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STRESS
SLEEP
AND ALLERGY

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ABSTRACT

Allergic diseases have recently increased dramatically in the western world, now affecting about 30% of the Swedish population. The reasons for this increase are unclear, but some of the suspects are behavioral factors, such as stress and sleep. Problems with stress are also common today, and stress may change the set-points for the functioning of the body, for instance in the immune system. Sleep, on the other hand, is important for recuperation, and disturbed sleep acts a stressor in itself. Allergic patients often report stressful situations to cause allergic symptom exacerbations, and experience increased fatigue and disturbed sleep, especially when exposed to allergen. However, most aspects of the relations between stress, sleep, and allergy are still obscure. Therefore, this thesis aimed at increasing the understanding of these links.

The thesis is based on three studies. The first is a quasi-experimental study of medical students with or without atopy, who were observed at two occasions, i.e. during a calmer study period and during a potentially stressful examination period (papers I & II). Assessments included blood sampling, lung function testing, and questionnaires and diaries on allergic and psychological symptoms and sleep. The results show that both atopic and non-atopic students increased ratings of stress and negative mood, had altered sleep patterns and changes in immune parameters, e.g. a marked increase in regulatory T-cells, during examination. Atopic participants also showed specific responses to stress, such as a shift towards T-helper 2 dominance, increased anxiety and disturbed sleep. Despite these changes, allergic symptoms were not affected.

Paper III is based on a prospective epidemiological study, using parent report questionnaire data on aspects of disturbed sleep and allergy from the Twin Study of Child and Adolescent Development (TCHAD). Controlling for confounding effects of several factors, including gender, birth weight, and socioeconomic status, results from this study show that being overtired in childhood (age 8-9) predicts development of rhinitis in adolescence (age 13-14), but also that having asthma in childhood is predictive of becoming overtired in adolescence. Controlling for gender only, it also replicates findings from cross sectional studies of associations between allergy and disturbed sleep.

The findings from paper I-III suggest that treatment of sleeping problems that are co-morbid with e.g. allergies is an important issue. Therefore, paper IV is a randomized controlled trial of the efficacy of a CBT-based self-help treatment for insomnia with co-morbid problems, including allergy. Assessments with questionnaires and sleep diaries took place at pre-treatment, post-treatment and three-month follow-up. The study shows that participants in the treatment groups display much improved sleep, and that the sleep of allergic individuals improved to the same extent as that of non-allergic individuals, despite co-existing allergic symptoms.

In conclusion, stress is involved in allergy relevant immune changes, and the cumulative negative effects of stress (i.e. allostatic load) seem to be increased in atopic individuals as compared to non-atopics. The results thus speak for stress as a co-factor in an allergic reaction when exposed to allergen. Aspects of disturbed sleep may be involved in the development of allergy and vice versa, but disturbed sleep, also in individuals with allergy, can be treated efficiently with a CBT-based self-help treatment. The results of the thesis confirm a link between stress, sleep, and allergy, and suggest future studies to test if successful treatment of stress and sleep may decrease symptom expression or even diminish the risk for developing allergic disease.

LIST OF PUBLICATIONS

I. Caroline Olgart Höglund, Jennie Axén, Cecilia Kemi, Susanna Jernelöv, Johan Grunewald, Charlotte Müller-Suur, Yvonne Smith, Reidar Grönneberg, Anders Eklund, Pontus Stierna & Mats Lekander.
Changes in immune regulation in response to examination stress in atopic and healthy individuals.
Clinical and Experimental Allergy, 2006; 36, 982–992.

II. Susanna Jernelöv, Caroline Olgart Höglund, John Axelsson, Jennie Axén, Reidar Grönneberg, Johan Grunewald, Pontus Stierna & Mats Lekander.
Effects of Examination Stress on Psychological Responses, Sleep and Allergic Symptoms in Atopic and Non-Atopic Students.
International Journal of Behavioral Medicine, 2009; 16, 305-310.

III. Susanna Jernelöv*, Mats Lekander*, Catarina Almqvist, John Axelsson & Henrik Larsson.
Development of allergies and sleep disturbances in childhood and adolescence– a longitudinal population-based study of Swedish twins.
Submitted manuscript

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IV. Susanna Jernelöv, Mats Lekander, Kerstin Blom, Sara Rydh, Brjánn Ljótsson, John Axelsson & Viktor Kaldo.
Efficacy of a behavioral self-help treatment with or without therapist guidance for insomnia with co-morbid problems.
Submitted manuscript

CONTENTS

LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
AD	Atopic dermatitis
ANOVA	Analysis of variance
AR	Atopic rhinitis
CA	Catecholamine
CAD	Coronary artery disease
CATS	Cognitive activation theory of stress
CBT	Cognitive behavioral therapy
CI	Confidence interval
CNS	Central nervous system
CRH	Corticotrophin releasing hormone
CVD	Cardiovascular disease
E	Epinephrine
ELISA	Enzyme linked immunosorbent assay
FEV_1	Forced Expiratory Volume 1 second
GC	Glucocorticoid
HPA	Hypothalamic pituitary adrenal
IFN	Interferon
IgE	Immunoglobulin E
IL	Interleukin
ISI	Insomnia severity index
MANOVA	Multivariate analysis of variance
NE	Norepinephrine
NK	Natural killer
NO	Nitric oxide
NREM	Non rapid eye movement
OR	Odds ratio
PNI	Psychoneuroimmunology
PNS	Parasympathetic nervous system
REM	Rapid eye movement
SAM	Sympathetic adrenal medullary
SD	Standard deviation
SES	Socioeconomic status
SNS	Sympathetic nervous system
SRH	Self-rated health
SWS	Slow wave sleep
TCHAD	Twin Study of Child and Adolescent Development
Th	T-helper
TNF	Tumor necrosis factor
VAS	Visual analogue scale

1 INTRODUCTION

Allergic diseases, such as asthma, eczema, and rhinitis, increased dramatically in the western world during the last third of the 20th century, and today, about 30% of the Swedish population has one or more allergic symptoms. The reasons for this dramatic increase are unclear, and several culprits have been hypothesized, including less exposure to bacterias (the hygiene hypothesis), changes in the microbiota of the guts, and stress.

Problems with stress are common in today's society, with stress related problems being the most common reasons for sick-leave in Sweden 2010. Although the commonly held belief that stress always leads to negative consequences is held as incorrect within the scientific community, it is clear that stress changes the set-points and alters the conditions for the functioning of bodily systems, including the immune system. Sleep, on the other hand, is important for restoring the balance of homeostatic and allostatic systems in the body. Disturbed sleep equals impaired recuperation, and can also be seen as a stressor in and of itself.

Many allergic patients make a connection between stressful situations and allergic symptom exacerbations. In addition, allergic individuals often experience fatigue and disturbed sleep, especially when exposed to allergen. Whether the fatigue is a consequence of disturbed sleep, or if it is related to for instance alterations in the immune system is not clear.

