was negatively correlated with trait anxiety in women. No significant association in men	In women ACR are negatively correlated to trait anxiety
Taylor 2008 [57]	STAI	Design: C-S. No.: 28 M/W: 28/0 Age: 21.6 years (SD 2.3) Group: Young military men Excl: Previous head injury, reporting having PTSD, substance dependence, medication	Days: 2 (+1 day under military stress, data not reported here) Samples per day: 5 Times for sampling: 07:30, 08:30. 09:00, 16:00 and 19:30 h Setting: Ambulatory	Radioimmunoassay No caffeinated food or beverage, alcohol, tobacco or medication 12 h before data collection period	Measurement(s): a2, a3, a4. Single measure all measurements a5. Mean of several measurement b3. Diurnal profile Cortisol data: Log transformation	a5, b3. ANOVA repeated measure. Adjusted for wake time a2, a3, a4. t-test	a5. Total cortisol level not different between the groups b3. A significant overall difference with the cortisol level remaining high in the mid-morning anxiety group a. For each time point, significantly higher at 08:30 h and 09:00 h	Although total cortisol did not differ between the groups, discrepant diurnal cortisol profiles were observed. In the high anxiety group the mid-morning levels remained increased
Hlavacova 2008 [58]	STAI	Design: EXP No.: 40 M/W: 0/40 Age: 20-30 years	Days: 1 Samples per day: 2 samples (before and 15 min following stress procedure)	Radioimmunoassay	Measurement(s): b4. Cortisol levels as an effect of time × group × menstrual cycle (MC) a2. Baseline levels (pre-	ANOVA repeated measure	No difference between the groups in baseline levels (pre-stress). Salivary cortisol levels were affected by stress in anxious women only in the follicular phase	A combined mental and physical stress procedure of mild intensity induced a significant cortisol increase only in women with high trait anxiety in the follicular

		Group: healthy students Excl: Any somatic or mental disease, BMI >25 kg/m^2, BP >140/90 mmHg, pregnancy and lactation. Selection of subjects with high (20) and low anxiety (20) scores	Setting: Stroop test and physical component test (handgrip exercise), performed between 07:30 and 11:30 h		stress). 07:30 h Cortisol data:		(interaction effect of time × group × MC phase)	phase of the menstrual cycle
Préville 2008 [59]	STAI	Design: C-S, EXP No.: 315 M/W:131/184 Age: 60-75 years Group: Healthy older Excl: Probable major depression, severe cognitive problems, endocrine disorder, cancer, antidepressant, estrogen, GC replacement during the previous month P rate: 62% of selected subject participated	Days: 2 exp days (solving math problem during 6 min) Samples per day: 3/exp Times for sampling: before, direct after stress and 40 min after relaxation. All experiments started at 08:30 h Setting: Two experimental days. Subjects divided into high and low anxiety	ELISA? (cortisol concentration was determined in duplicate by enzymatic dosage using an automated device Ìmmuno I by Bayer Instructed not to smoke, or drink coffee or alcohol after 00:01 h the previous evening	Measurement(s): a2. Mean baseline cortisol secretion for the two different days (T1/T2) b4. Mean of the experimental related cortisol reactivity b4. The cortisol curve during the experimental situation Cortisol data:	Structural equation modelling	a2. No significant difference in mean baseline cortisol secretion between the groups at either T1 or T2 b4. No significant difference in the mean of the experimental related cortisol reactivity. The response was different with a positive response in the non-anxious group compared with lack of response in the anxious group	The magnitude of the experimental related physiologic reactivity level, 40 min after the test was higher in the non-anxious group. The anxious group showed no significant positive gradient which appears to be in agreement with the helplessness reaction hypothesis in individuals presenting a high level of anxiety

Table 2d: Studies on burnout and exhaustion sorted by year of publication

Reference	Outcome	Study design/group characteristics	Sampling	Laboratory method and standardization in sampling	Statistical approach for cortisol measure	Statistical analysis, cortisol in relation to outcome	Results	Discussion
Kristenson 1998 [73]	VE MQ	Design: C-S No.: 183 M/W: 183/0 Age: 50 years Group: Population-based random selection of 50-year-old men from Lithuania and Sweden Excl: Serious disease, SBP >180 mmHg, DBP >105 mmHg, AMI <3 months, unstabilized angina pectoris, insulin-treated diabetes P rate: 85%	Days: 1 weekday Samples per day: 4 Times for sampling: At baseline (after rest) before, and 16, 20 and 40 min after start of stress test Stetting: Laboratory, at 07:30 h or 09:30 h Stress test: 1. Anger recall 2. Mental arithmetic 3. Cold pressor	Salivette cotton swabs (Sarstedt, Sweden) RIA (CoatA-Count, LA USA) Participants arrived at laboratory fasting after a night's sleep	Measurement(s): b4. Before and after stress test Cortisol data: Samples used: +40 min	Repeated measures analysis of variance	b4. No significant association between VE and cortisol at 40 min after start of stress test Effect marginally significant p=0.09	Main aim to investigate cardiovascular and cortisol responses to acute stressors Sign negative relationship between VE and serum cortisol at 30 min. p<0.001
Melamed 1999 [8]	BO SMBQ Subgroups: HCB high	Design: C-S No.: 111 (37+22+52) M/W: 107/4	Days: 1 Samples per day: 2 Times for	Saliva collected in 2 mL test tubes RIA (Coat-A-Count)	Measurement(s): a5. One morning sample (08:00 h) and one	Subgroups by median splits of SMBQ scores, and chronicity scores. Repeated ANCOVA:	a5. Subjects with high chronic BO had higher salivary cortisol than	BO is associated with heightened physiological arousal

	chronic (n=37), HNCB high non-chronic (n=22) Low burnout (LB) (n=52	Age: 43.1 years (SD 9.0) Group: Fulltime non-shift employees Excl: Self-reported CVD, and incomplete data P rate: 111/152 (73%)	sampling: 08:00 h, 16:00 h Setting: Ambulatory		afternoon sample (16:00 h) b3. Slope morning-afternoon Cortisol data: Log transformed Samples used: Both samples	main effects and interaction (group × time) Confounders: smoking, medication	subjects with low BO. The difference was significant only for those with high chronic BO b3. There was no significant group by time interaction effect	May disclose mechanism underlying the link between BO and CVD risk
Pruessner 1999 [64]	BO MBI+TBS	Design: C-S No.: 66 (HB 30, LB 36) M/W: 24/42 Age: 43.6±9.5 years Group: Medication-free teachers (except for use of oral contraceptives) Subgroups: HB (z>0), LB (z<0)	Days: 3 Samples per day: 4 Times for sampling: Awakening, +15, +30, +60 min Setting: Ambulatory. Instructed to sample during working days Dexamethasone: 0.5 mg by mouth, on 2nd evening, between 22:00 and 23:00 h	Salivette (Sarstedt) Time-resolved fluorescence immunoassay Instructed to strictly follow time schedule for sampling, stay in bed until after 2nd sample, and not brush teeth or have breakfast until sampling was completed	Measurement(s) a2. Mean awakening response b1. Awakening response: interaction BO by time tested d. Cortisol data: - Samples used: All four samples	Seven subscales of two questions were z-transformed and aggregated into a single variable with HB defined as z>0, and LB defined as z<0 ANOVA (group × day × time) Confounders: gender, perceived stress, oral contraceptive use	a2. High BO was associated with lower morning cortisol levels on all 3 days ($p<0.001$) (based on MBI+TBS, MIB, and TBS, respectively) b1. There was no significant interaction (BO × time): (based on MBI+TBS) d. Stronger cortisol suppression after DST, in HB than in LB group (based on MBI+TBS)	Teachers with HB levels showed blunted cortisol levels the first 2 h after awakening and increased suppression after DST Combined BO measures resulted in the most marked differences
Nicolson 2000 [74]	VE MQ, MIVE	Design: C-S No.: 59 (29+30) M/W: 59/0 Age: VE, 51.1 years (SD 4.5); C, 52.2 years (SD 5.1) Group: Healthy, non-smoking VE subjects and controls Excl: CVD, pulmonary disease, GI complaints, major depression, diabetes, rheumatoid disorder, hypercholesterolemia, Parkinson, thyroid disorders, etc., smoking P rate: 577/1600 screened; 29 VE subjects, and 30 controls selected	Days: 2 Samples per day: 5+6 Times for sampling: Day 1: 21:30 h, +20, 40, 60 min after SIST and at 22:35 h Day 2: 06.55 h +20 min, 11:00 h, 16:00 h, 17.40 h, 19:00 h Setting: Laboratory, Stress test: SIST	Salivette cotton rolls (Sarstedt) RIA, using HPLC-purified preparation of cortisol-3-CMO-histamine and antiserum made by 3-CMO-BSA conjugate Morning sampling was performed at awakening while prone in bed, with second sample before breakfast	Measurement(s): a1. 06:55 h a2. 11:00 h a3. 16:00 h, 17.40 h a4. 19:00 h, 21.30 h, 22.35 h a5. Basal cortisol levels: mean of 7 samples (06:55 h, 11:00 h, 16:00 h, 17:40 h, 19:00 h, 21.30 h, 22.35 h) b1. Awakening response: awakening, +20 min b3. Basal cortisol levels: interaction tested group by time b4. Cortisol in relation to stress test: baseline at 17.30 h, +20, 40, 60 min after beginning of SIST Cortisol data: Log transformed Samples used (time): All samples	t-test Repeated ANOVA Multiple linear regression Confounders: covariates: perceived stress, sleep quality, current daily fatigue	a1, a2, a3. No significant difference between groups in cortisol levels at any time point a4. Significantly lower cortisol levels in VE subjects at 21.30 h and 22:35 h, but no significant differences at 19:00 h a5. No significant main effect of VE on overall cortisol levels ($p=0.08$) b1. No significant difference in CAR between groups b3. Significantly lower cortisol levels at 21:30 h and 22:35 h in post hoc analyses performed b4. VE-subjects had significantly lower cortisol levels throughout SIST, but no significant group by time interaction in	Results showed few differences between groups. However, the observed power of the test of group differences in reactivity was low Samples for determining morning response were obtained only at awakening and 20 min later; the interval may be too short for detecting differences between groups

							response to SIST	
Morgan 2002 [65]	BO MBI	Design: C-S No.: 41 M/W: - Age: - Group: Soldiers participating in combat diver qualification training Excl: -	Days: 1 Samples per day: - Times for sampling: Morning and evening Setting: -	-	Measurement(s): a2. a4. Cortisol data: - Samples used: -	- Confounders: -	a2. Subjects with higher levels of BO had significantly lower morning cortisol levels a4. Subjects with higher levels of cortisol had significantly higher evening cortisol levels	Adds weight to argument that disruption of HPA axis regulation exists in individuals with BO
De Vente 2003 [66]	BO MBI Group: BO: Patients on sick leave (2 weeks to 3 months fulltime, or 6 months part-time) work-related complaints Controls (C): Healthy working controls	Design: C-S No.: 45 (BO 22, C 23) M/W: 24/21 Age: BO, 42 years (10); C, 31 years (7.6) Excl: Medical disease that could explain fatigue BO: Primary axis I disorders, and severe depression (BDI<25) C: trauma, psychiatric illness, pregnancy, current sick-leave, and scoring within clinical range of MBI+fatigue scores	Days: 1 Samples per day: 9 Times for sampling: 1. Morning samples: Awakening, +30, +60 min, 12:00 h 2. Laboratory session (TSST) -4, +5, +19, +33, +47 min (in relation to stress test, starting at 13:30 h, final sample taken at 15:00 h) Setting: Laboratory and ambulatory. Sampling on weekday	1. Non-coated Salivette (Sarstedt) 2. Spit into cup (Navazesh) Enzyme-immunoassay (DSL) 1. No breakfast or brush teeth within 15 min of sample being collected 2. Asked to refrain from coffee and cigarettes for at least 60 min before TSST	Measurement(s): a2. Mean awakening response: awakening, +30, +60 min a3. 12:00 h, 15:00 h b1. Awakening response: interaction tested (group by time) b4. Laboratory session samples Cortisol data: - Samples used: all samples	Repeated ANOVA (time × group) Covariates entered in analyses: age, gender	a2. Significantly higher cortisol levels in BO patients a3. No significant difference in cortisol levels between groups at 12:00 h or 15:00 h b1. No significant interaction effect (group by time) b4. Significant time by group interaction, probably due to steeper decrease in cortisol levels in BO group, No significant difference between groups during TSST	Results suggest dysregulat-ion of HPA axis in BO as indicated by increased early morning cortisol levels BO patients might not have recovered fully during the night which may be a sign of sustained activation of the HPA axis
Ekstedt 2004 [61]	BO SMBQ Subgroups identified based on SMBQ-scores: High burnout (HB) (n=12), LB controls matched for age, gender and employment duration (n=12)	Design: C-S No.: 24 (HB 12, LB 12) M/W: 10/14 Age: 30.5 years (SD 1) Group: Fulltime, non-smoking employees, non-sedentary life-style, with moderate alcohol intake Excl: - P rate: 414/676 (61%) screened, 24 selected	Days: 1 Samples per day: 9 Times for sampling: Awakening, +15, 30, 60 min and 11:00 h, 15:00 h, 19:00 h, 21:00 h and at bedtime Setting: Ambulatory, workday	Salivette (Sarstedt) RIA (ORION)	Measurement(s): a1. Awakening sample (07:00 h ± 1 h) a2. Mean cortisol: Awakening, +15, 30, 60 min b1. Difference between samples at awakening and +60 min Cortisol data: Variables with skewness >±2 were log transformed Samples used: Awakening, + 15, 30, 60 min	Stepwise multiple regression Pearson correlation Confounding: Excl: CVD, lung disease, diabetes or metabolic disease in the past 12 months. No anxiolytics, beta-receptor stimulants, ACE inhibitors, or antidepressants. 9 used oral contraception	a1. BO was positively associated with cortisol at awakening a2. No significant associations between BO and mean value of all four cortisol samples after awakening b1. The difference in cortisol sampled at 0 and 60 min after awakening, did not correlate significantly with group	Association between cortisol and sleep was primary focus of study. BO was treated as a potential confounder
Grossi 2005 [62]	BO SMBQ HB (n=22): sick leave due to burnout, white collar Moderate burnout (MB) (n=20) + LB (n=22): White-collar, median split	Design: C-C No.: 64 (22+20+22) M/W: 29/35 Age: HB, 42 years (SD 9); MB, 39 years (SD 9); LB, 41 years (SD 10) Excl: HB, no comorbid	Days: 1 Samples per day: 4 Times for sampling: awakening, +15, 30, 60 min Setting:	Salivette, cotton rolls (Sarstedt) RIA (ORION) Instructed not to brush teeth or consume any food before having completed sampling, to	Measurement(s): a1. Awake a2. Mean awakening response: awakening, +15, +30, +60 min b1. MnInc: $(cortisol_{15+30+60\ min})/3$ -	Repeated ANOVA Linear regression Spearman rank correlations Covariates in regression analyses: antidepressant medication, time of awakening, sleep variables,	Women (n=35): a1. Sign higher cortisol levels at awakening a2. Sign higher cortisol at +15 min, +30, +60 min, in HB vs LB	Results indicate dysregulation in HPA axis activity by increased morning salivary cortisol, among female BO patients Among males highest cortisol levels in

	of SMBQ scores	depression; MB+LB, insufficient saliva; HB, 22 patients with HB selected from 93 consecutive patients MB+LB: 330/450 (73%) screened, 45 selected, 42 included	Ambulatory, weekdays	collect first two samples while remaining prone in bed	$cortisol_{awake}$ c1. AUC_{ground} Cortisol data: Log transformed Samples used: All four samples	negative mood	b1. No significant difference MnInc c1. HB patients higher AUC_{ground} than LB group Men (n=29): a1. No significant difference a2. No significant difference. Sign higher cortisol levels in MB vs LB group at +60 min. No other significant difference b1, c1. No significant difference in MnInc or AUC_{ground}	intermediate levels of BO
Söderström 2006 [63]	BO SMBQ HB (n=12) and age- and gender-matched controls with LB (n=12)	Design: C-S No.: 24 (12+12) M/W: 10/14 Age: HB, 30 years (SD 2); LB, 31 years (SD 2) Group: IT-employed grouped based on SMBQ scores Excl: - P rate: 414/676 screened, 24 sampled	Days: 2 Samples per day: 9-10 Time of sampling: Awake, +15, +30, +60 min, 11:00 h, 15:00 h, 19:00 h, 21:00 h, 23:00 h and/or at bedtime Setting: ambulatory, 1 workday, 1 day off	Salivette (Sarstedt) RIA Instructed not to eat or brush teeth for at least 30 min before sampling. Morning saliva sample was sampled between 05:30-07.30 h before breakfast	Measurement(s): a1. Awakening, a2. Awakening response: awake, +15, 30, 60 min a2. 11:00 h a3. 15:00 h a4. 19:00 h, 23:00 h a5. Diurnal cortisol pattern: awakening, 11:00 h, 15:00 h, 19:00 h, 23:00 h b3. Diurnal amplitude: difference between morning peak and bedtime value Cortisol data: Samples used: awakening, morning peak, 11:00 h, 15:00 h, 19:00 h and 23:00 h	*t*-test Repeated ANOVA Diurnal amplitude was calculated as difference between post-awakening peak value and the bedtime value	a1, a2, a3, a4. No significant differences in cortisol levels between groups. HB had higher cortisol at awakening on work day compared with weekend a5. No main effect of group on diurnal pattern of cortisol b3. No significant difference (difference post-awakening peak and evening values) between groups during either day	Lacked information on the duration of BO symptoms. It is possible that a longer duration of BO may have yielded different results
Langelaan 2006 [67]	BO MBI Subgroups: BO (n=29), work-engaged (WE) (n=33), controls (C) (n=26)	Design: C-S No.: 88 (29+33+26) M/W: 88/0 Age: BO, 45.3 years (SD 8.1); WE, 45.1 years (SD 7.9); C, 42.9 years (SD 7.7) Group: Male managers Excl: Cortisone medication, asthma, diabetes, RA, CVD, BMI >30 kg/m^2, abuse, metabolic or endocrine abnormalities P rate: 338/450 (75%) screened, 88 selected	Days: 4 Samples per day: 4 Times for sampling: awakening, +15, +30, +60 min Setting: Ambulatory, 3 consecutive workdays+1 non-workday Dexamethasone: 0.5 mg on second evening at 22:30 h	Cotton roles (Sarstedt) Immunoassay (DELFIA) Instructed to follow time schedule strictly, report sampling time, to complete sampling before breakfast, and refrain from drinking coffee or tea and brushing their teeth before sampling	Measurement(s): a2. Mean of morning samples b1. Interactions group × time d. DST Cortisol data: Log transformed Samples >3SD from mean were excluded from analyses Values of 2 workdays were pooled for analysis Samples used: All four samples	BO was defined based on MBI cut-off-scores Repeated ANOVA (interactions day × group and time × group tested)	a2. No significant differences in morning cortisol levels between groups b1. No significant group by time interaction effect d. There were no significant differences in suppressed cortisol levels between BO and reference groups (main effect or group by time interaction)	There is no convincing evidence for HPA axis abnormalities functioning in BO