Both stress and impaired sleep have been shown to cause shifts in physiological systems that would be of clear relevance for allergies, but the relations between stress, sleep, and allergy have not been extensively studied. Opinions about stress and its effects are abundant, but firm knowledge is still lacking about many (if not most) aspects of the stress process, not least in allergic individuals. In relation, the function of sleep in allergic disease and its potential role in development of allergic diseases is very poorly understood. Moreover, although sleep in allergic individuals is often impaired, no trials have been conducted that test the possibility to treat insomnia in allergic individuals.

Therefore, this thesis is concerned with the relations between stress, sleep, and allergy, and in increasing the understanding for links between stress and allergy, and sleep and allergy.

2 BACKGROUND

2.1 THEORETICAL FRAMEWORK

2.1.1 Behavioral Medicine

I first started working as a clinical psychologist in the area of behavioral medicine. Behavioral medicine to me means working with individuals with medical problems from a psychological perspective, and other behavioral or social sciences can also be involved. My first job was in rehabilitation of patients with coronary artery disease (CAD), and individuals at risk for developing CAD or other stress related problems. That is where I got interested in stress.

An early definition of the field of behavioral medicine is "… the field concerned with the development of behavioral-science knowledge and techniques relevant to the understanding of physical health and illness and the application of this knowledge and these techniques to diagnosis, prevention, treatment and rehabilitation. Psychosis, neurosis and substance abuse are included only insofar as they contribute to physical disorders as an end point." [1]. Behavioral medicine is concerned with a big picture; it is not enough to just look at the sick part – for instance the heart – of the person, but in order to understand and help as best as we can, we need to take into account the person's behaviors and cognitions, the situation the person is in, perhaps the whole society and culture they live in. This project includes a study evaluating a psychological treatment for insomnia. Although treatment of insomnia is not the main focus of this project, this is a typical behavioral medicine approach. In many traditional medical problems, there are aspects that can be understood and treated with knowledge from behavioral sciences.

This PhD thesis is a work within the behavioral medicine area. It is concerned with developing knowledge in the psychological area, contributing to the understanding of a medical problem; allergy.

2.1.2 Psychoneuroimmunology

Although the assumptions that events outside the body (e.g. stressors) can affect events inside it (e.g. heart beat, vigilance), and that different processes within the body can affect each other (e.g. psychological and immunological processes) can be seen as part of a behavioral medicine view of the world, these relations are also part of a more basic research area, namely psychoneuroimmunology (PNI). PNI has been described as "the study of behaviorally associated immunological changes and immunologically associated behavioral changes that result from reciprocal interactions among the nervous, endocrine, and immune systems" [2]. Rather than looking at changes in one system at a time, PNI research focuses on these interactions among the body's systems, and relates these interactions to a person's behaviors. In later sections, different aspects of these relations will be described more thoroughly. Here I just want to emphasize that such interactions are well established and of utmost importance for what is commonly referred to as stress. This project investigates relations between event in the environment, event inside the body, and overt behavior.

This PhD thesis is thus also a work within the PNI-area; it is concerned with the relation between behaviors and the systems within the body.

2.2 STRESS

Since stress in the everyday use of the word has a very negative ring to it, I here want to make clear that stress is not all bad. On the contrary, one main effect of the stress response is to free energy and re-distribute it to increase breathing frequency, heart rate, blood-flow etc. This is obviously advantageous in certain extreme situations, for instance each and every time one needs to run away from roaring lions. In addition certain cognitive functions are enhanced on a short term [3, 4], which make us able to focus exclusively on the lion until we are safe. After having escaped, they help us remember important aspects of the situation, such as where the lions were hiding.

But the so called stress systems (or allostatic systems, see below) in the body are not only responsible for preparing the body in situations of acute danger, the same systems are also involved in everyday functions, such as adjusting the blood pressure upon anticipation of changing position from horizontal to up right, and for keeping us awake and alert during the day. In these functions, activation of the stress systems is far from bad.

Thus, well-adjusted activations of the stress systems are adaptive and particularly well suited for short-term situations where high demands are placed on the organism. However, if the stress response is activated for too long, or too often it increases the risk for a number of problems [3], where some pertain to the function of the individual, and some to the direct health of the individual. This will be discussed in more detail below.

The nature – duration and course – of stressors have implications for the effects on the body [5]. In their meta-analysis of stress effects on human immune function, Segerstrom & Miller [5] adopt a taxonomy for characterization of stressors, (developed by Elliot and Eisdorfer in 1982), which includes five categories. Laboratory challenges, such as public speaking, and other similarly stressful, but short and passing, events are denoted *acute time-limited stressors*. Academic examinations or other events when a person confronts a real-life short-term challenge, are called *brief naturalistic stressors*. In *stressful event sequences*, one major event, such as the loss of a spouse or a major natural disaster, gives rise to subsequent related challenges, the end of which may be difficult to foresee, but is expected. Challenges that are not expected to end, or where it is highly uncertain when they will end, such as traumatic injury, being a refugee, or caring for a chronically ill child, are called *chronic stressors*. Such stressors also force changes to a person's identity or social roles. Finally, *distant stressors*, examples of which are childhood sexual abuse or war, have occurred in the distant past, but continue to have an impact on stress system and immune system function long after the event took place. Different effects of these different types of stressors will be discussed below.

2.2.1 Definitions of stress

Three broad traditions in stress research each have their own focus. The environmental tradition focuses on environmental events that are ("objectively") associated with substantial adaptive demands and their relation to disease; the psychological tradition has its focus on the individual's subjective evaluation of the demands and resources at hand and the relation between the subjective experience and disease; and the biological tradition is most interested in changes in different biological systems in response to demanding conditions [6].

The three traditions have their own definitions of stress, and since I am a psychologist, I first looked into definitions of stress from the psychological tradition. Psychological definitions of stress have often focused on consciously perceived discomfort, and the

process from appraisal to discomfort (see below), whereas the relation between any event and the appraisal of the event is oftentimes ignored [7], as are any (biological) mechanisms leading to disease. For instance Richard Lazarus, one of last century's most influential psychological stress researchers, states that: "Stress arises when individuals perceive that they cannot adequately cope with the demands being made on them or with threats to their well-being."[8] Or as he later put it, simply: "Stress results from an imbalance between demands and resources" [9]. Others have had even more focus on the psychological aspects, and as I understand it, exclude any event that is not consciously appraised: "Stress, it is argued, can only be sensibly defined as a perceptual phenomenon arising from a comparison between the demand on the person and his or her ability to cope. An imbalance in this mechanism, when coping is important, gives rise to the experience of stress, and to the stress response." [10] Interestingly, the same author later explains that stress cannot be measured biologically, only correlates of stress can [11]; by this definition, stress IS the experience.

Although the Lazarus definition definitely has some merit, I cannot easily accept a definition of stress that appears to preclude non-consciously evaluated events, and my interest in PNI put me into contact with the more biologically oriented definitions. For instance, George Chrousos states that stress is "a condition threatening homeostasis, which can be restored by a complex repertoire of physiological and behavioral responses of the organism" [12]. Firdaus Dhabhar claims that stress is "a constellation of events, consisting of a stimulus (stressor) that precipitates a reaction in the brain (stress perception), which activates physiologic fight-or-flight systems in the body (stress response)" [13]. In both these definitions the translation from external to internal events is implicitly recognized, but the emphasis is not on this process.