Mommersteeg 2006 [68]	BO MBI (UBOS) Groups: BO: Burnout on sick leave, C= age- and sex-matched relatives/acquaintances of researchers	Design: C-C No.: 43 (22+21) M/W: 14/29 Age: BO, mean 43 years; C, mean 50 years Excl: Outliers (z-scores >3SD; BO, corticosteroid medication P rate: -	Days: 2 Samples per day: 7 Times for sampling: Awakening, +15, 30 min Day curve: 12:00 h, 18:00 h, 22:00 h	Salivette, cotton role (Sarstedt) Time-resolved immunoassay with fluorescence detection Collection time was registered in paper diary Smoking, use of oral contraceptives, prescribed medication were registered	Measurement(s): a2. Awakening response: awake, +15, 30 min a5. Day curve: 12:00 h, 18:00 h, 22:00 h b1. Awakening samples: interaction (group by time) b3. Day curve: interaction (group by time) c1. $AUC_{increase, ground}$ Cortisol data: - Samples used: All samples	Repeated ANOVA with interactions tested Spearman rank correlations Outliers (z-scores >3SD) were excluded from analyses	a2. BO group had significantly lower cortisol levels after awakening (CAR) a5. No significant differences in cortisol levels during the day b1. No significant difference in cortisol rise after awakening (interaction group × time) b3. No significant difference decline during day (interaction group × time) c1. No consistent pattern of correlations	Hypoactivity of the HPA axis suggests that BO may be associated with exhaustion/ fatigue rather than with depressive mood
Mommersteeg 2006 [69]	BO MBI-GS	Design: C-S No.: 94 (56+38) M/W: 44/50 Age: BO, 43.0 years (SD 9.3); C, 44.8 years (SD 8.6) Group/subgroups: BO patients (n=56) on sick-leave, and at initial stage of treatment. Selection was based on cut-off scores indicating clinical burnout; controls (n=38), - Excl: BO, symptom check list scores (SCL90) within psychopathological range, asthma, RA, diabetes, and antidepressant medication; C, - P rate: -	Days: 3 Samples per day: 3 Times for sampling: Awakening, +15, +30 min Setting: Ambulatory, weekdays Dexamethasone: 0.5 mg on the second evening	Salivette, cotton roll, (Sarstedt) Chemiluminescence assay (LIA)	Measurement(s): a2. Mean awakening response: awake, +15, +30 min b1. Awake, +15, +30 min (interaction effects tested) d. Awake, +15, +30 min after DST Cortisol data: Positively skewed data were log transformed Samples used: All samples	Repeated ANOVA (interaction group by time tested)	a2. No significant differences in morning cortisol levels between groups b1. No significant differences in morning cortisol increase (slope) between groups d. No difference in cortisol levels or increase in cortisol (slope) between groups	Absence of difference in cortisol levels between groups is in accordance with previous study from the same group
Mommersteeg 2006 [70]	BO MBI-GS	Design: C-C No.: 109 (74+35) M/W: 78/31 Age: BO, 43.9 years (SD 8.7); C, 44.9 years (SD 10.5) Group/subgroups: BO (n=74); C, age- and gender-matched controls (spouses and coworkers of researchers) Excl: - P rate: -	Days: 3 Samples per day: Day 1+2: 6 samples Day 3: 3 samples Times for sampling: Awake, +15, +30 min Day curve: 12:00 h (before lunch), 18:00 h (before dinner), 22:30 h Setting: Ambulatory, weekdays Dexamethasone:	Cotton role (Sarstedt) Immunoassay (DELFIA) Instructed not to brush teeth, eat or drink coffee or alcohol 30 min before sampling. Sampling time reported by participants in paper diary	Measurement(s): a2. Awakening response: awake, +15, +30 min a5. Day curve: 12:00 h, 18:00 h, 22:30 h b1. Awakening response: interaction group by time b3. Day curve: interaction group by time c1. Awakening response: AUC_{ground}, $AUC_{increase}$	Cortisol data that deviated >3SD of the mean were excluded from further analyses Pre-dexamethasone samples (day 1+2) were pooled, mean data used for further analyses Repeated ANOVA (interactions group by time tested) correlations No effects on results of gender, age, BMI, smoking, oral contraceptive use, sick leave, work status, seasonal effect, activity and perceived stress during the day, coffee/alcohol/food intake per	a2, a5. No significant difference in morning cortisol levels, or cortisol levels during the day, between groups b1, b3. No significant difference in cortisol rise after awakening, or cortisol decline during the day, between groups c1. There were no significant correlations between level of complaints and non-	There are no clear disturbances in HPA axis functioning in clinically diagnosed BO patients and within the BO group no association between the cortisol parameters and any of the indicators of severity of complaints Saliva samples or low-dose DST may not be sensitive enough to reveal subtle dysregulations in the HPA axis

			0.5 mg by mouth at 22:30 h on second evening		d. Awakening samples after dexamethasone: mean levels and interaction group by time Cortisol data:	measurement, time of awakening and sampling time, flat CAR, sleep, and in BO group: complaint duration, sick leave, history of work-related problems	suppressed or suppressed morning AUC_{ground} or $AUC_{increase}$ in either the BO or the control group d. No significant differences in suppressed cortisol levels, or in cortisol increase after awakening between groups	
Mommersteeg 2006 [77]	BO MBI-GS Exhaustion subscale	Design: L No.: 74 M/W: 53/21 Age: 43.9 years (SD 8.7) Group: BO patients from outpatient clinic Excl: Cortisol data that deviated > 3SD over mean were excluded from analyses. The longitudinal part was not designed as an RCT intervention study	Days: 3 days at 3 time points Samples/day: Day 1+2: 6 samples Day 3: 3 samples Times for sampling: Awake, +15, +30 min, 12:00 h, 18:00 h, 22:30 h Setting: Ambulatory, weekdays Sampling at 3 time points: Before treatment (baseline), 8.5 months later and at follow-up 6.3 months post-treatment Dexamethasone: 0.5 mg at 22:30 h on second evening	Luminescence immunoassay (LIA) Instructed not to brush teeth, eat, drink coffee or alcohol 30 min before sampling	Measurement(s): b1. Awake, +15, 30 min b3. 114:00 h, 18:00 h, 22:30 h Cortisol data: square root and log transformed values Samples used: all samples from all three measurement points *Cortisol data which deviated > 3SD over mean were excluded from analyses*	Multilevel regression analyses Covariates sampling time, food/coffee/nicotine activity level 30 min before sampling sleep, perceived stress, medication, smoking, sick leave/work complaints, age, gender. BMI, education	b1. CAR was positively associated with exhaustion at baseline b3. No association between exhaustion at baseline and cortisol day curve d. There were no significant associations between exhaustion at baseline and cortisol after dexamethasone intake	Results imply that variability of cortisol within persons is larger than variability between persons
Sjögren 2006 [75]	VE MQ	Design: C-S No.: 257 M/W: 129/128 Age: 30-64 years Group: Random sample population based P rate: 61% response rate in population-based health survey. Random sample of 400 individuals invited. 64.5% responded (257/400)	Days: 3 Samples per day: 3 Times for sampling: Awakening, +30 min, before going to bed Setting: Ambulatory, workdays	Salivette Time-resolved fluorescence detection *Instructed to fill in exact time of sampling. Instructions on fasting (first two samples before breakfast)*	Measurement(s): a1. Awakening a2. +30 min after awakening a4. Before going to bed b1. Awakening response (awakening +30 min) b3. Diurnal deviation calculated as arithmetic difference between morning samples (awakening and 30 min, respectively) and evening value Cortisol data: log transformed	Partial correlation analyses Cortisol values 2SD from the arithmetic mean were excluded Mean values of each sampling time over 3 days was calculated. Confounders: age gender, awakening time, regular medication, smoking, alcohol entered	a1, a4. No significant correlation between awakening or evening cortisol and VE a2. Negative correlation between cortisol 30 min after awakening and VE b1. No significant associations between CAR and VE b3. Significant negative association between awake/evening and cortisol, and between 30 min/evening	Scale scores of VE are related to flat diurnal cortisol rhythm

					Samples used: All samples		cortisol, respectively, and VE	
Wirtz 2007 [76]	VE MEQ-S	Design: C-S No.: 50 M/W: 50/0 Age: 42.5 years (SEM 2.0) Group: Healthy medication-free men Excl: Acute somatic or psychiatric disorders, heavy exercise, smoking, CVD, RA, allergies, atopic, current infectious disease, increased blood-sugar or cholesterol	Days: 1+1 Samples per day: 8+9 Times for sampling: 1. TSST: immediately before stress test, and 0, 10, 20, 30, 40, 50, 60 min after completion of TSST 2. Circadian profile: awake, +15, 30, 40, 60 min, 08:00 h, 11:00 h, 16:00 h, 20:00 h Setting: Laboratory, Ambulatory, TSST-start between 14:00 h and 16:00 h	Salivette, cotton rolls (Sarstedt) Chemiluminescence immunoassay (LIA) Participants abstained from food and drink for 2 h before the experiment and exercise, alcohol and caffeinated beverages the evening before the stress test	Measurement(s): c4. $AUC_{increase}$ in response to TSST Cortisol data: z-transformed Samples used: TSST samples	Linear regression All regression parameters were z-transformed before analyses Covariates: age, BMI, MAP	c4. No significant association between VE and $AUC_{increase}$ (cortisol increase in response to TSST)	Main aim to investigate association between perfectionism and cortisol
Sonnenschein 2007 [78]	BO MBI-exhaustion subscale ESM exhaustion subscale (aggregated and same-moment measures, respectively)	Design: C-S No.: 42 M/W: 18/24 Age: 42.7 years (SD 8.3) Group: BO patients on sick leave, with complaints >6 months Excl: Primary psychiatric disorder, use of antidepressants or anxiolytics, pregnant women P rate: 209/293 (71%) screened, 42/47 selected met inclusion criteria	Days: 3 Samples per day: 3 Times for sampling: Awakening, +15, +30 min Setting: Ambulatory, weekdays Dexamethasone: 0.5 mg by mouth, at 22:30 h on second evening	Cotton roll, Salivette (Sarstedt) Chemiluminescence assay (LIA) 1-h instruction to explain use of electronic diary and saliva sampling Instructed not to brush teeth, eat, or drink coffee or alcohol from awakening until last sample	Measurement(s): b1. Awakening response: level and increase d. Cortisol data: - Samples used: All samples	Multilevel regression analyses (interaction tested symptoms × time) ESM scores: same moment and aggregated 2 week scores, respectively Confounding/entered covariates: time of awakening, BMI, depressive mood, sleep quality, age, smoking, gender, oral contraceptive use, sick leave Analyses were rerun without participants with negative morning cortisol awakening response, and without participants with comorbid psycho-pathology	b1. No significant association between MBI-exhaustion or aggregated 2-week ESM-exhaustion scores and awakening cortisol level or cortisol increase after awake Significant negative association between same-moment ESM scores and awakening cortisol level and increase. d. Significant negative association between aggregated ESM scores and suppressed cortisol increase after awakening, but no association between MBI-exhaustion score or ESM same moment assessments, and DST suppressed cortisol increase after awakening No significant association between MBI-exhaustion score, aggregated or same-	Findings of no association between general severity of exhaustion assessed with retrospective questionnaires and cortisol confirms prior research. However, association between same-moment general severity of symptoms and cortisol levels was observed. Difference in findings suggests that ESM same-moment assessments are a more reliable way of assessing symptom severity. The presumption that BO symptoms and endocrine values fluctuate in individuals across days was confirmed

							moment ESM scores and awakening cortisol levels	
Bellingrath 2008 [60]	BO MBI, TBS VE MQ	Design: C-S No.: 135 M/W: 40/95 Age: 46.1 years (SD 9.2) Group: Employed teachers Excl: Psychiatric disorder, diabetes, pregnancy, corticosteroid or psychotropic medication, non-compliance with sampling P rate: 149/190 (78%) recruited participants were screened, 135 different participants contributed to cortisol data in analyses	Days: 4 Samples per day: 7 Times for sampling: Awakening, +30, 45, 60 min, 11:00 h, 15:00 h, 20:00 h Setting: Ambulatory, 2 work days + 1 leisure day + 1 day after DST Dexamethasone: 0.25 mg by mouth at 23:00 h on 3rd evening	Native saliva in 2 mL reaction tubes (Sarstedt) Time-resolved fluorescence immunoassay (DELFIA) Participants were informed in one-to-one interviews about importance of accurate timing of sampling + that sampling time would be electronically monitored. Participants recorded exact sampling times in paper diary Instructed not to brush teeth before morning sampling was completed, not to smoke, eat, or drink beverages (alcohol, caffeine or fruit juice) for 60 min before sampling	Measurement(s): a5. Cortisol day profile: all samples d. All samples Cortisol data: Log transformed Samples used: Non-compliant profiles were excluded from analyses	GLM Effects of age, BMI, waist/hip ratio, sleep quality, awakening time, gender and smoking on cortisol were tested: gender and smoking status yielded significant effects and were entered in analyses, as well as HADS (in the final analysis)	a5. No significant association between MBI exhaustion subscale, or VE scores, and cortisol day profile d. Higher MBI-EE subscale and VE scores were associated with lower suppressed cortisol levels. When HADS depression scores were entered simultaneously, no significant associations remained	Results suggest that subtle dysregulation can be found in school teachers with high levels of BO and VE, but only after application of DST High BO/VE, with chronically increased cortisol levels or repeated cortisol peaks, may have increased sensitivity of the GR, which in turn may lead to an especially sensitive regulation of the negative feedback loop
Österberg 2009 [71]	BO MBI-GS	Design: C-C No.: 220 (46+174) M/W: 71+168 Age: BO, 48.2 years (SD 9.5), C, 47.5 years (SD 10.2) Group/subgroups: BO patients (n=65) on recent sick leave with work strain as probable cause; C, external reference group (n=174): blue- and white-collar workers in different professions, with similar age, and gender distribution Excl: BO, non-work related exhaustion or other disease; C, - P rate: BO, 101/729 (14%) screened, 68 eligible, 65 agreed to participate; C, -	Days: 1 Samples per day: 3 Times for sampling: Awakening, +30 min, 21:00 h Setting: Ambulatory	Salivette (Sarstedt) BO: cotton swabs; C, polyester swabs RIA (Spectria) Instructed to keep swabs in mouth until fully hydrated, avoid smoking and eating heavy meals 60 min before sampling, and not to brush teeth or eat until after morning sampling	Measurement(s): a1. Awake a2. +30 min after awakening a4. 21:00 h b1. Difference cortisol awakening to +30 min, (absolute and proportional change) b3. Diurnal deviation: Decrease in cortisol concentration from cortisol morning peak (highest concentration found in either of the two samples) and the 21:00 h evening sample Cortisol: Log transformed Samples used: All samples	Univariate ANOVA Confounding:	a1, a2. No significant differences at awakening or +30 min between groups a4. Significantly lower evening cortisol values in BO group b1, b3. No significant difference between groups	Main aim was to investigate cognitive performance in patients with BO, in relation to flexibility of the HPA axis. Suggested that, concerning lower cortisol in evening in BO patients, most of whom were on sick leave to some extent, may reflect being more relaxed in the evening due to lower demands
Wingenfeld 2009 [72]	VE MQ	Design: C-S No.: 279 (181+77+18+3) M/W: 65+214 Age: 37.5 years (SD 10.9) Group: Nurses	Days: 1 Samples per day: 4 Times for sampling: 07:00 h, 11:30 h, 17:30 h, 20:00 h	Salivette (Sarstedt) Immunoassay with chemiluminescence detection (IBL, Hamburg) Instructed not to brush	Measurement(s): a2. 07:00 h a3. 11:30 h, 17:30 h a4. 20:00 h a5. Mean all four	Pearson's correlation Repeated ANOVA or ANCOVA Three VE subgroups created Potential confounders tested: gender, smoking, use of oral	a2, a3, a4. There was no significant correlation observed between VE and cortisol a5. There were no	VE was not associated with altered cortisol secretion

		Subgroups compared: 2a. No VE (n=46) 2b. Moderate VE (n=122) 2c. Severe VE (n=109) Excl: - P rate: -	Setting: Ambulatory, working day	teeth, eat or drink other than water, or smoke, 30 min before sampling	samples b3. Deviation all four samples Cortisol data: Log transformed Samples used: All four samples	contraceptives, age. Covariates and between-subject factors entered: age, smoking, depression score, vital exhaustion	significant differences in cortisol levels over the day between VE-subgroups b3. No significant time by group interaction effect observed, for VE	

DISCUSSION

The aim of the present chapter was to analyze associations between several measures of mental health related to chronic stress and cortisol in saliva. Relatively few papers met the final inclusion criteria. Many papers have studied the relationship between depression and salivary cortisol, but the studies often includes patients with other somatic or mental diseases, making it difficult to conclude whether a true association exists, not related to other diseases.

MDD and Depressive Mood

For depression, when looking at the overall picture, there seems to be some consistency that salivary cortisol is increased in clinical populations of patients with MDD, particularly in the single morning and evening measures. The results from the laboratory stress studies also suggest that MDD is related to poorer ability to respond with cortisol to acute stress. Measures of salivary cortisol after dexamethasone administration demonstrated that patients with MDD showed less suppression, usually calculated as the number of non-suppressors with a larger proportion among the patients.

No time point, however, among the single measures showed full consistency regarding salivary cortisol levels in patients with MDD. In an attempt to explain the discrepancy between different studies, 4 main explanations are given: the type of the depression, the power of the study in terms of participants, the reliability of cortisol measures in terms of one or more days of sampling, and confounding factors, mainly medication.

Discussions about the type of depression seem to mainly involve melancholic or non-melancholic depression. Seven out of 21 papers studying MDD did not find any significant differences between patients and controls for measures of salivary cortisol [16, 19-21, 23, 25, 30]. The authors of 3 of these studies mentioned that one possible explanation could be that the HPA axis abnormalities are usually found in either melancholic or psychotic depression [19, 23, 25], but Copolov and coworkers did not find any differences between non-melancholic and melancholic patients regarding salivary cortisol levels [13]. Unfortunately, most of the papers do not specify the type of depression and thus it is difficult to speculate to what extent this explains the discrepancy between the studies. One study showed changes in the opposite direction and the authors suggest that this could be because the patients were a healthier population of individuals recruited from a general population through advertising [28] compared with other studies in which *e.g.* inpatients with MDD were included.

The power of the studies in terms of small sample size may be another important explanation for several non-significant findings [16]. The sample sizes in studies not showing significant differences between patients and controls are, in general, small, ranging from 14 to 44 patients. However, among those studies in which significant differences between the groups were found, small samples sizes were also found. Small sample size could in many cases, depending on the type of depression, increase the difficulty in identifying difference between patients and controls. However, power is also dependent on the reliability of the measures taken. Among the present studies, the number of days for ambulatory sampling was lower (median 2 measures) for studies without significant results compared with those with significant findings (median 3 measures). The combination of few cases and few days of sampling may add to this loss of power.

The authors of 3 of the 7 papers not showing any difference in salivary cortisol levels between patients and controls also suggested use of antidepressants as a possible explanation, because the HPA axis function has been normalized using antidepressants [19, 21, 25]. The fact that 3 of the studies showing hypercortisolemia in the patient group included patients taking antidepressants does not entirely support this notion. However, there are too few studies to draw firm conclusions but most of the studies showing hypercortisolemia did include antidepressant-free patients. Possibly, a combination of all these factors could explain much of the discrepancy between studies. Among the MDD studies, 5 of those showing hypercortisolemia in patients with MDD attempted to relate to salivary cortisol levels with scale scores in the patient group by using different measures of self-reported depression or, in one case, symptom severity; none of these correlations were found to be significant.

There are fewer studies on depressive mood and salivary cortisol measures included in our analysis. Of 9 studies, measuring single values or the mean of several measures at any time point during the day, 4 showed that depression was related to increased levels, and 2 of these divided the groups into high and low depression instead of relating to scale scores.

The results regarding depressive mood are less consistent for higher levels in singles measures and this is confirmed when the authors studying MDD attempted to relate cortisol level to scale scores. However, with regards to deviation measures, the results seem to be consistent with MDD; 3 of 6 studies showed flatter diurnal deviation. The only study measuring acute response to stress confirms the data from the MDD groups showing poorer response.

Anxiety

An overall conclusion for all single measures is that cortisol levels do not seem to be related to anxiety. In the 2 studies measuring ambulatory salivary cortisol during the morning hours, only 1 study showed a significant relation to anxiety. This was also the only study that measured cortisol at several time points during the morning (3 time points) at fixed hours. The fact that the study group consisted of men on military service, where stressors are often more prominent than in ordinary life, may be more important [57].

Similarly, there were few significant findings regarding measurements of deviation, but also few studies. Only 1 study examined anxiety in relation to CAR and this showed lower CAR among more anxious men but not among women [45]. Diurnal deviation was studied in 4 studies. In 3 of these, significant relationships were found, 2 of which were positive and 1 negative. These differences occurred in different contexts; positive relations occurred among healthy and/or military men [34, 57], and negative relations among women attending a breast clinic [38]. However, the changes where relatively small and the results are not explained by a difference in the awakening or evening levels but rather a slight change in the diurnal profile during mid-morning or afternoon showing either higher or lower levels in relation to anxiety.

Six of the 17 studies assessed saliva cortisol in relation to a laboratory stress test. Among these, only 1 had a significant effect on baseline levels, *i.e.*, standardized rest before stress.

Three of 7 studies found significant results but in opposite directions. Thus, 2 showed a positive relationship (male students and healthy elderly men and women) and 1 showed a negative relationship (female students during the luteal phase). In addition, 1 out of 3 studies measuring AUC in response to stressors showed significant findings; with lower salivary cortisol among male students with high anxiety.

In 6 of 9 studies showing a significant relationship between anxiety level and salivary cortisol, regardless of the measure of cortisol, the groups were split into high and low anxiety [38, 50, 53, 57-59], either by median split (3 studies), using a cut-off (1 study) or by only including extreme groups, *e.g.*, subject with high or low anxiety (2 studies). All except 1 of the studies not showing any significant relation with anxiety treated the anxiety measure as a continuous variable using scales scores. This is in parallel with the conclusion drawn from the depression section that differences are more likely to be detected when the subjects are stratified into groups with higher contrasts. An additional explanation for non-significant findings could be low power and few sample days, because many studies included 1 day sampling. In conclusion, very few significant findings were found for single measurements. Using deviation measures, although there were only a few studies, most showed a significant relationship between anxiety and salivary cortisol, but in opposite directions. The observed divergence of results may be dependent on contextual factors, *i.e.*, the amount of external stressors and population characteristics. Thus, our paper may extend the previous conclusion made by Chida *et al.* that the HPA axis does not seem to be strongly affected by anxiety levels [2, 4].

Burnout

The overall conclusion regarding BO is that most of the statistical analyses (59 analyses in 13 papers), irrespective of the character of the analysis, do not show any significant relationship between BO and measures of cortisol in saliva. However, most of the studies (9 out of 13) found a significant relationship

between BO and at least one of the cortisol measures. Only four studies did not show any relationship with BO [63, 67, 69, 77]. Consequently, most of the authors rightfully concluded that BO is associated with dysregulation in HPA axis functioning. Clearly, however, when analyzing the findings, there seems to be a large discrepancy between different studies regarding the measures used and there is no salivary cortisol measure that clearly demonstrates the character of this supposed HPA axis dysregulation.

Full consistency in terms of non-significant findings is seen for the single midday measures, for all deviation measures from ambulatory saliva sampling, the morning deviation measures (9 studies), and for diurnal deviation measures (6 studies).

No clear picture regarding methodological issues can be seen, that could explain the discrepancy between different studies. There are probably several contributing factors, including the groups studied and the possible influence of comorbid conditions such as depression and in some cases PTSD that could explain some discrepancy between the studies. Differences related to the BO measure used are another possible explanation. It is clear that the BO concept is defined differently depending on which BO measure is being used [7]. Scrutinizing all single measures of cortisol among the papers studying BO, it seems that the type of BO measure could partly explain the discrepancy among the papers showing significant relationships. When significant, studies using the SMBQ showed a positive relationship with cortisol [8, 62]; there were divergent findings among the papers using MBI, as those papers showed both positive and negative associations [64-66, 68, 71, 72].

Unfortunately, many papers include too little or different information about the subjects, comorbidity, medications, adjustments, etc., making it difficult to use the information in the articles to explain the results. Thus, we confirm the conclusion by Kudielka and coworkers that the inconsistency between studies on BO cannot be easily explained [11].

Vital Exhaustion

The pattern for VE is somewhat different from BO, as significant relationships were found for several deviation measures that were not seen for BO. Thus, 2 of 3 studies relating VE using MQ to diurnal variation showed significant relationships, both in the same direction, towards a flatter curve among VE subjects. The major difference between these 2 studies and the study not showing any relationship seems to be the subjects included. The studies showing a flatter day curve included subjects originally selected from a population sample; the study not showing a significant relationship included nurses with high levels of BO. The BO measures in that study were significantly related to increased levels of salivary cortisol, but no relation was seen with VE. Thus, there seems to be some differences between the VE measures and the BO measures regarding the relationship with changes in the HPA axis but this is also highly dependent on which subjects are included in the studies. In all 3 studies measuring VE with MBI-EE, BO was also present, and in 2 of these studies, the inclusion criterion was BO. Consequently, in these studies it is difficult to separate BO from VE, and it also shows that these measures are highly correlated. When significant, the DST suppression test shows consistent results, all pointing towards higher suppression among VE subjects. The only study showing a significant relation among the BO papers also showed higher suppression.

The findings for VE indicate that VE measured with MQ in the general population do suggests that exhaustion could be related to a flatter day curve and poorer response to stress test. The results for cortisol in relation to BO measured with SMBQ seem to be entirely the opposite as BO seems to be related to a higher level of cortisol. The results from the MBI BO measure are mixed and when exhaustion is measured with the MBI-EE subscale, BO is also present and it is thus difficult to conclude whether VE is related to cortisol independent from BO. From these results, BO and VE seem to some extent to be different entities, and the 2 BO measures also seem to be different entities. Even more interesting, 2 of the BO also seem to be different entities, strengtening what has been previously suggested [7].

CONCLUSIONS

The relationship between cortisol measures in saliva and mental health, and consistency of the results, varies depending on the mental health measure. However, for all measures studied, one important notion is that the final number of studies included is relatively few and the power of studies is, in general, small.

For MDD, there seems to be some consistency for higher mean cortisol levels, poorer response to stress, and loss of feedback. In most of the studies, single measures of salivary cortisol were increased in clinical populations of patients with MDD, particularly in the morning and evening measures. The pattern of response to stress in ambulatory settings and in laboratory stress testing does suggest that MDD is related to a poorer ability to respond with cortisol to acute stress. However, this is seen in terms of a flatter diurnal deviation and in poorer laboratory stress reactivity, but not in terms of CAR. Responses to dexamethasone administration showed less suppression among MDD patients. The results regarding depressive mood are less consistent for higher levels in singles measures and this is confirmed when the authors studying MDD attempted to relate cortisol level to scale scores. However, with regards to deviation measures, the results seem to be consistent with MDD; 3 of 6 studies showed flatter diurnal deviation. The only study measuring acute response to stress confirms the data from the MDD groups showing poorer response.

In contrast, the anxiety results show poorer consistency and few studies are included. For single measurements, very few significant results were found; for deviation measures, the results were divergent. Thus, our paper may extend the previous conclusion made by Chida *et al.* that the HPA axis is not strongly affected by anxiety levels [2, 4].

Similarly, the overall conclusion regarding BO is that most statistical analyses, irrespective of the character of the analysis, do not show any significant relationship between BO and cortisol measures, and when these are seen, the results are inconclusive. The two BO measures seem to be partly responsible for this and there is a better consistency within the studies using SMBQ compared with those using MBI. One possible explanation is that the BO concept is defined differently depending on the measures being used [7].

For VE measured using MQ, the pattern of results seems to be different, suggesting that this measure of exhaustion, particularly in the general population, is related to a poorer cortisol response to stress, which is only seen for diurnal deviation and laboratory stress testing but not for CAR. The coexistence of BO and VE in many studies makes it difficult to conclude how the different concepts are related to cortisol. However, an interesting difference appeared between MDD and VE in response to dexamethasone administration. MDD patients tended to show lower suppression and VE patients higher suppression, which suggests a difference in the biological (HPA axis) underpinning of these conditions.

A general impression is that the large proportion of non-significant findings is a function of low power; because of small study samples, few sampling days resulting in low reliability of saliva measures, but also less sensitive measures in terms of low contrast between study groups and within study populations. All mental health measures included in this chapter can be considered to be a chronic state of symptoms or illness, related to long term exposure of psychosocial stress. A cautious conclusion is that this seems be related to poorer cortisol response to stress and a flatter deviation day curve, but more studies are needed with larger power to confirm this as many studies also show non-significant findings. An overall conclusion is also that for several mental health outcomes, deviations measures are more valid than single time point measures

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CHAPTER 8

Somatic Disease and Salivary Cortisol

Margareta Kristenson[1,*] and Oskar Lundgren[2]

[1]Professor in Social Medicine and Public Health Science at the Department of Medical and Health Sciences, Linköping University, 58183 Linköping, Sweden and [2]Ph.D. Student in Social Medicine and Public Health Science at the Department of Medical and Health Sciences, Linköping University, Sweden

Abstract: Stress is a well-known predictor of somatic disease. Although most clearly demonstrated for Coronary Heart Disease (CHD), stress has also been shown to be involved in several other somatic diseases *e.g.* rheumatoid arthritis, cancer and for pain syndromes. The psychoneuroendocrine mechanisms of these effects have been examined in terms of cortisol levels and cortisol dynamics. The aim of this chapter is to investigate if there are associations between salivary cortisol and somatic disease in terms of cardiovascular disease (CVD), rheumatoid arthritis, cancer and pain, and whether divergent results can be explained by differences in the theoretic assumptions made and methods used. A literature research identified eight articles on CVD, four articles on cancer (all breast cancer), three papers on rheumatoid arthritis and 15 papers on the term pain. CVD, CHD and atherosclerosis were associated with low morning cortisol levels, high evening cortisol levels and a flat diurnal curve. Among patients with metastatic breast cancer, high evening levels and low diurnal deviation characterized patients compared with healthy controls, and low diurnal deviation predicted poorer survival. No relationships with salivary cortisol were found early in the breast cancer disease process. Patients with rheumatoid arthritis, especially with high disease activity, had higher evening levels and a poorer reactivity for laboratory stress. In most studies on pain, low morning cortisol, high evening cortisol, low cortisol awakening response and low diurnal deviation were associated with more pain. Fibromyalgia and pelvic pain among men were an exception. We found few studies where the relationship between salivary cortisol and somatic disease/illness was analyzed. However, among these, a relatively large proportion showed significant findings. The results suggest that, across outcomes, low morning cortisol levels, high evening cortisol levels and a low dynamic cortisol response to stress are related to poorer somatic outcome.

Keywords: Salivary cortisol, cardiovascular disorders, breast cancer, rheumatoid arthritis, pain, single time point measures, deviations measures, area under the curve, laboratory test, dexamethasone.

INTRODUCTION

Psychosocial stress is a well-known determinant for several somatic outcomes [1]. This has especially been demonstrated for CHD, where both the incidence of disease and the risk of recurrent disease have been shown to be related to psychosocial factors and stress [1-3].

However, this has also been demonstrated for other somatic diseases *e.g.*, cancer [1, 4, 5], rheumatoid arthritis [1, 6, 7] and pain disorders [8, 9]. Generally, stressful events are thought to influence the pathogenesis of disease by causing negative affective states (*e.g.*, feelings of anxiety and depression), which, in turn, exert direct effects on biological processes or behavioral processes that influence disease risk [1].

One of the endocrine systems that is particularly reactive to psychological stress is the Hypothalamus-Pituitary-Adrenal (HPA) axis. Cortisol as the primary effector of HPA activation regulates a broad range of physiologic processes including anti-inflammatory responses. Although this stress hormone is important for survival, a disrupted balance of response may lead to higher vulnerability to disease [10] and have the potential to influence a variety of diseases including CHD, autoimmune diseases and cancer [1].

***Address correspondence to Margareta Kristenson:** Professor in Social Medicine and Public Health Science at the Department of Medical and Health Sciences, Linköping University, 58183 Linköping, Sweden; Tel: +46 10 103 5075; Fax: +46 10 103 1865; E-mail: margareta.kristenson@liu.se

Margareta Kristenson, Peter Garvin and Ulf Lundberg (Eds)

Alternations in cortisol dynamics have been associated with tumor growth [5] and rheumatic disease [6-8] and have also been shown to affect the prognosis of treatment among cancer patients [4] and patients with pain [8, 9].

In several of the somatic diseases mentioned earlier, cortisol assessments have been performed to try to establish possible mechanisms for the observed relations to stressors. However, as for other measures in this book, the results have been conflicting, and both high and low cortisol have been found in relation to different somatic outcomes, if any relationship exists at all. One possible explanation for these inconsistencies in results is the differences in hypotheses and basic assumptions behind different studies. Such differences can be seen in terms of whether researchers look for high overall cortisol output or dynamic responses to acute stress.

AIM

To investigate if there are associations between salivary cortisol and somatic disease, more specifically in relation to measures of CVD, cancer, rheumatoid and pain morbidity, and whether divergent results can be explained by differences in the theoretic assumptions made and methods used.

METHOD

Search Strategies

A literature search was conducted using the PubMed database for articles published before October 1st, 2009. The search was limited to human studies on adults more than 19 years of age in the English language. The search terms were truncated saliva* AND cortisol AND somatic disease/diagnosis for the following diagnoses: CVD, Coronary Artery Disease (CAD), CHD, Acute Coronary Syndrome (ACS), atherosclerosis and stroke. The search for cancer included breast cancer, prostate cancer, intestinal and cervical cancer. The search for inflammatory disease was done for RA and rheumatoid arthritis. A search was also done for the term pain.

The search result was skimmed for relevant articles by reading the title and abstract from each hit. Articles that met the inclusion criteria with measures of salivary cortisol related to the measures of somatic disease or pain were then selected for inclusion in the literature review. These articles were then read in detail.

RESULTS

The number of hits and final number of papers used in the review were: for cardiovascular disease; CAD (15 and 5), CHD (15 and 5), ACS (3 and 2), CVD (86 and 8); atherosclerosis (12 and 4), and stroke (10 and 1). Several of these findings were the same studies and the final number of articles on CVD was 8. For cancer diagnoses, the number of hits and final number of papers were: breast cancer (32 and 4), prostate cancer (3 and 0), intestinal cancer (3 and 0), and cervical cancer (1 and 0); in summary 4 studies met the inclusion criteria. For rheumatoid arthritis, 3 papers met the inclusion criteria, selected from the following search results: RA (9 and 2) and rheumatoid arthritis (8 and 3). The search term pain resulted in 76 hits and 14 papers. A total of 29 articles met the inclusion criteria and were hence included in the literature review.

CVD

A total of 8 papers were found [11-18]. Four of these studies used measures of atherosclerosis as outcomes, *i.e.*, Intima Media Thickness (IMT) [11, 13], coronary calcification [14] and plaques in arteria carotis [18]; the other four used measures of symptomatic CVD, CAD or ACS [12, 15-17]. A brief summary of the results (indicated as a positive association, a negative association or a nonsignificant finding) is presented in Table **1a** and the details are given in Table **2a**.

Four of 14 results on cortisol measurements from single time points showed significant findings: none at awakening, 1 of 4 morning levels and 1 of 2 midday levels (both a negative relationship) and 2 of 4 evening levels (both showing positive relationships). Hurwitz Eller *et al.* [11] reported, in cross-sectional analyses,

lower morning levels among women (not men) with higher Intima Media Thickness (IMT) and Rosmond *et al.* [12] reported lower mean cortisol at 12:00 h to characterize a pattern predicting CVD, diabetes and hypertension over 5 years. Nijm *et al.* [15] found high evening levels among CAD patients, compared to healthy controls, and Dekker *et al.* [18] found that high levels at 17:00 h predicted more plaques.

Among deviation measures, 8 of 13 results showed significant findings. Six CAR results were reported from four studies and three of these showed significant findings. Two studies came from the same population from which Hurwitz Eller *et al.* reported significant relationships to IMT (for women only); a negative relationship in cross-sectional analysis [11] and a positive relationship in a prospective analysis [13]. Bhattachyyra *et al.* [17] reported higher CAR among CAD patients compared with patients investigated for suspected CAC without CAD; Matthews *et al.* [14] found no relationship between CAR and different severity of ACS among patients within 5 days of admission.

Five studies reported 6 measures on deviations throughout the day and 4 of these showed significant findings. In a prospective study Rosmond *et al.* [12] reported a significant relationship for low cortisol deviation at midday and higher CVD incidence over 5 years. Matthews *et al.* [14] reported that a flatter diurnal decline was related to a higher risk of coronary calcification and Nijm *et al.* [15] reported a flatter diurnal decline among CAD patients compared with controls. Bhattachyyra *et al.* [17] reported that, with no significant relationship between diurnal deviation and the presence of CAD, CAD patients with depression had a flatter diurnal decline compared with those without depression. As for measures of CAR, Whitehead *et al.* [16] found no significant relationship between diurnal deviation and severity of ACS.

Only one study reported results from laboratory stress test reactivity: Nijm *et al.* [15] reported a significantly poorer response to the stress test among CAD patients compared with controls. Two studies examined the Area Under the Curve (AUC). Matthews *et al.* [14] found no significant relationship between AUC and coronary calcification. Dekker *et al.* [18] demonstrated that a higher area under the daytime curve (AUC) was related to more carotid plaques.

These findings can be summarized as follows: CVD seems to be associated with low morning cortisol levels, high evening cortisol levels and lower deviation throughout the day.

Table 1a: Summary of the main findings of associations between salivary cortisol parameters and CVD, sorted by year of publication

References	Year	Outcome	Design	No. cortisol	M/W	Single time points (or sum/mean of two or more time points)					Deviation (difference/slope between two or more time points)					AUC								Supplementary test
						a1	a2	a3	a4	a5	b1	b2	b3	b4		c1		c2		c3		c4		d
Hurw Eller [11]	2001	IMT	C-S	121	37/84	0	0/↓				0/↓													
Rosmond [12]	2003	CVD	Prosp	141	141/0			↓				↓												
Eller [13]	2005	IMP-p	Prosp	95	32/63	0					0/↑													
Matthews [14]	2006	Cor Ca	Prosp	718	305/413	0	0	0	0				↓							0				
Nijm [15]	2007	CAD	CC/CS	60	50/10				↑				↓	↓										
Whitehead [16]	2007	ACS	CS	72	66/6						0		0											
Bhattachar. [17]	2008	CAD +/- depression	CS	84	58/26						↑		0 *											
Dekker [18]	2008	Plaque	CS	1866	~50%	0	0	0	↑											↑				

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory test increase/ground; d, DST, dexamethasone suppression test; CC, case-control; CS, cross-sectional; Prosp, prospective.

*Significant lower diurnal deviation among CAD patients with depression compared to CHD patients without.

Breast Cancer

Four studies were identified [19-22] (see Table **1b** and Table **2b**). Of these, one studied survival of patients with metastatic breast cancer; the other three were case-control studies of breast cancer at different stages (one metastatic cancer, one newly diagnosed breast cancer and one at stage I-III).

Two results from single measures were reported; both were summary measures over the day; in one case a significant positive relationship (case- control study of metastatic breast cancer) was found; the other had nonsignificant findings (stage I-III).

One measure of CAR was reported and showed nonsignificant findings for a relationship for patients with newly diagnosed breast cancer.

All four studies reported results from diurnal cortisol deviations. Two of these showed significant relationships. Both were studies of metastatic breast cancer and in terms of a flat curve: Sephton *et al.* [19] as a determinant of survival over 7 years and Abercrombie *et al.* [20] as the difference between patients and healthy controls. The other two studies showed nonsignificant findings: Vedhara *et al.* [21] for patients with newly diagnosed breast cancer and Carlson *et al.* [22] for breast cancer stage I-III.

Three studies reported results from the AUC, both with respect to increase and ground, all with nonsignificant findings [19, 21, 22].

The findings for breast cancer can be summarized as follows: patients with metastatic cancer seem to be characterized by high evening cortisol levels and low diurnal deviation compared with healthy controls, and the low diurnal deviation predicted poorer survival. Among patients early in the disease process, no relationship with salivary cortisol was found.

Table 1b: Summary of the main findings of associations between salivary cortisol parameters and breast cancer, sorted by year of publication

References	Year	Outcome	Design	No. cortisol	M/W	Single time points (or sum/mean of two or more time points)					Deviation (difference/slope between two or more time points)					AUC								Supplementary test
						a1	a2	a3	a4	a5	b1	b2	b3	b4		c1		c2		c3		c4		d
Sephton [19]	2000	Survival	Prosp	104	0/104								↓							0	0			
Abercombie [20]	2004	BC metastasis	CC/CS	48	0/48					↑			↓											
Vedhara [21]	2006	BC newly detects	CC/CS	144	0/144						0		0							0	0			
Carlsson [22]	2007	BC stage I-III	CC/CS	66	0/66					0			0							0	0			

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory test increase/ground; d, DST, dexamethasone suppression test; BC, breast cancer; Prosp, prospective; CC, case-control; CS, cross-sectional.