I prefer Dhabhar's definition because it implies a process, and explicitly includes perception and evaluation of events (happening in the brain), but this perception and evaluation need not necessarily be consciously experienced. From a PNI-perspective, it is hard not to include also non-consciously perceived events such as immune challenges in the term stressor, since they threaten the stability of the internal milieu - that is, "a stimulus to the organism that activates the stress system to help reattain homeostasis" [14]. This also rhymes well with theories of emotion; for instance in a resent symposium on feelings and emotions, several leading emotion theorists such as Arne Öhman and Antonio Damasio [15, 16] underline the ability of non-consciously appraised stimuli to elicit emotional responses, and emotional responses are defined as "bioregulatory reactions that aim at promoting […] the sort of physiological states that secure [...] survival […]" [16].

This is an important aspect, and the Dhabhar definition unfortunately has nothing to say about emotional responses. In addition, it does not explicitly mention reactions to stress other than the fight-or-flight response (which can to a large extent be seen as equivalent to the fear response [15, 17]).

Despite these shortcomings (which may be account for in models of stress (see below) rather than plain definitions), it makes use of some of the premises of this thesis, which are that *stressors* are events (whether in the environment, in the body or in the mind) which cause stress – or rather a *stress response*. A stress response involves changes that can be observed psychologically as well as biologically. Psychological changes involve health behaviors, fight-flight-freeze-reactions, and increased vigilance with anxiety and feelings of worry. Biological changes involve activation of stress hormones, (autonomic) neural functions and function of the immune system.

These are then some basic premises, but how do these aspects relate to one another? That, of course, is not entirely clear yet, but several models and theories of stress have been developed.

2.2.2 Theories and models of stress

2.2.2.1 *Earlier models*

The question of how an event in the environment (a stressor) "enters" the body has been discussed over the years. In 1950, a book entitled "Life Stress and Bodily Disease" [18] summarized a symposium concerning man's reaction to stress. Evidence was put forward "that stressful life events, by evoking psychophysiological reactions, played an important causative role in the natural history of many diseases" [in 19]. The idea was quite crude; an event in the environment causes psychophysiological reactions in the body. For some time, the debate was on the nature of the event; Holmes & Rahe [19] and others were mostly interested in larger life events, such as death of a spouse, marriage or change in economic situation, a view that was questioned by for instance Kanner [20], who were more interested in the effect of daily hassles. Kanner and others [21] theorized that disrupted daily routines and minor daily annoyances could also be mediating the effects of larger life events. In 1976, Richard Lazarus noted that something was lacking – individuals reacted differently in similar situations – and entered psychological processes into the equation [22]. The Lazarus & Folkman theory of stress [9] emphasizes appraisal of a situation to be central to whether a stress-response will be evoked or not; not the situation in and of itself. Coping was a concept introduced to cover what we do to handle stress. Over the years in research on stress and appraisal, Lazarus came to appreciate emotion as central to stress, and in 1999 he concluded: "The three concepts, stress, emotion, and coping, belong together and form a conceptual unit, with emotion being the superordinate concept because it includes stress and coping" [23]. And, as Margaret Kemeny recently noted in a chapter on emotions and the immune system [24]: "Today, many stress and coping theorists consider affective states to be the final link in the chain from the environmental perturbation to the biological response. In other words, coping processes and social support, for example, are posited to act on physiology by modifying the affective response to a given context." Kemeny distinguishes between different types of affective experiences, and defines *emotions* as short, intensely felt affective states that are associated with distinctive facial expressions and behavioral dispositions (much similar to Damasio's definition above); *moods* as longer-term affective states that do not necessarily have a specific trigger, may involve a blend of different affective responses, and are often accompanied by persistent and distinctive cognitions. This bears similarities to Damasio's definition of *feelings*; "Feelings depend on the perception of a changed body state alongside the perception of a certain style of mental processing of thoughts with themes consonant with the emotion" [16]. Kemeny further denotes *affective traits* as a dispositional tendency to experience specific emotions and moods across situations and over time; and finally *affective disorders* as pathological forms of affective experience that interfere with daily life, such as major depressive disorder or generalized anxiety disorder [24].

Early on, and partly independent of these psychological models of stress, the more physiologically oriented models developed. An early model was Walter Cannon's fight-or-flight [25]. Cannon also used the term "homeostasis" – stability – meaning the relatively constant internal milieu that was upheld, despite external changes. Later, Hans Selye described "the general adaptation syndrome" [26]. In the 1940's he observed that animals

exposed to different stressors showed similar patterns of physiological damage; thickening of the adrenal cortex, shrinkage of thymus and lymph tissue, and gastric ulcer. He found that this response could be divided into three phases: 1) the alarm phase where the organism attempts to adjust to meet the demands by secreting corticosteroids, 2) the resistance phase where the organism adapts to the stressor and symptoms disappear but with remaining elevation of corticosteroids, and 3) if the stressor is prolonged, the exhaustion phase where the organism is no longer able to secrete sufficient amounts of corticosteroids and thus looses its ability to adapt to the stressor [26].

2.2.2.2   Stress as a process

As disparate as these approaches may seem, they do share an interest in the process by which events in the environment – and not pathogens directly – result in increases risk for disease. Cohen, Kessler & Gordon [6] described this as "an interest in a process in which environmental demands tax or exceed the adaptive capacity of an organism, resulting in psychological and biological changes that may place persons at risk for disease". They attempted to integrate the different perspectives into one theoretical model. Basically, Cohen et al. see the three traditions (environmental, psychological and biological) as focusing on different stages of the stress process "through which environmental demands are translated into psychological and biological changes that place people at risk for disease" [6], and each of the stages, is of course of interest for the understanding of this process. So while the earlier models mentioned may be seen as focusing on separate stages of the process, the "unifying model" of Cohen et al. can be seen as a model of the process as a whole.

A newer and potentially interesting theory of stress as a process is the "The Cognitive Activation Theory of Stress (CATS)" by Ursin & Eriksen [27]. It differs from the other models mentioned in that it is a more formalized theory, and in that it certainly aims at including every step of the stress process. One of the main differences is in the appraisal part of the process. Rather than seeing appraisal of the situation as central to whether a stress response occurs, as the Lazarus appraisal model states, these authors underline that "the stress responses are normal activation responses leading to an increase in arousal, and corresponding changes in behavior as well as in most or all parts of the body".

2.2.3   The allostasis model

For my work in this project, however, the most influential model has been the allostasis model [28]. The allostasis model came out of the observation from many researchers that stress is not always bad, and was developed in an effort to account for the findings that stress sometimes seems to improve immune functioning – and health. The model is based on the definition of stress involving challenge to homeostasis, and is also a model viewing stress as a process. It states that challenge to homeostasis causes so called allostatic responses [29] aimed at protecting the organism from deviations from homeostatic set-points. For example, in order to keep constant oxygen tension in the brain (homoeostasis), blood pressure needs to be variable (allostasis), depending on for instance if we are lying down, anticipate to rise, or start running [3]. Bruce McEwen explains that: "The maintenance of homeostasis is an active process that requires the output of mediators such as those of the autonomic nervous system, and the neuroendocrine and immune systems. This process is called 'allostasis', or 'maintaining homeostasis through change'" [30]. Many (if not all) systems in the body are included in the allostasis network, and the mediators of

allostasis need to be tightly regulated. The regulation of the allostatic mediators is reciprocal – i.e. the mediators regulate each other – and non-linear, within the network. If the regulation is disturbed so that too much or too little of a mediator is at work, the entire network may be perturbated, with harmful consequences [30]. Potentially damaging effects of allostasis are denoted allostatic (over)load, which thus connects exposure to stressors with health outcomes (e.g. expression of disease) [28]. According to the model, there are four conditions that, over time, lead to allostatic load; "1) Repeated hits, a normal response to novel stressors, repeated over time 2) a lack of adaptation 3) prolonged response due to delayed shut down, or 4) inadequate response that leads to compensatory hyperactivity of other mediators, e.g. inadequate secretion of glucocorticoid, resulting in increased levels of cytokines that are normally counter-regulated by glucocorticoids."

In this model, behaviors of the individual are also considered important for protection or damage. Behavior and cognition are seen as important for determining what is stressful, and based on individual differences, including (but not limited to) both experience and genetic variations, people differ in their appraisal of what is stressful, and in the physiological response mounted in response to the potential threat. Individual differences also include the condition of the body itself; a physically fit person will be more resilient to stress and the response to challenge will have less adverse effects. Finally, responses to challenge can take the form of altering behaviors, for instance increasing smoking or drinking, which have perceived soothing effects in the short run, but add to the physiological allostatic load in the longer run.

Although the function of affective states is implied and sometimes talked about explicitly, their exact place is somewhat unclear within the allostasis model. My preliminary understanding is that they could be conceptualized as differing according to the stage in the process. Perception and interpretation is involved in the encounter with the stimulus, and in the allostasis model, threat, helplessness and vigilance are included in stress perception [28]. Stress perception is posited to initiate behavioral and physiological responses. An emotion, as categorized by Kemeny [24] can thus be seen as part of the response itself [16]. Affective disorders are clearly stated as parts or signs of allostatic overload [31]. However, exactly where (negative) moods, or feelings come in is a bit obscure, but they could be seen as part of an ongoing stress response, since for instance worry and anxiety are seen as resulting in allostatic load [32]. If a further distinction needs to be made, (negative) affective traits could tentatively be seen as signs of allostatic load, since feelings of fatigue, lack of energy, irritability and hostility has been referred to as "chronic stress" [28].

In sum, in this thesis stress is seen as a process, rather than a state or stage. The process starts with 1) an event – *the stressor* – 2) being (sometimes non-consciously) perceived and evaluated by the organism – *stress perception* – and possibly only if evaluated negatively 3) eliciting a *stress-response* or *allostasis* aimed at protecting the organism, but which may under certain circumstances 4) heavily charge the systems – *allostatic load* – and 5) ultimately compromise mental and physical health – *allostatic overload*. The line between allostasis and allostatic load is not entirely clear, but one option is to view allostasis as a process, while allostatic load is a state, albeit a changing state. What is clear is that "Allostatic load refers to the cumulative cost to the body of allostasis" [33], so while allostasis may refer to short term alterations, allostatic load has to do with more long term or chronic alterations.

According to the model, not only physiological changes are included in the stress response and allostatic load, but also behavioral and emotional changes, which may then feed into the process and either perpetuate or help terminate the stress response. Whether a

response has been effectively mounted and appropriately terminated also affects the ability to respond to subsequent stressors. However, exactly how stress responses are altered under the influence of allostatic load reminds to be clarified. Also the questions of how the evaluation happens, and what aspects of the event are being evaluated, are still under debate, although some kind of threat-appraisal is often understood. Likewise, the exact biological pathways leading to disease and moderating circumstances are only to a little extent understood.

2.2.4 The biology of stress

The entire central nervous system (CNS) is directly or indirectly involved in a stress response; the brain is initially responsible for perception of events, and for the interpretation and evaluation of the importance of events, and it is the brain which then initiates the appropriate (physiological as well as emotional) responses to the particular stimulus. How does the brain then convey its wishes to the rest of the body? There are several ways, of course, as always when something important is going on.

2.2.4.1 Basic neuroendocrine signaling

The nervous and the endocrine systems are intimately connected. The main connections from the nervous system to the endocrine systems are from the cortex to other areas of the brain, including the hippocampus, the hypothalamus, different areas in the brain stem, and the amygdala which seems to have a central role in the stress response. Neurons in the amygdala release corticotrophin releasing hormone (CRH) which has two major effects:

1) The activation of the brain stem, which stimulates the sympathetic nervous system (SNS). The SNS then has two major pathways; a) nerve cells that reach target organs throughout the body, where the neurotransmitter norepinephrine (NE) is released, stimulating the organs directly, and b) via the adrenal medulla where the SNS nerve cells stimulate secretory cells to secrete a mixture of epinephrine (E, about 75%) and NE (about 25%). E and NE may be secreted in slightly different proportions depending on the stimulus, but they may also have differing effects depending on which adrenergic receptor is stimulated (so called alpha or beta adrenergic receptors). However, activation of the SNS generally results in changes that characterize the fight-or-flight response such as increased heart rate and blood pressure, elevated breathing rate, increased blood flow to muscles, released energy stores into the blood, etcetera. This is the sympathetic adrenal medullary (SAM)-system, the maximum physiological effect of which occurs a few minutes after stressor onset

The activity in the SNS is regulated and complemented by activity in the parasympathetic nervous system (PNS). I will not go into details about the PNS, but only mention the vagus nerve, or tenth cranial nerve, which is an important part of the PNS. The vagus innervates many tissues, including the heart and lungs where activity of the vagus lowers heart beat and breathing frequency – i.e. opposite actions compared to the SNS.

2) The amygdala also activates the hormone producing cells of the paraventricular nuclei of the hypothalamus. The nerve terminals of these neurosecretory neurons also release peptides, such as CRH. These peptides are in turn important for releasing adrenocorticotropic hormone (ACTH) from the anterior pituitary gland, which in turn increase the production and release of glucocorticoids (GCs; e.g. cortisol in humans) from the adrenal cortex. This is the hypothalamic pituitary adrenal (HPA)-axis, from which the physiological effect occurs about one hour after stressor onset.

Cortisol crosses the cell membrane and binds to receptors in the cytoplasm, from which the receptor complex enters the cell nucleus and activates or represses different genes [34]. As part of a stress response, cortisol has several different roles. Background levels of cortisol permit the actions of other stress-related systems [35], for instance in the early stages of the stress response when GCs augment the actions of catecholamines (CAs). Also, while CAs act quickly, for instance to elevate blood glucose levels, cortisol acts mores slowly, in this case maintaining the high levels of glucose, partly by increasing insulin resistance [34].

In the early phase of a stress response (acute stress), CRH and NE stimulate the secretion of each other, through reciprocal neural connections between the above mentioned neurons.

To turn off the stress response, there are "autoregulatory ultrashort negative feed back loops" in both the hypothalamic CRH and the brain stem catecholaminergic neurons, as well as several central regulatory pathways, for instance via the hippocampus and frontal cortex, the hypothalamus, and the pituitary gland. Importantly, GCs – the final effectors of the HPA-axis – are central not only for regulating basal HPA-axis activity but also for terminating the stress response [12].