Rheumatoid Arthritis

Three studies reported salivary cortisol in relation to rheumatoid arthritis [23-25] (see Table **1c** and Table **2c**). Two studies reported results from summary measures over the day. Dekkers *et al.* [23] and Catley *et al.* [24] both found significant positive relations, *i.e.*, higher mean cortisol among patients with rheumatoid arthritis [23, 24]. However in the study by Dekkers *et al.* [23] this finding occurred in patients with high disease activity only, and not for patients with low disease activity. All three studies reported deviation measures, but none with significant findings. Dekkers *et al.* [23] found no relationship with to CAR and neither Catley *et al.* [24] or Eijsbouts *et al.* [25] found any significant relationship with diurnal deviation.

In summary, patients with rheumatoid arthritis, especially those with high disease activity, seem to have higher mean levels of salivary cortisol.

Table 1c: Summary of the main findings of associations between rheumatoid arthritis sorted by year of publication

References	Year	Outcome	Design	No. cortisol	M/W	Single time points (or sum/mean of two or more time points)					Deviation (difference/slope between two or more time points)					AUC								Supplementary test
						a1	a2	a3	a4	a5	b1	b2	b3	b4		c1		c2		c3		c4		d
Dekker [23]	2000	RA	CC/CS	53	14/39					↑*	0													
Catley [24]	2000	RA	CC/CS	30						↑			0											
Eijsbouts [25]	2005	RA	CC/CS	70									0							0				

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory test increase/ground; d, DST, dexamethasone suppression test; RA, rheumatoid arthritis; CC, case-control; CS, cross-sectional.

*Valid for difference between RA patients with high activity, compared to RA patients with low activity and to healthy controls.

Pain

Fourteen papers were identified [26-39] (see Table **1d** and Table **2d**). They included sciatic pain, pain in interstitial cystitis, pelvic pain, pain in fibromyalgia, chronic widespread pain, pain in the shoulder and neck, acute and chronic lumbar pain, facial pain and experimental pain.

Eight out of 12 results (11 studies) from single time points showed significant findings.

Two studies reported awakening levels. Geiss *et al.* [26] reported that patients who had persistent pain after discectomy had a lower cortisol level on awakening compared with patients with low postoperative complaints and with healthy controls. McLean *et al.* [28] found that, among patients with fibromyalgia, current pain symptoms were related to higher cortisol levels on awakening.

Four studies reported results from morning levels: two with positive associations, one with a negative association and one with nonsignificant findings. Lutgendorf *et al.* [27] reported that, among patients with interstitial cystitis, those with lower morning cortisol levels had more pain, and McBeth *et al.* [31] reported that low morning salivary cortisol was associated with higher risk of chronic widespread pain 15 months later. In the study of McLean *et al.* [28] on patients with fibromyalgia, a positive relationship between current pain symptoms and higher cortisol level was also seen 60 min after awakening. Ehrström *et al.* [36] reported no relationship with morning cortisol levels in a case-control study of localized vulvodynia.

Two studies reported midday levels. McLean *et al.* [28] found no relationship to pain among patients with fibromyalgia while Shell *et al.* reported that, in a cross-sectional analysis of healthy population that midday/evening cortisol levels were higher in the group with higher pain, however only among men (35).Two studies reported evening levels of cortisol, one with significant positive relationships. McBeth *et al.* [31] reported that high evening cortisol levels were related to higher risk of chronic widespread pain 15 months later, while McLean *et al.* [28] found no relationship to pain among patients with fibromyalgia. One study reported a summary measure of cortisol. McBeth *et al.* [30] reported, in a cross-sectional case-control study using a summary measure of morning and evening levels, that subjects with, or at risk of, chronic widespread pain were more likely to have lower cortisol scores.

For deviation measures 12 results were reported from 9 studies and 7 of these with significant findings. Six out of seven studies on CAR showed significant findings: 5 with negative associations and 1 with a positive relationship.

Giess *et al.* [26] reported that patients with persistent pain after discectomy had lower CAR than those without pain. Gaab *et al.* [29] showed that patients with chronic widespread pain had lower CAR compared

with healthy controls. Ehrström *et al.* [36] reported that patients with localized vulvodynia had lower CAR than healthy controls. Sudhaus *et al.* [38] reported that patients with chronic low back pain and more nonverbal pain behavior had lower CAR and Fabian *et al.* [37] reported that a flattened CAR was related to greater pain intensity in experimental pain ratings. Anderson *et al.* [33] reported that men with chronic pelvic pain syndrome had higher CAR than age-matched pain-free controls.

Four studies reported results from diurnal deviation: one with marginally significant results and three with nonsignificant findings. Johansson *et al.* [34] demonstrated that among patients scheduled for lumbar disc surgery, those with low diurnal deviation had more leg pain and disability compared with the high diurnal variation group (p=0.10). Gaab *et al.* [29], using a short circadian profile found no difference between patients with chronic widespread pain compared with healthy controls and Galli *et al.* [39] found no differences in diurnal deviation between patients with chronic myogenous facial pain and controls and Anderson *et al.* found no differences between men with or without chronic pelvic pain syndrome (33).

One study reported data from laboratory stress testing. Wingelfeld *et al.* [32] found no difference between patients with chronic pelvic pain and fibromyalgia and healthy controls in the Trier Social Stress Test.

Three studies reported results in terms of AUC, all of them with nonsignificant findings; Wingelfeld *et al.* [32] found no difference between patients with chronic pelvic pain and fibromyalgia , Sudhaus found no relationship to low back pain (38) and Galli *et al.* found no significant relationship to chronic facial pain (39).

Four studies reported results from the dexamethasone test, all with significant findings (two with positive and two with negative associations). Gaab *et al.* [29] and Galli *et al.* [39] reported on prolonged/enhanced suppression among patients with whiplash-associated disorder and chronic myogenous facial pain, respectively. In a cross-sectional [30] and prospective (31) analyses of chronic widespread pain, McBeth *et al.* found higher serum cortisol levels the day after a dexamethasone test in the pain group.

In summary, in most of the studies low morning cortisol, high evening cortisol and low cortisol reactivity in terms of low CAR or low diurnal deviation were related to more pain. Exceptions were findings of higher cortisol being related to more pain among patients with fibromyalgia and men with pelvic pain.

Table 1d: Summary of the main findings of associations between salivary cortisol and pain sorted by year of publication

References	Year	Outcome	Design	No. cortisol	M/W	Single time points (or sum/mean of two or more time points)					Deviation (difference/slope between two or more time points)					AUC								Supplementary test
						a1	a2	a3	a4	a5	b1	b2	b3	b4		c1		c2		c3		c4		d
Geiss [26]	1997	Sciatica pain	CC/CS	23		↓					↓													
Lutgendorf [27]	2002	Pain	CC/CS	83	0/83		↓																	
McLean [28]	2005	FM	CC/CS	55	21/34	↑	↑	0	0															
Gaab *et al* [29]	2005	WAD	CC/CS	40	20/20						↓		0											↓
McBeth [30]	2005	CWP	CC/CS	429	160/269					↓														↑
McBeth [31]	2007	CWP	Prosp	241			↓		↑															↑
Wingelfeld [32]	2007	FM/CP	CC/CS	42	0/42									0					0					
Andersson [33]	2008	CPP	CC/CS	65	65/0						↑		0											
Johansson [34]	2008	Disc	CS	42	23/19								(↓)*											

Schell [35]	2008	Neck	Prosp	121	67/53			↑/0														
Ehnström [36]	2009	Vulva	CS	78	0/78		0				↓											
Fabian [37]	2009	Exp	CS	64	30/34						↓											
Sudhaus [38]	2009	LBP	CS	43	14/29						↓					0						
Galli [39]	2009	CMP	CC/CS	40	6/34						0		0			0						↓

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory test increase/ground; d, DST, dexamethasone suppression test; FM, fibromyalgia; WAD, whiplash-associated disorder; CWP, chronic widespread pain; Exp; Experimental pain ratings LBP, low back pain; CMP; Chronic myogenous pain. * Parenthesis denoting marginal significance of results.

Table 2a: Studies on cardiovascular disease sorted by year of publication

References	Outcome	Study design/group characteristics	Sampling	Laboratory method and standardization in sampling	Statistical approach for cortisol measure	Statistical analysis, cortisol in relation to outcome	Results	Discussion
Hurwitz Eller *et al.* 2001 [11]	IMT	Cross-sectional, 121 healthy participants	Number of days: 1 day 1998 Number of samples per day: 3 samples 1998 Times for sampling: awakening (S1), 20 min (S2)and 60 min after (S3) Setting: Ambulatory	RIA	Log-transformed; not specified a1. Saliva at awakening a2. Saliva after 60 min b1. Changes after 20 min Confounders: Not specified	Adjustment for age, Hb_{a1}, BMI (women), and age, physical activity (men)	Higher cortisol levels 1 h after awakening and cortisol reactivity was related to higher IMT among women	
Rosmond *et al.* 2003 [12]	Incidence of CVD, type 2 diabetes and hypertension	Design: Prospective 5-year follow-up study No.: 141 M/W, 141/0 Age: 54 years Group: Men born during the first 6 months in 1944 and living in Gothenburg	Number of days: 1 Number of samples per day: 7 Times for sampling: morning (08:00-09:00 h), 11:45 h, at 30, 45 and 60 min after a standardized lunch at 12:00 h, at 17:00 h and just before bedtime Dexamethasone: 0.5 mg at 22:00 h on day 2	RIA	Log-transformed: Not specified An algorithm classified the hormone pattern using b2, difference between the salivary cortisol in the morning and at 11:45 h (the slope) and a2, the mean of these two values (the level). Serum testosterone was included in the algorithm Confounders: Not specified	Data comparisons with Student's t-test, Fisher's exact test or chi-squared test Missing data excluded case wise	Group normal hormone pattern: cortisol slope 12.8 ± 6.0 nmol/l, mean cortisol 13.1 ± 3.1 nmol/l. The abnormal group: 3.8 ± 5.1, and 8.6 ± 3.1 Men with abnormal secretion pattern (n=73) had a significantly higher ($p<0.001$) incidence of CVD, type 2 diabetes and hypertension	"… abnormal neuroendocrine secretory pattern is prospectively associated with an increased incidence of cardiovascular-related events and type 2 diabetes
Eller *et al.* 2005 [13]	Progression in IMT (IMT-p)	Prospective 4-year follow-up study [1998-2002) No.: 95 M/W: 32/63 Age: 34-63 years Group: Volunteer participants in good health Participation rate: 73%	Number of days: 1 day 1998 and 1 day 2002 Number of samples per day: 3 samples 1998 and 3 samples 2002 Times for sampling: awakening (S1), 20 min (S2) and 60 min after (S3) Setting: Ambulatory	RIA	Log-transformed: not specified a1. Cortisol at awakening b1. Changes after 20 min (S1-S2), changes after 60 min (S1-S3). Cortisol reactivity defined as (S1-S2/S1]×100% was also used Both measures from 1998 (S1-3_{98}], 2002 (S1-3_{02}) and average of the 2 years (S1-$3_{average}$) were analyzed Confounders: Not specified	Regression analysis with IMT-p as the dependent. Simple and multivariate analysis Salivary cortisol	Among women significant bivariate association of slope an IMP-p ($p<0.001$) The best model for IMT-p for women included IMT mean 98 ($p=0.019$) and S1-2 average (positive association, $p=0.056$). The linear model explained 24% of the variation in IMT-p	"… awakening cortisol response of great importance to IMT-p in women but not in men" "The present study was very small …"
Mattews *et al.* 2006 [14]	CaC	Prospective 15-year follow-up study (1985/1986 to 2000/2001) No.: 718; 151/235 black men/women, 154/178 white men/women Group: Middle aged black and white men and women with different SES Participation rate: 62.6%	Number of days: 1 Number of samples: 6 Time for sampling: awakening, 45 min, 2.5 h, 8 h, 12 h after awakening and at bedtime Setting: Ambulatory	Cortisol level was determined by time-resolved immunoassay with fluorometric end point detection	Log-transformed measures: Yes b3. Diurnal slope c1. AUC Confounders: Sex-race group, age, treatment for diabetes, cigarette smoking, SBP, triglycerides, educational attainment and average cortisol levels measured across the day	AUC: cortisol values against collection times × 16 h and divided by the duration, first and last sample The slope; by linear regression line for each participant	Those with any CaC declined 6% per hour in cortisol over the course of the day; those with no CaC declined more than 8% per hour ($p=0.003$) No difference between groups in AUC Those persons with slope scores in the flattest quartile had a greater likelihood of any CaC than those in the remaining quartiles	"… relatively flat diurnal pattern is associated with risk of coronary calcification … independent of established cardiovascular risk factors … understanding psychosocial factors linked to coronary disease"

Nijm *et al.* 2007 [15]	CHD	Case-control, cross-sectional No.: 60 M/W: 50/10 Age: 54-68 years Group: 30 CAD patients and 30 randomly selected controls	Sampling over 3 consecutive days Number of samples per day: 2 Times for sampling: 30 min after awakening and right before going to bed Setting: Ambulatory. Saliva samples at baseline and after the two different stress tests	RIA	Log-transformed: Not specified 1. Cortisol deviation morning-evening 2. Cortisol response in stress test Confounders: Smoking, β-blocker therapy, and statin therapy	Student's t-test, Pearson's correlation. Linear regression model analysis was performed to assess the independent contribution of different factors to the increase in salivary cortisol during stress test	CAD patients had a flattened diurnal slope compared with controls ($p<0.05$), resulting from significantly higher cortisol levels at bedtime Exposition to stress test revealed a significant blunted cortisol response among CAD patients compared with controls	"… indicate that dysfunctional HPA axis involves failure of inflammatory activity in CAD patients, thus providing a possible link between stress and inflammatory in disease"
Whitehead *et al.* 2007 [16]	Severity of ACS. Score on Type-D Personality Scale Score on BDI Depression Scale	Cross-sectional No.: 72 M/W: 66/6 Age: 20-80 years Group: Patients within 5 days of admission for ACS Participation rate: 95%	Number of days: 2 Number of samples per day: 8 Times for sampling: 19:00 h, 21:00 h, bedtime, awakening (day 2), 15 and 30 min after awakening, 11:00 h and 16:00 h Setting: Hospital	High-sensitivity competitive salivary immunoassay	Log-transformed: Yes b1. CAR (difference between awakening and the highest of the +15 or +30 min sample) b3. Cortisol throughout the day (repeated measures analysis of variance, with sample time as a within-subject factor) Confounders: Age, gender, and BMI	Associations using Pearson correlation for continuous data, and t-test comparisons for dichotomous data	Neither CAR nor cortisol levels throughout the day were related to the severity of ACS or underlying coronary artery disease or to BDI scores (the CAR was positively associated with type D personality independent of age, gender, BMI)	"Type-D … disruption of HPA axis function in survivors of acute cardiac events … may contribute to heightened inflammatory responses influencing future cardiac morbidity"
Bhattacharyya *et al.*, 2008 [17]	CAD	Cross-sectional No.: 84 M/W: 58/26 Age: 62 years (mean) Group: Patients being investigated for suspected CAD Participation rate: 58%	Number of days: 1 Number of samples per day: 8 Times for sampling: Between 09:00 and 10:00 h (lab), at 11:00 h, 16:00 h, 19:00 h, just before bed, awakening, +15 and 30 min Setting: Laboratory, ambulatory	High-sensitivity chemiluminescence assay	Log-transformed: Not specified b1. CAR (measured on day 2) b3. Cortisol over the day (measured on day 1) Confounders: Age, gender, and medication with β-blockers	b3. Repeated measures analysis with clinical group as between-subject, and samples over the day as within-subject factors. CAR was analyzed using repeated measures analysis of the waking, 15-, and 30-min saliva samples	52 (62%) patients were found to have definite CAD on angiography. b3 was flatter in more depressed patients with CAD ($p<0.001$), lower cortisol early in the day and higher in the evening, but not related to depression in patients without CAD ($p=0.68$) CAR was greater in CAD than in non-CAD patients ($p=0.04$), but was not related to depression	"The flatter cortisol rhythms of more depressed CAD patients may contribute to the progression of coronary atherosclerosis"
Dekker *et al.* 2008 [18]	Plaque in carotid arteries (ultrasound)	Population-based, cross-sectional No.: 1866 M/W: 45 vs 55% Age: 74 years (mean) Group: 1 part of Rotterdam Study of risk factors for chronic disease in the elderly	Number of days: 1 Number of samples per day: 4 Times for sampling: Awakening, +30 min, at 17:00 h and at bedtime Setting: Ambulatory	CLIA Log-transformed: not performed; after exclusion of data above 98th percentile Data normally distributed	a1, a2, a3, a4. b3. Slope over day by regression over day excluding second sample to minimize impact of morning increase c3. AUC; cortisol level by times divided by hours between first and last sample Adjustments for age, sex, time from awakening, smoking, month, aspirin use, education, blood pressure, lipids and diabetes	Linear regression models between cortisol measures and plaque scores Relations between tertiles of AUC/slope distributions and plaque scores *via* ANOVA	After full adjustment, higher cortisol at 17:00 h, high AUC but not any other single measure, slope over day related to a higher plaque score	"… we showed, in an elderly population, higher total cortisol exposure was associated with number of atherosclerotic plaques in the carotid arteries, independent of CVD risk factors"

Abbreviations: ACS, acute coronary syndrome; BDI, Beck Depression Inventory; BMI, body mass index; CaC, coronary calcification; CAD, coronary artery disease; CHD, coronary heart disease; CLIA, chemiluminescence immunoassay; IMT, intima media thickness; RIA, radioimmunoassay; SBP, systolic blood pressure; SES, socioeconomic status.