Both the HPA-axis and the SAM-system have allostatic effects in the body; helping to maintain homeostasis through the initiation of physiological change. Increases in GCs and E as well as NE are part of the healthy acute stress response, helping individuals handle acute stressors effectively [3]. As these stress hormones provide feedback to the brain, they also influence neural structures that control emotion and cognition [17], which is then how stress affects affect.

Changes in these systems can also be seen in chronically stressed individuals, but long-term alterations are generally considered negative [3]. One example of negative alterations is the attenuated cortisol awakening response seen, for instance, in patients suffering from depression [36]. Such alterations include a decreased capacity to initiate a healthy increase in for instance cortisol following acute stressors, thereby hampering the ability of the organism to mount an adequate response in other systems.

2.2.4.2 The immune system

The immune system's main function is to protect the host from infection. There are different types of immune responses that work in concert to produce an optimal defense for the host. A distinction is made between innate and adaptive immune responses, where the innate response is fast and non-specific, whereas the adaptive response is somewhat slower, but has a type of memory enabling the cells to recognize specific antigens more quickly upon subsequent exposure. Within adaptive immunity a further distinction is made between the humoral and cell-mediated responses. A humoral response is mediated by antibodies and is directed to protecting against extra cellular pathogens and their toxic products. A cell-mediated response on the other hand is the function of T-lymphocytes, and is needed to control intracellular pathogens, and to activate B-cell responses to most antigens [37].

Cells of the immune system communicate with each other using so called cytokines which are crucial for mediating responses and for maintaining homeostasis within the immune system. Cytokines can signal back to the cell that secreted them, to nearby cells and to distant cells in the body. Cytokines activate immune cells for different purposes, and many cytokines have overlapping effects. Cells of the different branches of the immune system secrete different cytokines. For instance, cells of the innate immune system release

pro-inflammatory cytokines such as interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)-α, the effects of which are counter-acted by anti-inflammatory cytokines, such as IL-4, IL-10 and IL-1ra. Within the adaptive branch cellular immunity is promoted by T-helper (Th) 1 cells, which secrete cytokines such as interferon (IFN)-γ , IL-2 and TNF-α. Humoral immunity is promoted by Th2 cells which secrete for instance IL-4, IL-5, IL-10 and IL-13 [38].

2.2.4.3 Basic neuroimmune signaling

There are several ways for the central nervous system to signal to the immune system. Immune cells bear receptors for neurotransmitters, neuropeptides and hormones, and nerve endings reach the lymphoid organs (e.g. bone marrow and thymus) [39]. By these pathways, signals from the nervous system can be received by the immune system. This means that when the CNS detects changes in the environment or within the body, signals can be sent to the immune system.

In turn, the immune system can signal to the nervous system. Neurons both in the periphery and centrally, have receptors for cytokines [40].

Although cytokines are too large to pass the blood brain barrier, they can signal to the central nervous system through other pathways. One way is via peripheral nerves such as the vagus nerve, where efferent activation of the vagus nerve leads to inhibition of pro-inflammatory cytokines, the so-called "inflammatory reflex" [41]. Cytokines can also signal to the brain over the blood brain barrier by activating second messengers such as prostaglandin E2, or simply by passing into the brain via circumventricular organs which lack the tight junctions of blood brain barrier [40, 42]. This way, changes in the immune system can be monitored by the brain, and the brain can send signals to alter the actions of the immune system. Such signaling is of relevance in inflammatory disease, since pro-inflammatory cytokines (such as IL-1β and TNF-α) when signaling to the brain induces a so called sickness response [42] which includes fever, fatigue, malaise, and anorexia [43, 44]. In addition, some of those cytokines also promote sleep (see below), and alter cognitive functions.

2.2.4.4 Stress induced immune changes

Stress has been shown to induce changes in the immune system. However, depending on the nature of the stressor – acute time limited, brief naturalistic, or chronic – different immune alterations have been seen [5]. For instance, acute time limited stressors (e.g. public speaking and mental arithmetic, lasting between 5 and 100 minutes) have been shown to cause marked increases in the number of natural killer cells and large granular lymphocytes in peripheral blood [5], whereas chronic stressors (including dementia caregiving, and living with a handicap), like other nonacute stressors, show little effects on numbers of immune cells. Chronic stressors, however, show suppressing effects on almost all functional measures of the immune system [5].

These results concur with the findings of Dhabhar & McEwen [45] that "acute stress enhances while chronic stress suppresses immune function". Dhabhar & McEwen propose biphasic changes in blood leukocyte numbers, where the first surge of stress hormones – CAs – cause the body's "soldiers" (leukocytes) to exit their "barracks" (e.g. spleen, lung, and other organs) and enter the "boulevards" (blood vessels and lymphatics). The second part is when the HPA-axis is activated, and the release of GCs induce leukocytes to exit the blood and take position at potential "battle stations" (skin, mucosal lining of gastro-

intestinal tract, etc.). This would be a preparation for potential immune challenges imposed by the actions of the stressor.

It has also been shown that stress increases the production of pro-inflammatory mediators, such as the cytokines IL-1, IL-6, and TNF-α (for a review see [46]).

Dhabhar & McEwen suggest a stress spectrum, where acute stress helps the individual cope effectively with environmental challenges, through allostatic processes mentioned above, whereas chronic stress is (generally) not seen as beneficial, ultimately resulting in allostatic load and health complications. These effects on health are at least partly due to the effects of long term stress on the functioning of the immune system described above.

2.2.5 Measuring stress

In order to study something, the "something" often needs to be measured. Measuring stress, however, is not entirely straight forward. From my point of view, The Stress Measure, which will explain everything about stress, probably does not exist. Instead, depending on one's working model and the context of the research question, measurements will have to vary. For instance, if interest is in the relation between how appraisal relates to activity in the stress-systems, then appropriate measurements of these aspects are needed. Some stress researchers find that the differences in focus and approaches between disciplines should be maintained [47]. However, if "stress" is viewed as a process, and the overall aim is to understand all aspects of this process more fully, then measures can be made at any stage, some of the stages, or even all of them [6, 27].

An additional aspect is if the stress being measured is acute or chronic, since acute and chronic stressors differ in hormonal output, and also in the effects on the immune system [5, 13]. It is therefore important to define the stressors measured, for instance according to the taxonomy proposed above.

Few questionnaires have been developed which specifically examine the affective responses to stressors. In this project, I have used several different measures, including single item measures, such as a Visual Analogue Scale (VAS) for measuring stress experience, and a nine-step Likert-type scale to measure tension, both of which I conceptualize as measuring acute stress, or stress perception. I have also used measures of anxiety and depression, and psychological distress as part of the stress response – or allostatic load.

Behavioral responses to stress are sometimes conceptualized as coping based on the Lazarus & Folkman model, in which case the Ways of Coping Questionnaire (WCQ, Folkman & Lazarus 1980) is commonly used. When not conceptualized this way, as in this thesis, different behaviors may be studied individually. I have focused on stress behaviors (i.e. type A behavior pattern) and sleep, since increases in stress behaviors and impairment in sleep can be expected to perpetuate allostasis and thereby contribute to allostatic load.

Common ways of measuring biological aspects of the stress response are by measuring cortisol or CAs (see above), although several biological systems are involved and affect the activity of one another. Although it is not reasonable to think that one measure of one specific hormone would be a sufficient and objective measure of whether we are stressed or not, measurement of cortisol was included in study I.

Since the disease of interest in this project is allergy, immune-parameters relevant to allergy were measured. This included the Th1/Th2 cytokine balance, and numbers of cells of types involved in the allergic reaction.

In the methods section below I describe the measures used in this project.

2.3 SLEEP

Sleep can be behaviorally defined as "a reversible behavioral state of perceptual disengagement from and unresponsiveness to the environment" [48].

Sleep is essential, and provides us with rest and recovery from the day's "wear and tear", and is often seen as The Restoration – the most powerful way to balance the (negative) effects of activity. However, "wear and tear", or at least "normal use" is needed to induce sleep. Sleep and wakefulness is an example of the homeostatic or allostatic processes the body is constantly balancing. McEwen states that "Sleep deprivation, even for the course of the active period of the day, increases the homeostatic drive to sleep, with resulting changes in pro-inflammatory cytokines and glycogen levels." [30]. As most of us have experienced at some point, reduced or disrupted sleep results in increased sleepiness and reduced well-being the following day.

Sleep consists of different "types" of sleep, called sleep stages. The most obvious distinction is between rapid eye movement (REM)-sleep and non-REM (NREM). As the name implies, REM-sleep is characterized by rapid eye movements, but (usually) under closed eyelids. NREM then, is simply sleep without rapid eye movements. NREM sleep is divided into three (traditionally four) different stages defined according to electroencephalogram (EEG), but differences are also seen behaviorally. For instance, stage 1 is the "lighter" sleep, from which a person is easily aroused and may even feel he or she did not sleep at all, and stage 3, or slow wave sleep (SWS), is the deepest sleep stage, from which it is very difficult to wake a person. Stage 2, which is the intermediate stage, normally constitutes almost 50% of total sleep time.

Although quite a lot is known about sleep, the overall question of why we sleep at all is a topic for discussion. However, without going into all the different hypotheses proposed, most sleep researchers agree that the need for sleep indicates an essential restorative function [49]. It is also clear that sleep is important for the functioning of many bodily systems, including the immune system [50], and the nervous system – for instance for cognitive functioning [51].

Sleep regulation has been described as a two-process model, closely regulated by circadian ("process C") and homeostatic ("process S") factors [52]. The circadian factor increases the likelihood of sleep onset at certain times of the day, and is more or less independent of sleep and waking, whereas the homeostatic factor increases the likelihood of sleep after a period of wakefulness, but decreases the likelihood of sleep after prior sleep. Process S increases with waking time and if previous sleep periods have been short or disturbed, and is reduced only during (slow wave) sleep.

Circadian factors promote sleep around the so-called circadian trough (which occurs during the latter half of the habitual nocturnal sleep episode) and interferes with sleep during the peak. Through the interaction of circadian and homeostatic factors, sleep is consolidated to night-time, and wakefulness to day-time. Circadian factors are more important for REM-sleep timing, and homeostatic factors are more important for SWS timing. Interestingly, SWS is selectively increased in brain areas previously activated during wakefulness [53]. In addition, the immune system is an important homeostatic factor influencing sleep. Although the effects of central and peripheral cytokines may differ, the pro-inflammatory cytokines IL-1 and IFN-γ, that are also involved in the sickness response (see above), have been shown to be directly sleep inducing [54], and are likely involved in

the increase in SWS seen during infection [55]. Anti-inflammatory cytokines on the other hand inhibit sleep [50].

2.3.1 Sleep and stress

In relation to stress, sleep is vital, since allostatic load can occur when adequate recovery from stress responses does not take place [28]. Sleep is central for recovery; it is important for restoring homeostatic and allostatic systems in the body, including the immune system [50]. Sleep is also fundamental for the well-being of the individual and impaired sleep is tightly linked to psychological distress [e.g. 56]. McEwen [30] concludes that "The long-term consequences of sleep deprivation constitute a form of allostatic load—with consequences involving hypertension, reduced parasympathetic tone, increased pro-inflammatory cytokines, increased oxidative stress, and increased evening cortisol and insulin.".

Several studies have shown a relation between self-rated stress and impaired sleep [57, 58], and a majority of individuals with persistent sleep disturbances relate the onset to a stressful life event [59, 60]. In addition, stress is indicated in the maintenance of sleep disturbances [61], and activity in the stress systems is related to degree of objective sleep disturbance in individuals with chronic insomnia [62]. Thus, the development of insomnia, which is the most commonly occurring sleep disorder [63], is related to stress. In the general population, about one third suffers from one or more insomnia symptoms, and about 10% fulfill diagnostic criteria for insomnia [64].

Diagnostic criteria (e.g. the diagnostic and statistical manual of mental disorders (DSM) [65] and the international classification of sleep disorders (ICSD) [66]) include that the individual reports either difficulty initiating sleep, difficulty maintaining sleep, waking up too early, or having sleep that is chronically non-restorative or poor in quality. They also commonly include some form of day-time consequences related to the nighttime sleep difficulty, for instance fatigue; impaired attention, concentration, or memory; mood disturbance/irritability; motivation/energy/initiative reduction; and concerns or worries about sleep.

Stress thus seems to be important for both the development and maintenance of insomnia problems [61, 67], and the Cognitive Behavioral model for insomnia, includes stress – or arousal – as an important factor, especially at the earlier stages [68].

Stress and sleep are thus intimately linked, and through their mutual connection with immune regulatory processes, they are of high relevance for inflammatory diseases such as allergies [69].

2.4 ALLERGY

Allergy is a strong, "abnormal", immune-reaction to harmless substances. Allergic individuals are predisposed to a number of clinically expressed disorders, so called atopic diseases, e.g. atopic rhinitis (AR), atopic asthma and atopic dermatitis (AD). Such diseases are costly to society and often represent lifelong consequences to the individual. During the last decades, there has been a substantial increase in the prevalence of atopic diseases, mostly in the richer parts of the world, such as the western part of Europe and the US. In Sweden, almost 40 % of the children in school age have or have had allergic problems [70], and other studies have found as many as 1 in 3 individuals to suffer from some form of allergic disorder [71].

Obviously, finding out why allergic conditions have increased, and how to best help those suffering from allergic diseases, is of great concern.

My interest in allergy started when I realized that life-style factors influence the expression, and possibly even the development, of atopic disease. For instance, paternal smoking [72], dietary factors [73, 74], growing up in rural or urban areas [75, 76], and stress [77] have all been shown to influence atopy. From a behavioral medicine perspective, all of these aspects would be of interest, but the relation to stress (and sleep) is obviously the most interesting one to me. Before going in to this relation, some background information is needed.

2.4.1 Immune function in allergy

In allergy, reactions occur since the individual has previously become sensitized – started producing antibodies – to antigen such as food or pollen (then called allergens). Upon subsequent exposure to the same or a similar allergen, the adaptive immune response causes a production of antibodies [78], whereupon follows a number of additional events leading to the typical expressions of allergy (e.g. runny nose, or difficulties breathing).