Table 2b: Studies on breast cancer sorted by year of publication

References	Outcome	Study design/group characteristics	Sampling	Laboratory method and standardization in sampling	Statistical approach for cortisol measure	Statistical analysis, cortisol in relation to outcome	Results	Discussion
Sephton *et al.* 2000 [19]	Survival time, metastatic breast cancer	Prospective case-control study (7 years) No.: 104 M/W: 0/104 Age: 53 years (mean) Group: Metastatic breast cancer patients Participation rate: 83%	Number of days: 3 consecutive Number of samples per day: 4 Times for sampling: 08:00, 12:00, 17:00 and 21:00 h Setting: Ambulatory	RIA	Log-transformed: Yes b3. Diurnal slope c3. AUC. Adjustment: age, site, estrogen receptor status, disease-free interval, time since diagnosis, physicians' rating; medical treatments: psychosocial, marital status, sleep, pain, and depression; and two immune measures (NK cell counts and function (LU20))	Diurnal slope: regression on collection time (12 measures) AUC calculated over 3 days by trapezoidal estimation Survival analyses by the Cox proportional hazards regression model	Cortisol slope predicted survival up to 7 years later with earlier mortality among patients with flat rhythms (p=0.0036, hazard ratio=464.9) Flattened profiles also linked with low counts and suppressed activity of NK cells. There was no association between AUC and subsequent survival	"… highlighting cortisol slope, and not the morning cortisol level, as a unique indicator of survival in metastatic breast cancer"
Abercombie *et al.* 2004 [20]	Metastatic breast cancer	Case-control cross-sectional No.: 48 M/W: 0/48 Age: >30 years Group: 17 metastatic breast cancer patients and 31 healthy controls Participation rate: 73%	Number of days: 3 consecutive Number of samples per day: 4 Times for sampling: Awakening, 12:00, 17:00 and 21:00 h Setting: Ambulatory	EIA	Log-transformed measures Yes b3. Diurnal slope (regression of the 12 cortisol values on the time of sample) a5. Mean value of all the values across the sampling days (using all 12 log-transformed values) Adjustment for effect of educational attainment and marital status	Correlations between biological indicators and disease severity were tested with two-tailed tests	Breast cancer patients had significantly flatter diurnal cortisol rhythms than healthy controls (p<0.05) More severe disease status among patients was associated with higher mean cortisol levels (r=0.50, p<0.05) and was positively but not significantly related to flatter diurnal cortisol slopes (r=0.45, p=0.07)	"Cortisol diurnal slope may have important but different correlates in healthy women versus those with breast cancer"
Vedhara *et al.*2006 [21]	Newly diagnosed breast cancer	Cross-sectional case-control study No.: 144 M/W: 0/144 Age: 53 years (mean) Group: 85 patients with newly diagnosed breast cancer and 59 healthy controls	Number of days: 2 consecutive Number of samples per day: 4 Times for sampling: Awakening, +30 min, before lunch, and 20.00-22.00 h (>2 h after evening meal) Setting: Ambulatory	RIA	Log-transformed: Yes b1. CAR (2nd measure - 1st) b3. Diurnal slope c3g. AUC_{ground} c3i. $AUC_{increase}$ The slope was calculated by regressing the cortisol level onto the time of the sample, with the first sampling occasion treated as time = 0, and later times being the number of hours after this time	Confounders: Not specified	No group differences with regard to any of the four cortisol measures were evident	"Inconsistent with previous studies of breast cancer patients .diurnal pattern of cortisol. reflect differences in disease stage of the patients and/or their treatment modalities"
Carlson *et al.* 2007 [22]	Breast cancer, stages I-III	Case-control cross-sectional No.: 66 M/W: 0/66 Age: 52 years (mean) Group: 33 women with breast cancer (stages I-III, primary	Number of days: 1 Number of samples per day: 4 Times for sampling: Awakening, 12:00, 17:00 and 22:00 h Setting: Ambulatory	ELISA	Log-transformed measures: Yes a3. Mean of cortisol throughout the day b3. Diurnal slope, by regressing the cortisol values as the dependent variable on the time of day that each sample c3. AUC (ground and increase)	Mean cortisol slopes were compared between groups with Chow test AUC values compared between groups using independent samples t-test	The women with breast cancer were not significantly different from the control women on measures of cortisol production at any time of the day, on mean daily cortisol value, on slopes using the Chow test, or AUC_{ground} or $AUC_{increase}$ (women with breast cancer had significantly higher levels of disturbance on all the psychological indices)	"Results highlight importance of disease characteristics when investigating endocrine functioning" Changes may not be seen in early stages of breast cancer

		stage II) and 33 healthy controls			Confounders: Not specified			

Abbreviations: EIA, electroimmunoassay; ELISA, enzyme-linked immunosorbent assay; NK, natural killer; RIA, radioimmunoassay.

Table 2c: Studies on rheumatoid arthritis sorted by year of publication

References	Outcome	Study design/group characteristics	Sampling	Laboratory method and standardization in sampling	Statistical approach for cortisol measure	Statistical analysis, cortisol in relation to outcome	Results	Discussion
Dekkers *et al.* 2000 [23]	Recent-onset rheumatoid arthritis	Case-control, cross-sectional No.: 53 M/W: 14/39 Age: 55 years (mean) Group: 12 patients with recent-onset RA and high activity (ESR>20], 13 same with low activity, and 28 healthy controls	Number of days: 2 Number of samples per day: 9 Times for sampling: Awakening, +15, +30, +45 min, at 10:00, 12:00, 14:30, 17:00 and 19:30 h Setting: Ambulatory	Time-resolved immunoassay with fluorescence detection	Log-transformed: Not specified b1. Cortisol measures 1-3 (CAR) b3. Cortisol measures 4-9 Confounders: Age and sex Post hoc analyses of each time measure; a1, a2, a3, a4	To assess group differences in the early morning rise and afternoon cortisol levels, cortisol values from the first 3 time points and the last 6 time points of the day were subjected to repeated measures ANOVA	There was no differences in early morning rise between the RA patients and the healthy control group, RA patients with high activity showed significantly higher cortisol levels than RA patients with low activity and healthy controls during the remainder of the day (p<0.001) and single measures at 12:00, 14:30, 17:30, 19:30 h were higher (p<0.05)	"… dynamic early cortisol morning rise, not distributed in patients with recent-onset RA … indicates that the HPA axis is not defective" Afternoon cortisol levels in patients with high disease activity did not drop
Catley *et al.* 2000 [24]	Rheumatoid arthritis	18 RA patients (21 fibromyalgia patients) from a community rheumatology practice and 22 healthy controls 45% response rate	Daily life sampling, signaled pre-programmed wristwatch; 6 samples per day; from 08:00 to 09:00 h, on 2 days Exclusions; endocrine disorders, pregnancy, corticosteroid treatment, night shift work	Time-resolved immunoassay with fluorescence detection	a5. Average cortisol b3. Diurnal deviation	Multilevel random effects model (MIXED procedure) Control for demography, lifestyle, and psychosocial factors (mood, stress)	RA patients had higher cortisol but no differences in diurnal profile	Provides additional evidence that the HPA axis is disturbed in RA. No evidence of relation to ongoing stress
Eijsbouts *et al.* 2005 [25]	Rheumatoid arthritis	Cross-sectional case-control study No.: 70 M/W: 37/45 Age: 18-65 years Group: 50 patients with RA; 20 with recent onset, 20 with longstanding active and 10 with longstanding RA in remission; and 20 healthy controls Exclusions; any condition or medication known to affect the HPA axis	The activity of the HPA-axis was assessed under basal conditions and in response to stress (insulin tolerance test, ITT). In addition, patients with recent onset RA underwent a dexamethasone suppression test.		b3. Diurnal deviation c3. AUC		Diurnal salivary cortisol levels did not differ between RA patients and healthy controls nor did AUC During stress test (insulin tolerance test) plasma cortisol levels were consistently lower in RA patients compared with healthy controls	"Under the standardized conditions of ITT, patients with RA have decreased plasma cortisol levels compared with healthy controls, despite increased IL-6. The defect is probably located at the adrenal level and may be of pathogenic significance for the development of chronic arthritis"

Abbreviations: ESR, eosinophil sedimentation rate; IL-6, interleukin 6; RA, rheumatoid arthritis.

Table 2d. Studies on pain sorted by year of publication

Reference	Outcome	Study design/group characteristics	Sampling	Laboratory method and standardization in sampling	Statistical approach for cortisol measure	Statistical analysis, cortisol in relation to outcome	Results	Discussion
Geiss *et al.* 1997 [26]	Persistent sciatic pain in patients who underwent discectomy	Cross-sectional case-control study No.: 23 M/W: Not specified Age: ~40 years Group: 7 patients with ongoing sciatic pain (P+), 7 with low postoperative complaints (P-) and 9 healthy controls Participation rate: 59%	Number of days: 1 Number of samples per day: 5 Times for sampling: Awakening, +15, +30, +45 and +60 min Setting: Patients, clinic; controls, not specified	Immunofluorescent assay	Log-transformed: Not specified a1. Awakening cortisol value b1. CAR (0-30 min) analyzed as AUC Confounders: Not specified	Not specified (probably ANOVA between groups)	Cortisol concentration immediately after awakening was significantly lower in P+ group than in the two other groups (p<0.05) The P+ patients also exhibited a blunted increase of cortisol secretion in response to awakening (p<0.086)	"… the persistence of pain in many of the patients may be related to dysfunctional reciprocal relations between neural, endocrine and immune function"
Lutgendorf *et al.* 2002 [27]	Pain in interstitial cystitis	Cross-sectional No.: 83 M/W: 0/83 Age: 51 years (mean) Group: 48 patients with chronic interstitial cystitis and 35 healthy controls Participation rate: Not specified	Number of days: 3 Number of samples per day: 3 Times for sampling: At 08:00-09:00 h, 16:00-17:00 h and 21:00-22:00 h Setting: Ambulatory	Time-resolved immunoassay with fluorescence detection	Log-transformed: Yes a1, a2 a4. Morning, afternoon and evening values b3. Diurnal slope (based on means from each measure time from the 3 days) Confounders: Chronic fatigue syndrome, rheumatoid arthritis, fibromyalgia and tricyclic antidepressant treatment	Logistic regression analysis on level of morning cortisol that corresponded to specific patient symptoms Symptom levels were dichotomized OR was determined in patients with different cortisol levels	Mean urinary or salivary cortisol did not differ in patients and controls Patients with interstitial cystitis and higher morning cortisol had significantly less pain and urgency	"These findings imply that regulation of HPA axis may be associated with interstitial cystitis symptomatology"
McLean *et al.* 2005 [28]	Pain in fibromyalgia patients	Cross-sectional case-control study No.: 55 M/W: 21/34 Age: 41 years (mean) Group: 28 patients with fibromyalgia and 27 healthy controls Participation rate: 65%	Number of days: 2 consecutive Number of samples per day: 5 Times for sampling: Awakening, +1 h, +5 h, between 15:00 and 16:00 h and 30 min before going to bed Setting: Ambulatory	Enzyme immunoassay	Log-transformed: No Single measures: a1, a2, a3, a4 b3. Differences in cortisol patterns analyzed through repeated measures analysis of variance with time as between-factor Confounders: Age, sex, number of symptoms of depression and self-reported history of physical or sexual abuse	Wilcoxon-Mann-Whitney test to compare categorical variables and Fisher's exact test for continuous variables Linear regression on the association between cortisol and pain, fatigue and stress symptoms	There were no significant differences in cortisol levels or diurnal cortisol variation between fibromyalgia patients and healthy controls In fibromyalgia patients a strong positive relationship between cortisol and current pain symptoms was observed at the waking point (p=0.008) and 1 h after waking (p=0.011)	"The results of this study indicate that pain symptoms in women with fibromyalgia are associated with cortisol concentrations during the early part of the day, but not at later time points"
Gaab *et al.* 2005 [29]	Chronic WAD	Cross-sectional case-control No.: 40	Number of days: 2 Number of samples per day: 9	CLIA	Log-transformed: Yes b1. CAR b2. Cortisol during the	ANOVAs for repeated measures were computed to analyze cortisol data, with clinical diagnosis as a	Compared with the controls, chronic WAD patients had attenuated CAR, normal cortisol levels during the day,	"Dysregulations of the HPA axis … The observed endocrine abnormalities could serve as a systemic mechanism of symptoms

		M/W: 20/20 Age: 36 years Group: 20 in-patients with chronic WAD and 20 healthy controls Participation rate:	Times for sampling: Awakening, +15, +30, +45 and +60 min and at 08:00, 11:00, 15:00 and 20:00 h Setting: Hospital/ambulatory Dexamethasone: 0.5 mg at 11:00 h day 1		day (short circadian profile) d. Dexamethasone test Confounders: No	grouping variable and time as the repeated measures factor. Correlations by Pearson product-moment correlation	and showed enhanced and prolonged suppression of cortisol after the administration of 0.5 mg dexamethasone	experienced by chronic WAD patients"
McBeth *et al.* 2005 [30]	CWP	Cross-sectional case-control study No.: 429 M/W: 160/269 Age: 25-65 years Group: 31 patients with CWP 267 subjects at risk and 56 controls Participation rate: 60%	Number of days: 1 Number of samples per day: 2 Times for sampling: At 22:00 h and between 08:00 and 09:00 h the morning after Setting: Ambulatory Dexamethasone: Serum cortisol levels were measured after an overnight 0, 25 mg dexamethasone suppression test and a pain threshold stressor	RIA	Log-transformed: Not specified PCA was used for combining the four sources of cortisol data a2. Between 08:00 h and 09:00 h in the morning a4. At 22:00 h d. Serum after dexamethasone test Confounders: Age and gender	OR for the at risk group compared with the reference group were calculated Linear regression for relationship between cortisol and psychological measures	Subjects in the CWP group or at-risk group were significantly more likely to have salivary cortisol scores in the lowest third (OR=3.1 and OR=1.8 respectively) CWP and at risk groups were more likely to have high serum cortisol (OR=1.9 and OR=1.6) after dexamethasone test None of the psychosocial factors were associated with cortisol	"Those with established, and at risk of, CWP demonstrate abnormalities of HPA axis function" "The occurrence of HPA abnormality is not fully explained by the accompanying psychological distress"
McBeth *et al.* 2007 [31]	New onset of CWP	Prospective cohort study No.: 241 M/W: Not specified Age: 25-65 years Group: Subjects identified to be at future risk of CWP at baseline. Follow-up after 15 months Participation rate: 52%	Number of days: 1 Number of samples per day: 2 Times for sampling: At 09:00 and 22:00 h Setting: Ambulatory Dexamethasone: Serum cortisol levels were measured after an overnight 0, 25 mg dexamethasone suppression test and a pain threshold stressor	RIA	Log-transformed: No a1. Morning saliva levels a4. Evening saliva levels d. Serum (post-dexamethasone levels) Confounders: Age, sex, baseline pain status, life events, illness behavior, and Hospital Anxiety and Depression Scale depression subscale score	Relationship between new-onset CWP vs no CPW and cortisol levels was expressed as the OR To identify factors that independently contributed to the onset of CWP, multivariate logistic regression was used	28 (11.6%) of subjects reported new onset of CWP High levels of cortisol after dexamethasone (OR=3.53), low levels in the morning saliva (OR=1.43) and high levels in evening saliva (OR=2.32) were all associated with CWP. The 3 factors were independent and additive predictors of CWP	"Among a group of psychologically at-risk subjects, dysfunctions of the HPA axis help to distinguish those who will and will not develop new-onset CWP"
Wingenfeld *et al.* 2007 [32]	Pain Fibromyalgia syndrome and chronic pelvic pain	No.: 42 M/W: 0/42 Age: xx years Group: 15 patients with CPP, and 16 patients with FMS. 21 healthy controls	TSST Number of days: 1 Number of samples : 8 Times for sampling: 15 min before test, during test (+15 min) and then after (+30, +45, +60, +75 and +90 min) Setting: Laboratory test	Time-resolved immunoassay with fluorometric detection	Log-transformed: Not specified b4. Salivary cortisol after stress test Confounders: Not specified	Salivary cortisol was analyzed by ANOVA with repeated measures with time as between-factor	TSST: There were no main effects of the group factor or a group by time interaction effect, reflecting nearly identical salivary cortisol release before, during, and after the TSST in all groups	"Our results suggest normal HPA responses to stress in patients with CPP"
Anderson *et al.* 2008 [33]	Chronic pelvic pain	Cross-sectional case-control study No.: 65 M/W: 65/0 Age: 43 years (mean)	Number of days: 2 consecutive Number of samples per day: 9 Times for sampling: Awakening, +15, +30, +45,	Luminescence immunoassay	Log-transformed: Yes b1. CAR b3. Diurnal slope Confounders: Not specified	Diurnal, or daytime slopes were calculated by regressing values at time of awakening as baseline. Correlations with Spearman's rank test	Men with chronic pelvic pain syndrome had significantly higher awakening cortisol responses than controls (p<0.05). (Men with chronic pelvic pain syndrome had more perceived stress and	"Men with CPP scored exceedingly high on all psychosocial variables and showed evidence of dysfunctional HPA axis function reflected in augmented awakening cortisol response"

		Group: 45 men with chronic pelvic pain syndrome and 20 age-matched pain-free controls Participation rate: 100%	+60 min, and then 4 samples at 3 h intervals Setting: Ambulatory				anxiety than controls (p<0.001))	
Johansson *et al.*2008 [34]	Pain in patients scheduled for lumbar disc surgery	Cross-sectional No.: 42 M/W: 23/19 Age: 41 years (mean) Group: Patients scheduled for lumbar disc surgery	Number of days: 1 Number of samples per day: 4 Times for sampling: Awakening, +30 min, before evening meal and at bedtime Setting: Ambulatory	RIA	Log-transformed: No b3. Diurnal cortisol variability (bedtime sample (4) - morning peak (2)) c3. $AUC_{ground1}$ (total AUC including on all 4 samples) c3. $AUC_{ground2}$ (AUC including only the 2 morning samples) Confounders: Not specified	Correlations with Spearman's correlation coefficient Patient dichotomized into 2 groups based on cortisol variability (high/low cortisol)	The low diurnal cortisol variability group had more leg pain at activity (p=0.10), significant more disability (p=0.03), lower coping scores (p=0.01), higher catastrophizing scores (p=0.04) and lower physical function (p=0.02)	"Patients with lumbar disc herniation and a low diurnal cortisol variability were more prone to catastrophize, than patients with lumbar disc herniation and a high diurnal cortisol variability"
Schell *et al.* 2008 [35]	Pain in the neck, shoulder and back	Cross-sectional No.: 121 M/W: 67/53 Age: 44 years (mean) Group: Healthy media workers Participation rate: 95%	Number of days: 1 Number of samples per day: 4 Times for sampling: At awakening, at lunch, at dinner and before going to bed Setting: Ambulatory	Not specified	Log-transformed: Yes a1, a3, a4 Longitudinal analyses were conducted with one-way ANOVA Confounders: Age and gender	Longitudinal analyses were conducted with one-way ANOVA Linear regression analyses were conducted to test if any stress biomarker at baseline predicted pain 12 months later	Only salivary cortisol nr 3 ("dinner") concentration in males in the pain group was significantly higher (p=0.020) than in the no pain	"Individuals in working life … decreased regenerative/anabolic activity is associated with increasing pain"
Ehrström *et al.* 2009 [36]	Pain-localized provoked vulvodynia	Cross-sectional No.: 78 M/W: /78 Age: 18-40 years Group: 43 women with localized provoked vulvodynia and 35 healthy controls Participation rate: 100%	Number of days: 1 Number of samples per day: 4 Times for sampling: Awakening, +15, +30, and +45 min Setting: Ambulatory	Time resolved fluorescence immunoassay	Log-transformed: Not specified b1. CAR (first 4 values) Confounders: Not specified	Linear regression analysis was used to calculate the slope, *i.e.*, the k-value of the equation of the graph of morning rise in salivary cortisol. A comparison between the k-values in patients and controls was performed with Student's t-test	The k-value (slope) was 1.68 in patients and 3.73 in control women. This means that morning increase in cortisol was significantly attenuated in women with localized provoked vulvodynia (p<0.05), indicating chronic stress Mean levels of salivary cortisol at awakening, at 15, 30 and 45 min did not differ between groups	"In conclusion, according to the pattern of morning cortisol and reported stress-related symptoms, the women with localized provoked vulvodynia of this study suffer from chronic stress"
Fabian *et al.* 2009 [37]	Experimental pain ratings	Cross-sectional No.: 64 M/W:30 /34 Age: 20 years (mean) Group: Individuals of diverse ethnicities recruited from a university	Number of days: 1 Number of samples per day: 7 Times for sampling: Awakening, +15, +30, and +60 min, and at 11:00, 16:00 and 22:00 h Setting: Ambulatory	High-sensitivity enzyme immunoassay	Log-transformed: A base 10 transformation was used b1. CAR c1. $AUC_{increase}$ (CAR), AUC_{ground} (CAR) Following $AUC_{increase}$ (CAR) calculation, a median split was used to create participant	The normal and flattened CAR groups were compared on negative affect, pain tolerance, AUC_{ground} (CAR), and ratings of pain intensity and pain unpleasantness using t-tests	Flattened $AUC_{increase}$ (CAR) was related to greater pain intensity and unpleasantness ratings The normal and flattened $AUC_{increase}$ (CAR) groups did not differ in their report of pain tolerance or negative affect	"a flattened CAR may be part of a diathesis relating to dysregulation of the HPA axis, placing individuals at increased risk for acute and chronic pain, although bidirectional associations are certainly possible"

		Participation rate: 80%			groups with either a normal or flattened CAR Confounders: No			
Sudhaus *et al.* 2009 [38]	Acute versus chronic low back pain	Cross-sectional No.: 43 M/W:14 /29 Age: 39 years (mean) Group: 24 patients with CLBP, 19 patients with ALBP Participation rate: 61%	Number of days: 2 Number of samples per day: 5 Times for sampling: Awakening, +15, +30, +45 and +60 min Setting: Ambulatory	Time-resolved immunoassay with fluorescence detection	Log-transformed: A base 10 transformation was used b1. CAR c1. AUC_{ground} Confounders: No	For analyzing subject group differences in the time courses of the cortisol awakening response, a two-way ANOVA for repeated measurements (group by time-point) was performed Subject group differences concerning the AUC_{ground} were analyzed by an independent samples *t*-test	There were no significant differences between the groups, neither concerning the consecutive measures (CAR), nor the AUC_{ground} Among CLBP patients, fear avoidance coping, and nonverbal pain behavior, was negatively associated with the cortisol awakening response	"The results indicate that pain-related coping strategies which are expected to be successful appear to lower the adrenocortical activity among ALBP patients, whereas affective distress may enhance the level of cortisol in this group" Among CLBP patients, long-term maladaptive coping strategies might contribute to hypocortisolism"
Galli *et al.* 2009 [39]	Chronic myogenous facial pain	Cross-sectional case-control No.: 40 M/W: 6/34 Age: 35 years (mean) 20 patients with chronic myogenous facial pain and 20 healthy controls Participation rate: 100%	Number of days: 2 Number of samples per day: 9 Times for sampling: Awakening, +15, +30, +45 and +60 min and at 08:00, 11:00, 16:00 and 20:00 h Setting: Ambulatory Dexamethasone: 0.5 mg at 11:00 h day 1	CLIA	Log-transformed: Yes b1. CAR b3. Cortisol during the day (short circadian profile) d. Dexamethasone test Confounders: No	ANOVAs for repeated measures were computed to analyze cortisol data, with clinical diagnosis as a grouping variable and time as the repeated measures factor. Correlations were computed by Pearson product-moment correlation	Unstimulated cortisol response (before dexamethasone-intake) to CAR and cortisol levels during the day did not differ between the groups Chronic myogenous facial pain patients showed enhanced suppression of cortisol after the administration of dexamethasone (CAR and cortisol levels during the day), compared with controls	"… the results showed that patients with chronic myogenous facial pain have enhanced negative feedback sensitivity after the intake of a low-dose of dexamethasone, whereas the CAR as well as the secretion of cortisol over the course of the day appear normal"

Abbreviations: ALBP, acute low back pain; CLBP, chronic low back pain; CLIA, chemiluminescence immunoassay; CPP, chronic pelvic pain; CWP, chronic widespread pain; FMA, fibromyalgia syndrome; OR, odds ratio; PCA, principal component analysis; RIA, radioimmunoassay; TSST, Trier Social Stress Test; WAD, whiplash-associated disorder.