There are different kinds of allergic immune reactions, but the most common is the so-called type I hypersensitivity resulting in diseases such as allergic rhinitis and asthma. Oftentimes, a so-called type IV hypersensitivity, or delayed type reaction, is also present especially in the chronic forms of asthma and rhinitis [78].

As mentioned above, these diseases are often referred to as atopic diseases, and atopy is defined immunologically as a genetic tendency to form immunoglobulin E (IgE)-antibodies towards common antigens in the surrounding environment [79]. In addition, a dominance of Th 2 cytokines over Th1 is by many considered a central feature [80]. Th1 cytokines include IL-2, IL-12, IFN-γ, and TNF-β. Th2 cytokines include IL-4, IL-5, IL-9, IL-10, IL-13. [81], and IL-6.

The immune response in allergy is also characterized by mast cell activation and degranulation. Mast cells have strong receptors for IgE-antibodies, and when the receptors of the mast cells bind to the antibodies, the cells become active and empty their granules. The granules of mast cells contain an array of inflammatory mediators, such as histamine (causing an immediate increase in local blood flow), different enzymes (causing tissue destruction) and the pro-inflammatory cytokine TNF-α (promoting influx of inflammatory leukocytes and lymphocytes into tissues). In addition, Th2 cytokines such as IL-4 and IL-13 are released, which perpetuate the Th2 response (by for instance inducing IgE production, and so called isotype switching to IgE), and signal to other important cell types, such as eosinophils and basophils, B-cells and dendritic cells, which come to the site and add to the inflammatory cascade [78, 82].

In addition to these well-established "allergic" immune processes, two other cell types need to be mentioned in the present context. The first is regulatory T-cells, whose role is usually one of moderating inflammation, and producing inhibitory cytokines to spare surrounding tissues from collateral damage [83]. However, regulatory T-cells in atopic individuals have been shown to be deficient in downregulating Th2 mediated inflammation [84]. Finally, natural killer (NK) cells that are usually involved in the defense against cancer cells, have been suggested to play a role in atopy and asthma, not least due to their capacity to secrete Th1 cytokines such as IFN-γ [85]. However, NK cells may also produce Th2 cytokines, such as IL-5 and IL-13 [86], and in a model where NK cells promoted allergic disease, they produced high levels of the Th2 cytokine IL-5 [87].

2.5 STRESS, SLEEP, AND ALLERGY

2.5.1 Stress and immuno-endocrine parameters relevant to allergy

Acute stress then, is associated with an increase in stress-system activity; arousal through the SNS (i.e. the SAM system) and HPA-axis (se above), and corresponding increases in CAs and GCs. GCs are generally anti-inflammatory [88], suppressing pro-inflammatory actions, and augmenting anti-inflammatory agents in the immune system.

In their 1999 review, Elenkov & Chrousos conclude that both GCs and CAs promote a Th2-cytokine (allergy-related) profile. CAs suppress the production of Th1-cytokines such as IL-12 and IFN-γ, thereby promoting a Th2-profile, whereas GCs in addition to suppressing IL-12, also up-regulate the production of Th2-cytokines, e.g. IL-4, IL-10 and IL-13 [89]. Thus physiological levels of GCs may be immunomodulatory rather than solely immunosuppressive, causing a shift in cytokine production from a Th1 to a Th2-type pattern.

Due to their anti-inflammatory properties, GCs represent standard care for persistent allergic diseases [78]. However, increased serum IgE-levels can be seen in allergic individuals treated with GCs [90, 91]. Recent studies have shown both pharmacological and physiological levels of GCs to increase production of IL-4 and suppress IFN-γ [81], and IL-4 is a Th2-type cytokine which among other things induce IgE-production [78].

In addition to the increased systemic levels of GCs seen during stress, CRH may be produced by sympathetic neurons and by immune cells locally in inflammatory sites, activating mast cell degranulation, and increasing allergic activity [92].

Stress, then, alters the level of circulating stress hormones. In addition to these alterations, the sensitivity of target organs (e.g. neurons of the hippocampus, blood vessels, white blood cells) may differ between individuals [93], and both steroid levels and target cell sensitivity are important to determine the physiological net effect [94]. The feed-back in the HPA-axis is crucial to a well-balanced immunological response [95], and studies in both animals and humans indicate that pathological consequences of altered regulation in the stress systems are relevant in allergic disease [96-99].

Stress has several effects on the immune system important for allergy. Many studies have shown stress to cause a shift from Th1 to Th2 profile [e.g. 100], and this is also the conclusion of a recent review on the subject [101]. In the previously mentioned meta-analysis by Segerstrom & Miller on effects of stress on the immune system, this shift is consistently seen in studies investigating the effects of "brief naturalistic stressors", such as exam-stress [5].

In atopic individuals, both IgE-levels and eosinophil numbers in blood samples have been shown to increase in response to an acute time-limited stressor [102]. Stress has also been shown to increase eosinophilic airway inflammation to antigen challenge [103]. In the same line, a number of studies on stress- and allergy-relevant immune parameters have been performed by Hajime Kimata. He found that computer stress and frequently ringing mobile phones enhanced skin wheal and IgE-responses in patients with AD, whereas both emotions with tears, kissing, and laughter (watching a Charlie Chaplin movie) significantly reduced skin wheal and IgE responses to allergens in AD-patients [104-109].

In sum, evidence is accumulating that a broad range of immune parameters important in allergy are affected by stress, in ways that would enhance an allergic response.

2.5.2 Stress and allergic symptoms

Patients, both with asthma and AD, often report that stress and worry contribute to exacerbations in allergic symptoms [110, 111]. For instance, the stress associated with experiencing an earthquake was shown to exacerbate AD symptoms in over 35% of patients [112]. In the same line, high levels of stress have been shown to predict higher asthma morbidity in children [113]. Finally, acute stress against the back-drop of chronic stress has been shown to exacerbate asthma symptoms more and with a sooner onset, than acute stress with no chronic stress, or chronic stress only (which did not have an effect on the risk for asthma exacerbations) [114]. However, other studies have found no association between stress and allergic symptoms [115], or functional measures of allergic disease (e.g. lung function) [116].

From an opposite viewpoint, interventions aimed at reducing stress and anxiety, can improve symptom severity. Data suggest that anxiolytics may alleviate stress-associated itching in patients with AD [117], and a recent review on psychological interventions concludes that "psychological interventions had a significant ameliorating effect on eczema severity, itching intensity and scratching in atopic dermatitis patients" [118].

In sum, there is evidence that allergic symptoms are affected by stress and that interventions aimed at reducing stress or anxiety may alleviate allergy symptom severity. However, there are also studies that have not found such a connection, pointing to a need to further clarify the specific circumstances associated with stress related allergic symptom exacerbations.

2.5.3 Allergy as a stressor

We have now seen that stress can affect a number of allergy relevant systems in the body. Based on the allostasis model, disease could also be viewed as a stressor, since disease is a threat to homeostasis requiring changes in the body's systems. However, allergic disease has not been discussed in this context.