DISCUSSION

The main impression from this literature review is that few studies have examined salivary cortisol in relation to somatic outcomes. Several nonsignificant findings were seen among these studies, however there was a relatively large proportion of significant findings and a parallel pattern of findings across diseases: *i.e.*, low morning cortisol, high evening cortisol and low cortisol reactivity related to disease or ill health.

However, not all findings followed this pattern. In a cross-sectional analysis CAR was negatively related to IMT among women [11], but in a follow-up of the same population the opposite finding (*i.e.*, a positive relationship) was reported [13]. Matthews *et al.* [14] found, in a young population with 8% prevalence of atherosclerosis, that an attenuated diurnal deviation was related to more coronary calcification, and Dekker *et al.* [18] found, in an elderly population with 75% prevalence of atherosclerosis, that high evening values and high AUC were related to more plaques in arteria carotis with no relationships for diurnal deviation.

These studies all used measures of atherosclerosis. This was also the case for the study of Bhattacharyya *et al.* [17], which examined patients being investigated for suspected CAD and definitive CAD defined by results from angiography. Patients with definitive CAD had higher CAR compared with patients without CAD. Thus, high evening levels and high AUC were positively related to measures of atherosclerosis and a possible summary of these findings is that the amount of atherosclerosis is positively related to high total cortisol.

Bhattacharyya *et al.* [17] also found that CAD patients with depression had flatter diurnal deviation and the authors concluded that this "flatter cortisol might contribute to the progression of coronary atherosclerosis".

Three studies had symptomatic CVD disease as the outcome: one prospective study of CVD [12], one case-control study of CAD [15], and one cross-sectional study on the severity of ACS [16]. For CVD event/CAD, the findings were consistent; the prospective study on CVD found low diurnal deviation related to more CVD [12]; the case-control study found low diurnal deviation and poor laboratory stress response among cases with CAD [15]; no relationship was seen for severity of ACS [16].

An acute coronary event is a result of a rupture of plaques and there is evidence that plaques characterized by more inflammation and higher levels of degrading enzymes are more vulnerable. In the study of Nijm *et al.* [15] the attenuated cortisol stress response was accompanied by higher levels of inflammatory markers and higher levels of degrading enzyme matrix metalloproteinase. Thus, the flat diurnal curve and/or attenuated response may be related to CAD *via* the quality of atherosclerosis, *i.e.* to more vulnerable plaques.

Four studies were found for cancer, all breast cancer. Two were on patients with metastatic cancer [19, 20] and two on early stage cancer [21, 22]. Results suggest that salivary cortisol is related to breast cancer disease at the metastatic stage but not in earlier stages. The two studies on metastatic breast cancer were in agreement that low diurnal deviation was related to patients with metastatic breast cancer and/or prognosis of disease [19, 20]. In addition, in the case-control study, patients had higher all-day cortisol [20].

High all-day cortisol was also seen in patients with rheumatoid arthritis compared with healthy controls. However, in one of these studies, this was seen only for patients with high disease activity in terms of sedimentation rate [23], and this was a result of higher levels in the afternoon and evening, with no differences in morning levels. In the second study, Catley *et al.* [24] concluded that the results provide additional evidence that the HPA axis is disturbed in rheumatoid arthritis, and also that there was no evidence that the differences were effects of differences in ongoing stress. In the third study, no significant relationship with salivary cortisol was seen [25].

The 14 papers on pain included a variety of patient groups; however, all studies had pain as outcome. The overall pattern suggests that low morning, high evening cortisol, flat CAR and/or diurnal deviation are

related to more pain in interstitial cystitis, risk of chronic widespread pain, risk of pain in the neck and shoulders, localized vulvodynia, chronic low back pain, experimental pain ratings and sciatic pain.

Low morning cortisol was related to more pain among patients with interstitial cystitis [27], and higher risk of chronic widespread pain 15 months later [31]; high evening levels of cortisol were related to higher risk of chronic widespread pain 15 months later [31], and high midday/evening cortisol predicted pain in the neck and shoulders 12 months later, however, among men only [35].

In case-control studies on patients with chronic widespread pain [29], localized vulvodynia [36], chronic low back pain [38] compared with healthy controls and in experimental pain ratings, a flattened CAR was related to greater pain intensity [37]. In two studies, patients with sciatic pain showed better outcome after surgery if they had a low morning cortisol level and/or morning/diurnal deviation of cortisol [26, 33], which in both cases was suggested to be a function of the anti-inflammatory effect of cortisol being related to steep diurnal deviation.

Contrasting findings were seen in a study on fibromyalgia in which current pain symptoms were related to higher cortisol at awakening and 60 min after awakening [28] and for men with chronic pelvic pain syndrome who had higher CAR than age-matched pain-free controls [33]. A possible interpretation is that cortisol levels in these latter cases are reactions to pain and/or the stressful situation for these patients.

Most studies were cross-sectional; two were prospective. Most had small numbers of participants, which may have influenced the results. The two studies reporting only nonsignificant findings had the smallest number of participants (n=40 and n=42, respectively) [32, 39].

A summary of the findings suggests that, depending on whether the pain is a result of an inflammatory disease or a chronic pain process, different results are found.

CONCLUSION

Few studies were found where the relationship between salivary cortisol and somatic disease/illness was analyzed. Among these, a large proportion showed significant findings which, across outcomes (CVD disease, metastatic breast cancer, rheumatoid arthritis or pain), suggest that low morning cortisol levels, high evening cortisol levels and a low dynamic cortisol response to stress are related to poorer somatic health.

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CHAPTER 9

Discussion and Concluding Remarks Based on the Scancort Group Review

Ulf Lundberg[1,*], Peter Garvin[2] and Margareta Kristenson[3]

[1]Professor Emeritus of Biological Psychology at the Department of Psychology and Centre for Health Equity Studies (CHESS), Stockholm University, 106 91 Stockholm, Sweden and [2]Post-doc at the Department of Medical and Health Sciences, Linköping University, Sweden and [3]Professor in Social Medicine and Public Health Science at the Department of Medical and Health Sciences, Linköping University, Sweden

Abstract: The aim of this book was to evaluate the usefulness of salivary cortisol as a biomarker in various settings. Our hypothesis was that observed diversities in results can be a function of different kinds of assessments. In this chapter, we try to respond to this aim by giving a summary of the results from different cortisol measures in relation to the health-related variables and conditions investigated in this review. The overarching pattern shows a predominance of non-significant findings but also a couple of rather consistent trends emerged when comparing the results from different chapters. The most apparent is that single measures of absolute concentrations of salivary cortisol, for most health-related variables, seldom give significant findings; deviation measures, in terms of diurnal deviations and/or laboratory stress tests seem to be more strongly and consistently associated with a number of factors, such as Socioeconomic Status (SES), psychological characteristics, biological variables in terms of overweight and abdominal fat accumulation, and mental and somatic disease. Across disorders, the pattern related to ill-health/stress is generally characterized by a flatter diurnal cortisol curve, which in most cases is due to attenuated morning and/or increased evening levels, or a reduced response to a laboratory stress test. For some specific questions, single mean values seem to provide valuable information, but in all cases a careful design in terms of power and standardization is important. Thus, salivary cortisol can be a useful biomarker in many settings, if caution is taken in the choice of methods used.

Keywords: Salivary cortisol, adult, conclusions, nonsignificant findings, deviation measures, diurnal curve, laboratory test, dexamethasone, biomarker, disease.

INTRODUCTION

As emphasized in Chapter **1**, salivary cortisol is a very popular measure in research on stress, health and disease. However, results from different studies are sometimes contradictory and confusing and there has been frustration over diverging findings where both high and low cortisol levels have been associated with the same condition. Our hypothesis was that the observed diversities could be a function of different frames of reference, and especially different kinds of assessments. The aim of this book was to perform a critical review of the existing empirical literature on salivary cortisol, to evaluate the usefulness of salivary cortisol as a biomarker in various settings. More specifically, we investigated the results that we found for different cortisol measures in relation to different health-related variables and somatic and mental conditions.

This review is based on articles published up to October 1, 2009. The analysis of the papers has been done by sorting the evidence for each variable and condition according to the methods used for different measures of cortisol: single time points, deviation/slope, Area Under the Curve (AUC) measurements and laboratory and dexamethasone testing according to standardized schemes. The variables and conditions in this review comprise demography (age, sex, SES, ethnicity), psychosocial work stress, perceived stress, psychological resources, biological markers of Cardiovascular Disease (CVD) or inflammation and hormones, sleep, mental health, and somatic disease (CVD, rheumatoid arthritis, breast cancer, and pain).

***Address correspondence to Ulf Lundberg:** Professor Emeritus of Biological Psychology at the Department of Psychology and Centre for Health Equity Studies (CHESS), Stockholm University, 106 91 Stockholm, Sweden; Tel: +46 8 163 874; Fax: +46 8 167 847; E-mail: ul@psychology.su.se

Margareta Kristenson, Peter Garvin and Ulf Lundberg (Eds)

The presentation of the results of significant findings defines positive as an association with higher single cortisol mean levels, steeper deviation, larger AUC, or greater reactivity in a laboratory stress test and a higher level of cortisol after dexamethasone suppression. In concordance, negative associations are set as lower single cortisol mean levels, a less steep deviation, smaller AUC, or lower reactivity in a laboratory stress test and a lower level of cortisol after dexamethasone suppression.

SUMMARY OF THE RESULTS

The overarching pattern from this review shows a predominance of non-significant findings. For some measurements and settings, very few or no significant findings seem to arise, or significant findings were seen but with opposing messages; *i.e.*, high or low levels/deviations for the same variable or condition. In both cases these results suggest that this measure of biomarker, in these settings, is not informative. The results are summarized in the present chapter and the implications for choices of methods in different settings are discussed. In some cases non-significant findings were also common in cases where significant associations were expected on the basis of general physiology or assumptions. Possible explanations for these non-significant or noncoherent findings are also discussed in the present chapter.

However, a number of significant findings were also seen, which, after sorting the results according to comparable measures, did show a coherent pattern in several studies, suggesting that some types of cortisol measurements seem to be more informative for some types of correlates and conditions and even across different variables. On the basis of the conclusions from this discussion, some general recommendations are given for future studies on salivary cortisol as a biomarker in research on stress, health, and disease.

First a short summary of the main findings for each cortisol measure (see Table **1**) is given, followed by a brief summary of the findings for each health-related variable/condition.

Table 1: Overview of the findings in each chapter of the book

Chapter	Content	Single time points (or sum/mean of two/more time points)					Deviation (difference/slope two or more time points)					AUC								Dexamethasone test
		a1	a2	a3	a4	a5	b1	b2	b3	b4		c1		c2		c3		c4		
2	**Sex, women vs. men**																			
	Total number of associations studied	6	6	5	3	1	10	1	3	8	–	3	1	–	1	–	1	–	–	1
	Total number of significant differences women > men	0	3	1	0	1	3	1	1	1	–	1	0	–	0	–	1	–	–	0
	Proportion of associations (%) women > men	0	50	20	0	100	30	100	33	13	–	33	0	–	0	–	100	–	–	0
	Total number of significant differences men > women	0	0	0	0	0	0	0	1	4	–	0	0	–	1	–	0	–	–	1
	Proportion of associations (%) men > women	0	0	0	0	0	0	0	33	50	–	0	0	–	100	–	0	–	–	100
2	**Age, increasing**																			
	Total number of associations studied	4	5	5	2	1	5	–	4	2	–	3	1	–	–	–	1	–	–	1
	Total number of significant positive associations	1	0	2	1	0	0	–	1	2	–	0	0	–	–	–	0	–	–	1
	Proportion of positive	25	0	40	50	0	0	–	0	100	–	0	0	–	–	–	0	–	–	100

	associations (%)																			
	Total number of significant negative associations	0	1	0	0	0	1	–	0	0	–	1	0	–	–	–	0	–	–	0
	Proportion of negative associations (%)	0	20	0	0	0	20	–	0	0	–	33	0	–	–	–	0	–	–	0
2	**SES and ethnicity, high status**																			
	Total number of associations studied	14	21	17	14	3	16	3	14	7	–	4	1	–	–	–	8	1	–	2
	Total number of significant positive associations	1	5	1	0	0	1	0	9	4	–	0	0	–	–	–	0	1	–	0
	Proportion of positive associations (%)	7	24	6	0	0	6	0	64	57	–	0	0	–	–	–	0	100	–	0
	Total number of significant negative associations	0	2	5	6	0	3	0	0	1	–	1	0	–	–	–	3	0	–	1
	Proportion of negative associations (%)	0	10	29	43	0	19	0	0	14	–	–	0	–	–	–	38	0	–	50
3	**Psychosocial work stress**																			
	Total number of associations studied	37	13	27	27	17	26	10	17	–	–	22	11	2	12	2	9	–	–	–
	Total number of significant positive associations	2	4	3	1	3	5	3	2	–	–	3	2	0	1	0	0	–	–	–
	Proportion of positive associations (%)	5	30	11	3	17	19	30	11	–	–	13	18	0	8	0	0	–	–	–
	Total number of significant negative associations	2	2	2	0	1	1	1	2	–	–	1	1	0	0	0	0	–	–	–
	Proportion of negative associations (%)	5	15	7	0	5	3	10	11	–	–	4	9	0	0	0	0	–	–	–
4	**Perceived stress**																			
	Total number of associations studied	–	5	4	2	3	4	–	9	1	–	2	2	–	–	2	1	1	–	1
	Total number of significant positive associations	–	0	0	0	0	0	–	0	0	–	0	0	–	–	0	1	0	–	1
	Proportion of positive associations (%)	–	0	0	0	0	0	–	0	0	–	0	0	–	–	0	100	0	–	100
	Total number of significant negative associations	–	0	1	0	0	0	–	2	0	–	1	1	–	–	0	0	0	–	0
	Proportion of negative associations (%)	–	0	25	0	0	0	–	22	0	–	50	50	–	–	0	0	0	–	0
4	**Psychosocial resources**																			
	Total number of associations studied	3	2	1	3	–	5	–	4	7	–	–	1	–	–	1	2	1	4	–
	Total number of significant positive associations	1	0	0	0	–	0	–	2	0	–	–	0	–	–	0	0	0	1	–

	Proportion of positive associations (%)	33	0	0	0	–	0	–	50	0	–	–	0	–	–	0	0	0	25	–
	Total number of significant negative associations	0	0	0	0	–	0	–	0	2	–	–	0	–	–	0	1	0	1	–
	Proportion of negative associations (%)	33	0	0	0	–	0	–	0	28	–	–	0	–	–	0	50	0	25	–
5	**BMI, waist circumference and waist/hip ratio**																			
	Total number of associations studied	11	15	6	12	9	12	4	16	6	4	1	7	3	–	–	1	–	–	–
	Total number of significant positive associations	0	0	0	0	2	3	0	1	0	0	0	0	0	–	–	0	–	–	–
	Proportion of positive associations (%)	0	0	0	0	22	25	0	6	0	0	0	0	0	–	–	0	–	–	–
	Total number of significant negative associations	0	8	0	0	0	0	4	5	1	0	0	1	3	–	–	0	–	–	–
	Proportion of negative associations (%)	0	53	0	0	0	0	100	31	16	0	0	14	100	–	–	0	–	–	–
5	**Other cardiovascular risk factors** [a]																			
	Total number of associations studied	11	29	6	16	13	12	–	16	15	8	8	5	–	–	–	2	1	–	1
	Total number of significant positive associations	0	5	0	1	2	2	–	2	6	2	1	0	–	–	–	0	1	–	1
	Proportion of positive associations (%)	0	17	0	6	15	17	–	13	40	25	13	0	–	–	–	0	100	–	100
	Total number of significant negative associations	0	3	0	1	0	2	–	0	0	1	0	1	–	–	–	2	0	–	0
	Proportion of negative associations (%)	0	10	0	6	0	16	–	0	0	13	0	20	–	–	–	100	0	–	0
5	**Markers related to inflammation**																			
	Total number of associations studied	–	3	2	2	5	–	–	2	7	2	2	–	–	–	–	–	5	2	–
	Total number of significant positive associations	–	0	0	1	4	–	–	0	0	0	0	–	–	–	–	–	0	0	–
	Proportion of positive associations (%)	–	0	0	50	80	–	–	0	0	0	0	–	–	–	–	–	0	0	–
	Total number of significant negative associations	–	1	1	1	0	–	–	0	2	0	0	–	–	–	–	–	2	0	–
	Proportion of negative associations (%)	–	33	50	50	0	–	–	0	29	0	0	–	–	–	–	–	40	0	–
6	**Sleep (negative features such as disturbed sleep)**																			
	Total number of associations studied	5	6	5	4	3	3	1	5	4	–	2	2	–	–	–	–	–	–	–
	Total number of significant positive	1	1	1	1	0	0	0	1	0	–	1	0	–	–	–	–	–	–	–

	associations																			
	Proportion of positive associations (%)	20	16	20	25	0	0	0	20	0	–	50	0	–	–	–	–	–	–	–
	Total number of significant negative associations	1	0	0	0	0	0	1	2	3	–	0	0	–	–	–	–	–	–	–
	Proportion of negative associations (%)	20	0	0	0	0	0	100	40	75	–	0	0	–	–	–	–	–	–	–
6	**Sleep (positive features such as sleep quality)**																			
	Total number of associations studied	12	15	3	7	3	9	–	4	4	–	4	5	–	–	–	–	–	–	1
	Total number of significant positive associations	5	3	0	0	0	1	–	2	3	–	0	1	–	–	–	–	–	–	0
	Proportion of positive associations (%)	42	20	0	0	0	11	–	50	75	–	0	20	–	–	–	–	–	–	0
	Total number of significant negative associations	0	0	0	1	0	3	–	0	0	–	1	0	–	–	–	–	–	–	0
	Proportion of negative associations (%)	0	0	0	14	0	33	–	0	0	–	25	0	–	–	–	–	–	–	0
7	**Depression and depressive symptoms**																			
	Total number of associations studied	6	25	7	14	8	4	–	10	8	–	2	3	–	–	–	–	–	2	6
	Total number of significant positive associations	1	12	3	6	3	0	–	0	0	–	1	2	–	–	–	–	–	1	2
	Proportion of positive associations (%)	16	48	42	42	37	0	–	0	0	–	50	66	–	–	–	–	–	50	33
	Total number of significant negative associations	1	3	0	0	0	0	–	4	3	–	0	1	–	–	–	–	–	0	0
	Proportion of negative associations (%)	16	12	0	0	0	0	–	40	37	–	0	33	–	–	–	–	–	0	0
7	**Anxiety**																			
	Total number of associations studied	1	4	1	2	3	2	–	4	5	–	–	–	–	–	–	–	–	2	–
	Total number of significant positive associations	0	2	0	0	0	0	–	2	1	–	–	–	–	–	–	–	–	1	–
	Proportion of positive associations (%)	0	50	0	0	0	0	–	50	20	–	–	–	–	–	–	–	–	50	–
	Total number of significant negative associations	0	0	0	0	0	1	–	1	2	–	–	–	–	–	–	–	–	0	–
	Proportion of negative associations (%)	0	0	0	0	0	50	–	25	40	–	–	–	–	–	–	–	–	0	–
7	**Vital exhaustion**																			
	Total number of associations studied	1	3	2	4	4	6	–	4	2	–	–	–	–	–	–	–	1	–	7

	Total number of significant positive associations	0	0	0	0	0	1	–	0	0	–	–	–	–	–	–	–	0	–	0
	Proportion of positive associations (%)	0	0	0	0	0	16	–	0	0	–	–	–	–	–	–	–	0	–	0
	Total number of significant negative associations	0	1	0	1	0	1	–	2	1	–	–	–	–	–	–	–	0	–	3
	Proportion of negative associations (%)	0	33	0	25	0	16	–	50	50	–	–	–	–	–	–	–	0	–	42
7	**Burnout**																			
	Total number of associations studied	5	15	3	4	5	10	–	6	1	–	2	4	–	–	–	–	–	–	4
	Total number of significant positive associations	2	3	0	1	2	0	–	0	1	–	0	1	–	–	–	–	–	–	0
	Proportion of positive associations (%)	40	20	0	25	40	0	–	0	100	–	0	25	–	–	–	–	–	–	0
	Total number of significant negative associations	0	5	0	1	0	0	–	0	0	–	0	0	–	–	–	–	–	–	1
	Proportion of negative associations (%)	0	33	0	25	0	0	–	0	0	–	0	0	–	–	–	–	–	–	25
8	**Pain**																			
	Total number of associations studied	2	4	3	2	1	7	–	4	1	–	–	2	–	1	–	–	–	–	4
	Total number of significant positive associations	1	1	1	1	1	1	–	0	0	–	–	0	–	0	–	–	–	–	1
	Proportion of positive associations (%)	50	25	33	50	100	14	–	0	0	–	–	0	–	0	–	–	–	–	25
	Total number of significant negative associations	1	2	0	0	0	5	–	1	0	–	–	1	–	0	–	–	–	–	2
	Proportion of negative associations (%)	50	50	0	0	0	72	–	25	0	–	–	50	–	0	–	–	–	–	50
8	**All other somatic disease**																			
	Total number of associations studied	4	4	2	4	4	8	1	11	1	–	–	–	–	–	6	3	–	–	–
	Total number of significant positive associations	0	0	0	2	2	2	0	0	1	–	–	–	–	–	1	0	–	–	–
	Proportion of positive associations (%)	0	0	0	50	50	25	0	0	100	–	–	–	–	–	16	0	–	–	–
	Total number of significant negative associations	0	1	1	0	1	1	1	6	0	–	–	–	–	–	0	0	–	–	–
	Proportion of negative associations (%)	0	25	50	0	20	12	100	54	0	–	–	–	–	–	0	0	–	–	–
2–8	**Total, all chapters**																			

	Total number of associations studied	122	175	99	122	80	139	20	133	74	14	55	45	5	14	11	33	9	10	28
	Total number of significant associations	20	68	22	26	24	37	11	49	37	2	12	12	3	2	1	10	4	5	14
	Proportion of significant associations (%)	16	38	22	21	30	27	55	36	50	14	22	27	60	14	9	30	44	50	50

Abbreviations: a1, awake; a2, morning; a3, midday; a4, evening; a5, all day; b1, morning; b2, midday; b3, morning to evening; b4, laboratory test reactivity/recovery; c1, morning increase/ground; c2, midday increase/ground; c3, morning to evening increase/ground; c4, laboratory test increase/ground; d, DST, dexamethasone suppression test.

[a]Results for HDL cholesterol and heart rate variability have been inverted in this table as low HDL and low heart rate variability is considered a cardiovascular risk factor.

SUMMARY OF THE RESULTS WITH REGARD TO THE TYPE OF MEASURE (TABLE 1)

In total 1188 analyses were reported. Of these 598 were single measures (50%), 292 were deviation measures (25%), 130 were AUC measures (11 %), 107 were taken in laboratory tests (9%) and 28 followed a dexamethasone test (2%). The overall proportion of significant findings was 30%, ranging from 0% to 60% among the different measures.

Single Time Points

The overall proportion of significant findings for single time points was 27%, ranging from 16% (awakening) to 39% (morning levels).

Awakening

One hundred and twenty-two analyses were found. Overall, the proportion of significant findings (in any direction) was 16%, which is the lowest proportion amongst the five single time points evaluated. Levels at awakening seem to be of most relevance for measures of sleep, *i.e.*, sleep duration and overall sleep quality with a positive association with a single measure at awakening (42% significant). Also, burnout was positively associated with a single measure at awakening (40% significant). Otherwise only very few significant findings, or divergent findings were seen.

Morning Value

One hundred and seventy-five analyses were found. The overall proportion of significant findings (in any direction) was 39%, which is the highest proportion amongst the five single time points evaluated. The highest consistency for positive associations with morning values was found for depression (52%) and anxiety (50%). The highest consistency for negative associations with morning values was found for BMI and waist/hip ratio (53%) and pain (50%).

Midday

Ninety-nine analyses were found for cortisol levels around midday. The overall proportion of significant findings was 22%. The highest consistency for positive associations with midday values was found for depression (42%) and increasing age (66%). However, the latter is based on three studies only. None of the tested variables had clear results for negative associations with cortisol levels at midday.

Evening

One hundred and twenty-two analyses were found. The overall proportion of significant findings was 21%. The highest consistency for positive associations with evening values was found for depression (42%) and somatic disease (50%). The highest consistency for negative associations with evening values was found for high SES (43%).

Summary Measures Over the Day

Eighty analyses were found. The overall proportion of significant findings was 30%. The highest consistency for positive associations with all-day means was found for somatic disease (50%), depression (37%), burnout (40%), and inflammatory markers (80%). However, all these proportions are based on a low number of studies. In addition, a moderate proportion of significant positive associations (with no negative associations found in the studies evaluated) were found for BMI (22%).

Deviations

The overall proportion of significant findings was 36%, ranging from 26% (morning deviation/Cortisol Awakening Response (CAR)) to 55% (midday deviation, including peak and later).

Morning Deviation/CAR

One hundred and thirty-nine analyses were found. Overall, the proportion of significant findings (in any direction) was 26%, which is the lowest proportion amongst the evaluated deviations. There were no clear positive relationships between CAR and any of the factors evaluated. The highest consistency for positive associations with a high morning deviation was found in sex analyses; women had higher CAR than men in three out of ten studies (30%). The highest consistency for negative associations with CAR was found for pain (72%), although one analysis suggested that high perceived pain is associated with a higher CAR. In addition, a moderate proportion of significant negative associations (with no positive associations found in the studies evaluated) were found for BMI and waist/hip ratio (25%).

Midday Deviation

Twenty analyses were found. Overall, the proportion of significant findings (in any direction) was 55%, which is the highest proportion amongst the five deviations evaluated. The low number of studies makes it hard to fully interpret the results. However, the proportion of significant associations is to a large extent explained by analyses on BMI and waist/hip ratio, where all four out of four analyses showed a negative relationship, *i.e.*, lower deviation between two time points at midday associated with higher BMI and/or waist/hip ratio. Thus, there is a high consistency for BMI and waist/hip ratio with regard to midday deviations. A lower midday deviation was also associated with somatic disease and disturbed sleep, and the midday deviation was lower among men, but these are all based on single studies and should be interpreted cautiously.

Diurnal Deviation

One hundred and thirty-three analyses were found. Overall, the proportion of significant findings (in any direction) was 37%. The highest consistency for positive associations with diurnal deviations was found for high SES (64%), psychosocial resources (50%) and sleep quality (50%). The highest consistency for negative associations with diurnal deviations was found for somatic disease (54%) and depression (40%).

Laboratory Stress Test

One hundred and seven analyses were found for cortisol response/reactivity in a laboratory stress test setting. Overall, the proportion of significant findings (in any direction) was 45%. The highest consistency for positive associations with a cortisol response was found for sleep quality and sleep duration (75%). The highest consistency for negative associations with a cortisol response was found for disturbed sleep (75%), and depression (37%) and inflammatory markers (33%).

There were nine analyses found for cortisol recovery after a laboratory stress test. Overall, the proportion of significant findings (in any direction) was 44%. The low number of studies makes it hard to fully interpret the results. Two out of four studies reported that a poorer recovery is associated with higher levels of inflammatory markers, one out of four studies reported that a poorer recovery is associated with cardiovascular risk factors (other than BMI and waist/hip ratio).

AUC

The overall proportion of significant findings was 27%, ranging from 9% (AUC with respect to increase throughout the day) to 60% (AUC at midday, including peak and later).

AUC Morning/CAR

Fifty-five analyses were found for AUC with respect to increase following awakening. Overall, the proportion of significant findings (in any direction) was 22%. There are too few studies to evaluate consistency. The highest proportions of positive associations with AUC with respect to increase were found for disturbed sleep (50%) and depression (50%). However, both these proportions are based on single studies and should be interpreted cautiously. The highest proportions of negative associations with AUC with respect to increase were found for perceived stress (50%) and increased age (33%). Again, all these proportions are based on single studies and should be interpreted cautiously.

Forty-five analyses were found for AUC with respect to ground following awakening. Overall, the proportion of significant findings (in any direction) was 27%. There are too few studies to evaluate consistency. The highest proportions of positive associations with AUC with respect to increase were found for depression (66%), based on two significant associations. The highest proportions of negative associations with AUC with respect to ground were found for pain (50%) and perceived stress (50%). However, these proportions are based on single studies and should be interpreted cautiously.

AUC Midday

Five analyses were found for AUC with respect to increase during the early part of the day. Overall, the proportion of significant findings (in any direction) was 60%, which is the highest proportion amongst the AUC measures evaluated. These were fully explained by three analyses on BMI and waist/hip ratio in which a higher BMI was negatively associated with AUC.

Fourteen analyses were found for AUC with respect to ground during the early part of the day. Overall, the proportion of significant findings (in any direction) was 14%. The two significant findings were: one where higher BMI was associated with a lower AUC; and one where psychosocial strain in the work environment was associated with a higher AUC. In the latter, an additional 11 studies showed non-significant findings.

AUC Throughout the Day

Eleven analyses were found for AUC with respect to increase throughout the day. Overall, the proportion of significant findings (in any direction) was 9%. The only significant finding of the analyses was a higher AUC associated with somatic disease. However, there were five additional studies on somatic disease showing non-significant findings. Twenty-four analyses were found for AUC with respect to ground throughout the day. Overall, the proportion of significant findings (in any direction) was 25%. The highest consistency was found for a negative association between high SES and AUC (50%). The other two significant findings were a negative association between psychosocial resources and AUC (50%), and a positive association between perceived stress and AUC (100%).

AUC in Laboratory Stress Tests

Nine analyses were found for AUC with respect to increase in laboratory stress tests. Four of them were significant. The highest consistency was found for inflammatory markers, showing an inverse association with AUC for salivary cortisol during a stress test (40%).

Ten analyses were found for AUC with respect to ground in laboratory stress tests. Overall, the proportion of significant findings (in any direction) was 50%. There are too few studies to evaluate consistency. Positive associations with AUC with respect to ground were found for depression (50%) and anxiety (50%). Negative associations with AUC with respect to ground were found for high SES (100%). Psychosocial resources had a study reporting a positive significant association (25%) as well as negative

significant association (25%). However, all these proportions are based on single studies and should be interpreted cautiously.

Dexamethasone Suppression Test

Twenty-eight analyses were found for dexamethasone suppression tests. Overall, the proportion of significant findings (in any direction) was 50%. The highest consistency for positive associations with a high cortisol level after suppression with dexamethasone was found for depression (33%). The highest consistency for negative associations with a high cortisol level after suppression with dexamethasone was found for vital exhaustion (42%). Studies on pain were inconclusive, with one study reporting a positive association (25%) and two studies reporting a negative association (50%). There are too few studies to evaluate consistency for the other factors. A positive association with a high cortisol level after suppression with dexamethasone was found for increased age (100%), perceived stress (100%), and hypertension (100%). A negative association with a high cortisol level after suppression with dexamethasone was found for high SES status (50%) and burnout (25%). However, all these proportions are based on a low number of studies and should be interpreted cautiously.

SUMMARY OF THE RESULTS WITH REGARD TO HEALTH-RELATED VARIABLES AND CONDITIONS

Demographic Variables (SES, Age, Sex, Ethnicity) and Salivary Cortisol

SES was usually defined in terms of education and/or income. In general, the results indicate that high SES is associated with a steeper cortisol slope and participants with high SES tend to have slightly lower mean cortisol levels, mainly due to lower levels in the evening. Health behavior, smoking in particular, seems to play an important role in this relationship, but a similar pattern with a flatter diurnal rhythm among low SES individuals has also been found among nonsmokers. This suggests a more normal and healthy cortisol secretion in individuals with high SES, which is in line with their generally better health and longer life expectancy compared with individuals with low SES. However, inconsistent and non-significant findings were also reported. Studies on ethnicity followed a clear trend: Caucasian study populations had a higher diurnal variation than African American study populations. It is also suggested that Caucasians had higher diurnal variation than Hispanics, who, in turn, had higher diurnal variation than African Americans. This ethnicity ladder is in congruence with a translation to a socioeconomic ladder, and give further support to the findings on SES, emphasizing that a higher status seem to be associated with a higher diurnal variation.

A small increase in cortisol levels during the later part of the day was seen with age, but no consistent sex differences in cortisol levels or responses were found.

Psychosocial Work Stress and Salivary Cortisol

With regard to work related psychosocial stress, most analyses of the association with salivary cortisol were found to be non-significant, but some significant positive findings were found between high work stress exposure and high cortisol levels. No specific cortisol measure or statistical analysis could be related to more consistent significant findings. Furthermore, the two main measures of work stress, that is, the demand–control and the effort–reward–imbalance models, did not differ in terms of significant findings in relation to salivary cortisol. In Chapter **3**, a quality index was calculated for each study and it was found that the more recent studies tended to be of higher quality. However, a finding of concern was that low-quality studies tended to produce more significant findings than high-quality studies.

Only a limited number of studies has been performed on the possible influence of work stress on deviation measures, usually CAR. This means that no conclusions can be made regarding the use of such measures compared with single time point measures. A tentative stress-induced change in the diurnal pattern, in terms of decreased morning levels and increased evening levels, could lead to inconsistent findings from single measures of cortisol obtained at different times of the day (*e.g.*, morning vs evening measures) or if a mean cortisol level is calculated for the whole day.

Another possible explanations for the relative lack of consistent significant associations between work stress and single measures of salivary cortisol could be that the stress induced by regular work conditions was mild, and/or that most occupational groups were relatively homogenous with regard to stress levels (*e.g.*, by having the same occupation) and, therefore, there was too little variability to reveal possible associations. In addition, study groups were usually relatively small.

A third conclusion is that the effects of a stressful work environment are not mediated by cortisol, at least not as measured by saliva cortisol measures.

In summary, the main pattern from this review shows non-significant associations between salivary cortisol and work stress. This is consistent with previous studies on work stress and cortisol measures based on urine samples [1-3], which show that routine work conditions are associated with only a small increase or no increase at all in mean cortisol during the day, whereas another stress hormone, urinary adrenaline, is consistently increased 50–100% during ordinary work [4]. Only one study has used dexamethasone administration and found higher suppression in relation to high work stress (defined by low reward, high burnout, and high vital exhaustion). If vital exhaustion was an important factor in this relationship, this finding is consistent with results reported for the dexamethasone suppression test above (p. 15).

Perceived Stress, Psychological Resources and Salivary Cortisol

Despite the fact that cortisol is an important stress hormone and known to increase in response to acute experimental stress, more than half of the studies failed to find a significant association between perceived stress and salivary cortisol. Furthermore, some of the significant associations found seem to be inconsistent with general expectations. For example, some studies showed that perceived stress was related to lower AUC cortisol, especially in the morning. One study found significantly lower cortisol in the afternoon (single time point) among individuals reporting high perceived stress. One study of the effects of dexamethasone showed, as expected, less suppression on the Hypothalamo-Pituitary-Adrenal (HPA) axis (response after awakening) in teachers with high levels of perceived stress. In conclusion, measures based on the Perceived Stress Scale (PSS) are not consistently related to salivary cortisol. In view of this, it is important to remember that PSS represents perceived stress during a longer period of time (weeks), whereas cortisol generally has been measured at a single time point and, thus, reflects a momentary value.

For measures of psychological resources, such as sense of coherence, internal locus of control, mastery, and self-esteem, many non-significant findings were seen, especially for single time measurements. Also 5/5 associations with CAR were non-significant. However, significant findings were seen for other deviation measures, but were different depending on the resource measure. High external locus of control and low levels of a combined measure of self-esteem and locus of control were related to a stronger cortisol response to a laboratory stress test. High scale scores of sense of coherence and of mastery were related to significantly lower cortisol baseline levels and two out of three studies on diurnal deviation showed a significant positive relationship, *i.e.*, steeper deviation related to high mastery. However, no significant associations were found between self-esteem and cortisol. In conclusion, for measures of psychological resources, significant findings differed depending on the measure used; there were few significant findings for single measures and CAR, but there were significant findings in relation to diurnal deviations or laboratory conditions in terms of reactivity or baseline levels, however not for self-esteem.

Biological Markers and Salivary Cortisol

The chapter on salivary cortisol in relation to a number of other biological markers generated very few significant associations and, for most markers, no consistent pattern appeared. However, for some markers there seems to be a consistency, which is also in line with expectations.

As several biomarkers tested do represent different time periods compared with salivary cortisol, strong correlations with single measures of cortisol cannot be expected. For example, BMI and waist circumference are measures that are very stable over time, urinary adrenaline and noradrenaline represent mean measures over a couple of hours, whereas blood pressure and heart rate represent momentary values

that fluctuate considerably over time. Additional possible explanations for the lack of associations are the small number of studies performed with each of the biomarkers and the small study samples.

However, among the few significant findings found, higher BMI was related to lower cortisol in the morning (but not at awakening), higher total measure over the day, to a lower deviation and a lower AUC around midday. Similarly, higher waist circumference was significantly related to lower deviation in the daily slope of the cortisol curve in two out of three studies. Results from measurements of waist/hip ratio showed a similar pattern with lower cortisol level in the early phase of the diurnal cycle among participants with higher waist/hip ratio. Blood pressure and heart rate responses to a stress test indicate positive associations with cortisol deviation, whereas a negative association was found for heart rate variability. Total cholesterol, triglycerides, and glucose levels did not show any consistent association with any cortisol measure.

Thus, the response pattern for variables characterizing the metabolic syndrome (high BMI, abdominal fat accumulation, *etc.*) indicates a less dynamic activity of the HPA axis. Compared with single measures of blood pressure and heart rate, cardiovascular reactivity seems more associated with cortisol secretion under experimental stress. Low heart rate variability is a risk factor for cardiovascular disorders and tended to be associated with higher increase in cortisol levels.

With regard to inflammatory markers, there were fewer significant associations than expected, but the associations found were in line with the immunosuppressive function of cortisol. A couple of studies reported that a higher capability to react with cortisol secretion on a stress test and a good capability to recover after a stress test are associated with lower levels of inflammatory markers [5-7]. This, in combination with the reported positive association between a higher cortisol output throughout the day and interleukin-6 [8], are in line with earlier research on the role of cortisol in immunoregulation in studies on patient populations [9, 10]. Fanatidis *et al.* [9] have proposed that "inappropriately normal" cortisol levels due to limited capability to respond with increased cortisol levels may not be sufficient to limit an ongoing inflammation. Raison and Miller [10] describe a situation "when not enough is too much", with increased levels of cortisol due to downregulation of receptors on target cells, making the glucocorticoid signaling in immunoregulation insufficient.

The number of studies on salivary cortisol and plasma catecholamines is low. Adrenaline and noradrenaline cannot (so far) be measured reliably in saliva. Also, the stress response in terms of catecholamine secretion is more rapid (less than a minute after exposure) than the cortisol response (30–40 min to reach a peak after stress exposure). Moreover, studies using urinary levels of catecholamines and cortisol collected over a longer time adjacent to a laboratory stress test indicate only moderate positive correlations in response to stress [11,12], and it is suggested that there is sometimes a dissociation of the activation of these two systems [13,14]. In line with this, earlier studies on laboratory stress testing have demonstrated that long-term stress affects immunologic but not cardiovascular responsiveness to acute stress in humans [15] and Schommer *et al.* [16] found a dissociation between reactivity of the HPA axis and the sympathetic adrenal medullary system to repeated psychosocial stress. As noted earlier and in line with CATS (positive outcome expectancies), the HPA axis seems to deactivate quickly to normal repeated stress exposure, whereas the sympathetic nervous system continues to respond.

In summary, the number of well-controlled studies found on the relationship between salivary cortisol and other stress hormones and inflammatory markers is low. Although cortisol is known to be involved in many central biological processes of importance for health and disease, more studies are needed on other stress hormones and inflammatory markers to fully elucidate the feasibility and usefulness of salivary cortisol in that context.

Sleep and Salivary Cortisol

Surprisingly few studies have been performed on sleep and salivary cortisol and the measures of sleep vary considerably. The most consistent finding from the present review was a positive association between sleep

duration and a single measure of salivary cortisol at awakening. Sleep duration also tended to be associated with low evening cortisol levels, and self-reported sleep quality was positively correlated with cortisol in the morning. Disturbed sleep tended to be associated with higher cortisol over the day and a more flat diurnal cortisol pattern. The general pattern from these studies indicates that a more dynamic cortisol response (high morning and low evening levels) is positively related to sleep quality. However, as time of awakening usually was not controlled, it is possible that individuals who occasionally are sleeping longer get up later and, therefore, have a higher cortisol level, due to the typical morning increase, but a consistently later wakeup should not influence the cortisol morning response.

Because sleep in terms of quality and quantity is such an important prerequisite for long-term health and for protection against stressful conditions, the conclusion from this chapter is that more specific studies on different indicators of sleep quality and amount of sleep in relation to salivary cortisol, controlled for time of wake up, are badly needed. Reduced sleep to less than 6 hours per night is known to have important metabolic consequences and increase the risk of health problems such as type 2 diabetes, increased susceptibility to infections [17], and musculoskeletal disorders [18]. Cortisol is likely to play an important role in these relationships but the results from the present review on sleep deprivation and salivary cortisol were inconsistent.

Mental Health and Salivary Cortisol

A large proportion of non-significant findings were seen for measures of mental health. Some consistency is seen for Major Depressive Disorder (MDD), mainly higher mean levels. The results regarding single measures and depressive mood are less consistent, but the overall picture for depression shows poorer diurnal deviation and response to stress.

Inconsistency among papers studying depression seems to be related mainly to the study population, with stronger effects for more depressed individuals. A recent review study [19] showing a link between depression and HPA hyperactivity, but with great variation across patient groups, is in line with the present findings.

A recent study based on 408 population-based midlife women investigated the relationship between a measure of depressive symptoms and salivary cortisol obtained at 18:00 h, 21:00 h, and immediately on awakening the next morning [20]. It was found that the diurnal cortisol slope was significantly flatter for women with high depressive scores than for less depressed women, after adjustment for a number of possible confounders, except sleep. A flatter curve for individuals with higher depressive scores is consistent with the studies reviewed in Chapter **7**, but according to Knight *et al.* [20] their finding is mainly based on lower morning cortisol levels among women with more depressive symptoms rather than increased evening levels. This group of women represents individuals with relatively mild symptoms and very few individuals were suffering from MDD.

Anxiety was related to cortisol levels only in studies comparing groups with high versus low anxiety, but not when anxiety scores were treated as a continuous variable. However, very few significant findings were found for anxiety, and findings from different studies, and across different measures used, were not consistent. Thus, saliva cortisol does not seem to be strongly related to anxiety.

The proportion of significant relationships (in any direction) among the six articles studying Vital Exhaustion (VE) using the Maastricht Questionnaire was 2/14 (14%) for single measures, 3/7 (42%) for deviation measures, 0/1 study using AUC measures. Chapter **7** also indicates that VE was related to poor cortisol response to stress and/or a flatter diurnal deviation and to higher suppression after dexamethasone administration. Most of the statistical analyses do not show a significant relationship between burnout and cortisol, and when these are present, the results are inconsistent. One explanation seems to be the measures of burnout used, probably due to the different conceptual basis for burnout. VE measured using the Maastricht Questionnaire seems to be related to a poorer cortisol response to stress and poorer diurnal deviation. The coexistence of burnout and VE in many studies does make it difficult to conclude how the different concepts are related to cortisol.

A seemingly contradictory result arises between depression and vital exhaustion. Despite often being intra-correlated to a high extent, depression is positively correlated and vital exhaustion negatively correlated with high levels of cortisol after suppression with dexamethasone. It is reported that the higher cortisol values due to nonsuppression in depressed subjects is explained by a small proportion of the subjects. For most subjects, cortisol levels are suppressed by dexamethasone administration, even in the depressed groups. However, the differences might pinpoint a physiologic difference amongst depressed patients, where some patients with clinical depression exhibit generally higher cortisol levels throughout the day and an insufficient feedback on cortisol secretion. Vital exhaustion, on the other hand, is associated with lower levels throughout the day, and a prolonged suppression after dexamethasone administration. In view of these findings, it is of interest to note that Lindeberg *et al.* (2008) found a flatter diurnal cortisol curve (smaller deviation between morning peak and evening values) related to more exhaustion measured by the inverted SF-36 vitality scale [21].

A general conclusion for all mental health measures is that a large proportion of non-significant findings are due to low power and few sampling days combined with low contrasts between study groups and within study populations. Generally, deviation measures such as diurnal deviation seem to be more valid measures compared with single measures to capture possible changes in the HPA axis measured using salivary cortisol.

Somatic Disease and Salivary Cortisol

Few studies were found for somatic disease, however, among these a rather high proportion showed significant findings.

Salivary cortisol was related to CVD, breast cancer, Rheumatoid Arthritis (RA), and pain syndromes but, again, many non-significant findings appeared. The main pattern for CVD was associations with low morning and high evening cortisol and a flat diurnal curve and low stress reactivity. With regard to breast cancer, a similar pattern of associations was found, that is, high evening cortisol and low diurnal variation, however only for breast cancer patients with metastases. RA patients, especially those with high disease activity, were found to be characterized by higher evening levels of cortisol. Pain conditions were more inconclusive for single time points, but tended to be associated with high midday and evening levels, low CAR, and low diurnal variation in cortisol.

In conclusion, a low diurnal variation in cortisol seems to be the most typical finding in relation to somatic disease.

The present review was limited to the somatic diseases described above. However, cortisol is assumed to play an important role also in many other somatic diseases. For example, Cohen *et al.* [22] have shown that individuals with high cortisol reactivity to experimental stress are more susceptible to upper respiratory illness when exposed to stressful life events.

METHODOLOGICAL DISCUSSION

The most important aim of the present review was to investigate if inconsistent results from different studies could be explained by different theoretical assumptions or by different ways to obtain and statistically analyse cortisol measures (single values, deviations, AUC, with samples either collected in a natural setting with ambulatory sampling or in laboratory stress tests). From the pattern in each chapter in this book, it was not possible to detect any consistent differences in results depending on the type of measure or theoretical assumption, generally due to insufficient number of studies for each type of measure. A predominant finding in each chapter, except for MDD, was that associations investigated between salivary cortisol and other variables were non-significant. Most studies are based on single time point cortisol measures and relatively few on more dynamic measures, such as slope of the curve, AUC, or on repeated measures over several days or reactivity and recovery of cortisol activity in response to acute stress exposure or dexamethasone administration. This makes it difficult to find consistent patterns and make conclusions regarding the feasibility of these different methods.

However, combining results across methods and over the different chapters, the overall pattern of findings indicates a trend. Although less clear for work stress, deviation measures of cortisol, such as diurnal pattern, seem to reveal somewhat stronger and more consistent associations than single time point measures. This was seen for SES, psychological resources, biological markers, including overweight and abdominal fat accumulation, sleep, and mental and somatic disorders.

Thus, a pattern that seems fairly consistent is that high exposure (low SES), low psychological resources, poor sleep, and various mental and somatic disorders are associated with a flatter diurnal cortisol curve. In most cases this is due to attenuated morning and increased evening cortisol, but in terms of major depression, increased cortisol levels were also found in the morning (but not at awakening). A flatter diurnal curve or reduced slope over the day is generally considered to indicate a dysregulation of the HPA axis. In view of the two stress models presented in the introduction, this is consistent with the assumption that certain long-term stress conditions, characterized by helplessness and hopelessness according to CATS due to negative outcome expectancies, and chronic or repeated stress according to the Allostatic Load Model, initiate/reflect pathologic biological processes.

For some measures and some settings, few significant results were seen, but when seen the results were inconclusive. This was the case for most of the single time measures but also for several other measures. This was especially the case for awakening cortisol, and most of the single cortisol levels for all health measures except for major depression and sleep duration. These measures, therefore, do not seem to be informative biomarkers. For some measures, *e.g.*, the PSS scale and for work stress, few significant findings were seen.

A non-significant finding does not necessarily mean that no association exists. Methodological shortcomings can in some cases explain the lack of significant findings. Many studies were performed with single time point cortisol measures and samples of less than 50 individuals, which after taking sex, differences in stress levels, various confounders, and subgroups into consideration, often become too small to reveal possible differences even if they exist. Low statistical power also increases the risk of significant chance findings and, consequently, inconsistent results.

A major weakness of many studies included in this review is the low statistical power. In view of the great natural variability in cortisol levels during the day, between different days, and between individuals, a large number of measurements and individuals are necessary to be able to demonstrate associations that may exist but are of low effect size.

In most cases just a few or a single cortisol measure has been obtained, usually on one single day. Because of the large day-to-day variation of cortisol, measures from more than one day are needed for good reliability of the measure. This is also the case for good validity, when the research question is about the ability to respond and relax, in general, among people with chronic mental health conditions, such as depression, anxiety, burnout, psychological resources (sense of coherence, locus of control), or in relation to demographic variables. It seems likely that a more successful approach would be to relate these conditions to cortisol levels measured on several occasions over several days or weeks or in response to acute stress under carefully controlled conditions. As an example, the study by Cohen *et al.* [23] revealed small but significant differences in mean cortisol levels measured over several days in relation different levels of SES defined by education and income. An alternative approach to study possible associations between cortisol and stable conditions could be the use of urinary cortisol, which can be measured more easily by collecting urine over longer periods of time. However, such mean measures do not reveal information about the dynamics of the HPA axis.

In addition, strict control of confounders and compliance (*e.g.*, time of sampling) among participants is of critical importance for valid findings. As shown in the tables, many studies were performed without proper control of important confounders and of compliance and, in some cases, the possible influence of these factors was not even discussed. Some confounders are more important than others. For example, demographic factors such as age and even sex seem to have little or moderate influence on the relation

between cortisol and other variables, whereas other factors such as time of the day, medication and cigarette smoking may have more pronounced effects.

A particular problem is the compliance among participants when obtaining a saliva sample at awakening, when experimenter control is very unusual and difficult to arrange. Even small variations in the time of saliva sampling in relation to wake up cause large variations in cortisol levels, which may produce misleading results. This may be one reason for the inclusive results from CAR measures.

The comparability of study groups is also important, and comorbidity, which is very common in many disorders, may cause different results in different studies depending on the composition of the patient group. Somatic disease often causes mental disorders and mental disorders may cause somatic symptoms, which could make conclusions regarding associations with specific conditions unclear. For example, in Chapter 8 it was found that patients with coronary artery disease differed in cortisol levels depending on their amount of depression. Lack of control of certain forms of medication may also contribute to inconsistent findings among patient groups.

Severity of the condition could also be of importance as indicated by the somewhat different results reported in Chapter **7** between cortisol and depressive symptoms versus major clinical depression. Also the duration of the condition could be of importance. Possible associations with the HPA axis of certain mental and physical health conditions may vary over an early and a late phase of a disease. It has been hypothesized that under certain stressful conditions the HPA axis first responds with overactivity (hyper-responsiveness) which gradually changes to underactivity (hypo-responsiveness), depending on changes in regulatory mechanisms (*e.g.*, increased or decreased receptor expression or number of receptors) [24], but empirical evidence for this assumption is weak. Theoretically, if several mental conditions are characterized by a gradual change in HPA activity from hyper- to hypoactivity, studies based on patients who have suffered from their condition longer or shorter periods of time are expected to give different results. If patients with different levels of chronicity with regard to their conditions are mixed in the same study, non-significant findings would be expected.

Comparisons within too homogeneous populations are also unlikely to reveal significant findings. Weak positive or most often non-significant associations were found between psychosocial work stress and cortisol levels (Chapter **3**). As these measures are well known to predict cardiovascular and other disease, these results could be surprising. A possible explanation could be the characteristics of the study populations in terms of healthy worker effects. According to the CATS model, ordinary workers have positive expectancies about their ability to handle their daily work demands. This is assumed to initially induce a short-term (phasic) activation of the HPA axis, followed by a rapid return to baseline and successive reduction in the stress response with repeated exposure to the same conditions. This represents a normal, healthy, and economic response pattern, as also suggested by the Allostatic Load Model as well as the adaptation stage of Selye's GAS. This means that the investigations on single individuals who continue to respond (chronic strain) to repeated work stress, who do not respond at all, or are unable to relax in the afternoon, may be of particular interest in order to identify work conditions that may cause health problems.

Many of the studies were not designed for investigating correlations between salivary cortisol and other biological or psychological variables. The design of the study was in these cases aimed at other purposes, which may explain the low statistical power for the analyses of interest for the present review. This means that the general conditions for investigating the relationships with salivary cortisol were not optimal in these studies.

An additional important factor to consider in this context is the possibility of publication bias. Studies showing significant findings are assumed to be more likely to be published compared with studies showing non-significant findings. This would mean that the actual number of non-significant findings could be even greater than found in the present review. However, in several cases the correlations reported in this review were only one part of studies with other aims and, therefore, the non-significant findings did not influence

the decision to publish very much. Nevertheless, it is likely that even more non-significant results would have been obtained if unpublished studies had also been included in this review.

It is important to see that our method of comparing studies is pragmatic, and, to a large extent, the results are mainly dependent on significant findings and the direction of the results. The number of participants varies considerably between studies and, in a few cases, one single study was based on more participants than all other studies investigating the same association added together. An example is the Whitehall II study by Steptoe *et al.* [8] with 2873 participants. This means that one single study could outweigh all the others and makes a simple calculation of the number of significant findings in relation to the number of non-significant findings misleading. However, the way in which data are handled is also of importance. In the Whitehall study average cortisol levels were calculated from awakening to bedtime and, thus, do not reflect changes in diurnal pattern. For example, the same mean level could be obtained by high morning and low evening levels as by low morning and high evening levels. A steep curve represents a normal healthy diurnal secretion of cortisol, whereas a flatter curve indicates a dysregulation of the HPA axis. Thus, even a study based on a large number of participants may suffer from other weaknesses and, therefore, may not be more informative than smaller studies. Additional weaknesses in large studies could be lack of sufficient variation in the variables investigated, too few cortisol measurements, or insufficient control of confounders.

CONCLUDING COMMENTS AND RECOMMENDATIONS

Despite several shortcomings mentioned above, some general conclusions and recommendations for future studies can be made. The overall conclusion is that the studies reviewed in this book show very few significant associations between salivary cortisol and the psychological and biological variables and health conditions investigated. The significant associations found were not very strong but, assuming that publication bias was not a major issue, certain associations between salivary cortisol and health-related conditions seem to exist. A pattern that emerges when summarizing the findings from all chapters is that mental and somatic disorders tend to be associated with a rather flat diurnal cortisol curve, which was due to (except for major depression) lower cortisol levels in the morning and increased levels in the evening. These conditions, including depression, also tended to be associated with an attenuated cortisol response to stress exposure and in the few cases with the dexamethasone stress test, a lack of suppression of HPA activity (except for vital exhaustion). Psychological resources including high SES, on the other hand, seem to be associated with a steeper diurnal cortisol curve and in a few cases lower mean cortisol levels.

In order to use salivary cortisol as a biomarker of psychological conditions and mental and somatic health and in relation to other biological variables, it is concluded that single absolute levels are usually not very informative. Repeated measurements over several days, where means and measures of deviation/slope over the day can be obtained, and large groups of participants, are necessary to investigate these relationships in view of the great natural variability in cortisol levels during and between days and between individuals. For some associations, mean levels of cortisol can be relevant, but in most cases the dynamic properties of the HPA axis are likely to be more important, except perhaps for CAR.

For example, the diurnal slope measured over two or more days and the response to a stress test or dexamethasone administration are recommended as more useful measures of the dynamic function of the HPA axis. In addition, strict control of relevant confounders and compliance (*e.g.*, time of sampling) among participants is of critical importance for valid findings. As shown in the tables, many studies were performed without proper control of important confounders and of compliance and, in some cases, the possible influence of these factors was not even discussed. A particular problem is the compliance among participants when obtaining a saliva sample on awakening, when experimenter control is very unusual and difficult to arrange. Even if the participant reports that he or she took the sample immediately on awakening, it is known that a large proportion of the participants deviated considerably in their time of saliva sampling.

As indicated by this review, many of the associations calculated between salivary cortisol and other variables were not part of the main aim of the study, but rather the result of secondary analyses. In future studies, the design of studies aimed at investigating the association between salivary cortisol and stress, mental and somatic disorders and health-related biological variables should be based on established theoretical formulations such as CATS and Allostatic Load and be optimal with regard to the possibilities to accept or reject the hypotheses regarding the associations of interest. This includes sufficient number of participants to give enough statistical power, strict control over possible confounders and compliance among participants, and repeated measurements at specific hours of the day and, when relevant, measures of responsiveness and recovery of activity in the HPA axis during and after stress exposure and/or use of the dexamethasone suppression test.

Other important points to consider are that when patient groups are compared with healthy controls, patients should be diagnosed with reliable instruments and be rather homogeneous with regard to medication and how long they have had their symptoms.When studying the association between salivary cortisol and mental and somatic disease, the severity and duration of the condition could be of importance for the findings. Another problem is comorbidity, which is very common in many disorders, and which may cause different results in different studies depending on the composition of the patient group. Lack of control of certain forms of medication may also contribute to inconsistent findings among patient groups.

Investigations of the possible effects of different levels of work stress on cortisol secretion should be performed with individuals exposed to a great variety of stress levels and not with a homogeneous group of workers at a specific work place (having the same work tasks).

As pointed out in the introduction of this book, salivary cortisol is a very convenient measure and, therefore, has been used extensively. This could easily lead to misuse of this measure. The surprisingly great number of non-significant findings reported cannot be ignored and indicates that many of the associations investigated in this review do not exist or are very weak. However, lack of significant findings and misleading results could in some cases also be due to lack of control over a number of factors important for adequate testing of the hypotheses. It is obvious that the use of salivary cortisol as a biomarker of stress and health is more complicated than first assumed and, therefore, these associations need to be investigated under more optimal and carefully controlled conditions and be based on established theoretical models before valid conclusions can be drawn. However, this measure could be a very important tool in further understanding the links between stress, health and disease.

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Margareta Kristenson, Peter Garvin and Ulf Lundberg (Eds)