Evidence of a connection in this direction can be seen for instance in a recent animal experiment, which showed that allergy sensitization and allergen challenge of animals induced anxiety-like behavior as well as a Th2-cytokine profile [119], suggesting anxiety to be an effect of the allergic disease.

In human studies, such experiments obviously cannot be performed. Different approaches have therefore been used to look for differences between allergic and non-allergic individuals, and different stages of the stress process have been investigated. If allergy in itself is a stressor, then perhaps the perception of acute stressors would be altered? This is not evident, since several studies have not shown a difference in perceived stress in relation to an experimental or quasi-experimental stressor between allergic and non-allergic participants [e.g. 102, 120].

However, many studies have shown an association between allergy and psychological aspects of allostatic load – i.e. increased levels of distress [e.g. 117, 121, 122]. Recently, a meta-analysis of prospective studies found allergy to be strongly associated with future psychological distress (and also psychosocial factors to be predictive of future allergy) [123]. In line, clinical observations of increased risk of suicide among allergic patients, findings that suicide peaks in spring, and of Th2-cytokine expression in the orbito-frontal cortex of suicide victims has also led to a suggestion that allergic inflammation may increase the risk of suicide [124].

Allergic individuals have been shown to exhibit altered responses to stress in both the HPA and SAM systems [125], and alterations are seen already in infants with atopic disposition [126]. In children and adults (but not in infants), the HPA-response to stressors is blunted (see [88] for a review), especially when exposed to allergen [127]. This has consequences for the ability of the endocrine system to help control immune processes during inflammation. For instance, during an inflammation, pro-inflammatory cytokines, such as TNF-α, IL-1 and IL-6, are released. Pro-inflammatory cytokines stimulate the HPA-system [95]. This normally increases the levels of GCs, which have an anti-inflammatory effect (see above). However, the decreased response in the HPA-system could result in an inability to shut of the inflammation, leading to an ongoing inflammatory process (e.g. chronic allergic condition). Buske-Kirschbaum et al. propose that "…because of defective HPA axis, immunoregulation under stressful conditions is ineffective in patients with atopic conditions, leading to aberrant immune responses and subsequent exacerbation of the disease" [128].

The SAM-system, on the other hand, seems to be over reactive in allergic individuals [125]. As mentioned above, CAs mediate a Th2-shift, both by suppressing Th1 and up-regulating Th2 cytokine production [14], which could then also contribute to disease exacerbations.

Like the HPA-system, the vagus nerve is stimulated by pro-inflammatory cytokines. This stimulation normally turns on an "inflammatory reflex", i.e. activates the vagus' cholinergic anti-inflammatory pathway [41, 129]. In allergy, the effects of vagus nerve stimulation has been researched for several decades (at least since the 1970's [130]), although to my knowledge not in the context of altered anti-inflammatory function, but rather in terms of broncho-constriction and, for instance, mast cell activation [e.g. 131, 132]. However, one recent study that found a shift in autonomic balance toward parasympathetic predominance in patients suffering from atopic dermatitis, discusses the anti-inflammatory function as well [133].

Taken together, allergic inflammation may be seen as a stressor, adding to the allostatic load for individuals suffering from allergic diseases.

2.5.4 Impaired sleep and allergy

A considerable percentage of insomnia-sufferers present with co-morbid problems [134], and a considerable proportion of allergic patients have impaired sleep [135-140].

Several recent findings suggest that disturbed sleep may be a link between stress and atopy. As noted above, sleep is a highly important restorative process influencing homeostatic and allostatic systems in the body, and with great importance for the function of the immune system; fatigue and increased sleep are part of the sickness response seen during inflammation [141]; and sleep is often impaired in allergic individuals (but as with stress it is difficult to establish the causal direction of this relationship). Everyday stress is related to disturbed sleep in healthy individuals [58], and sleep disturbances, even in the absence of atopy, is related to a shift in the cytokine balance toward a Th2-response [142]. In addition, it has been hypothesized that sleep loss might be responsible for some of the immune system changes that accompany stressors [143, 144]. For instance Hall et al. [143] found that effects of stress-related intrusive thoughts and avoidance behaviors on lower numbers of circulating NK cells were mediated by greater time spent awake during the first sleep cycle.

In line, it has been suggested that sleep disturbances can worsen the course of chronic inflammatory conditions, such as asthma and allergic rhinitis [69], and sleep deprivation has been shown to enhance allergic skin responses [145].

Some studies have shown improvements in sleep after treatment of the allergic disease [146, 147], but it has also been suggested that some patients treated to full remission in allergic symptoms still suffer from impaired sleep [148].

Insomnia has well-known long term negative consequences, for instance in the increased risk for depression [149]. This could be due to long-term consequences of sleep deprivation which, as we have seen above, constitutes a form of allostatic load [30, 31]. One obvious possibility for diminishing the risk of allostatic overload could then be to try improving sleep in individuals with impaired sleep. Since sleep is consistently shown to be impaired in allergic individuals, it would make sense to find out if a treatment for insomnia could improve their sleep. Perhaps by improving sleep, allostatic load could be diminished, ultimately leading to less health problems?

2.5.4.1 *Psychological treatment of impaired sleep – CBT for insomnia*

Cognitive behavioral therapy (CBT) has been shown to be an effective treatment for insomnia [150, 151] and is considered treatment of choice [152]. The Standards of Practice Committee of the American Academy of Sleep Medicine therefore recommends CBT for the treatment of insomnia [153].

CBT for insomnia includes both behavioral [68, 154, 155] and cognitive techniques [156]. The most thoroughly evaluated of the techniques described in the literature are sleep restriction (sometimes referred to as sleep compression) and stimulus control, but today CBT for insomnia usually includes a number of techniques, all of which may have some merit. For an overview of CBT techniques used in insomnia treatment, see for instance [155].

Although earlier studies of insomnia treatment tended to only treat primary insomnia, recent findings suggest that it may be worthwhile to treat the sleep problem first or in parallel with the additional problem(s) [157-159]. CBT for insomnia has thus been shown to be effective also for individuals with co-morbid problems [160], but attempts to treat sleep problems in individuals with allergy have not been reported.

3 AIMS OF THE THESIS

This thesis aimed at investigating stress and sleep in the context of allergy. The main focus was on the stress response in allergy; sleep in times of strain and its involvement in the development of allergy, and the possibility to treat co-morbid insomnia.

The specific questioned asked were:

- How are allergy-relevant immune-parameters affected by a brief naturalistic stressor – an exam? (Paper I)

- How are psychological functioning (levels of anxiety, depression, and tension), sleep, and allergic symptoms affected by the same brief naturalistic stressor (exam)? (Paper II)

- Do sleep problems in childhood contribute to the development of allergic disease in adolescence, and vice versa? (Paper III)

- How do individuals with disturbed sleep (insomnia) and co-morbidities including allergy respond to a psychological treatment for insomnia? If sleep is improved, are there any effects on allergic symptoms? (Study IV)

4 PROJECT DESIGN

In order to answer the research questions in the project and to gain experience from different methodologies during my doctoral education, I have used studies with different approaches. The first two papers are based on a study with a quasi-experimental design, where medical students with or without atopy were observed at two occasions, i.e. during an ordinary study period and during a stressful examination period (Paper I and II). The third paper is an epidemiological study with a prospective design, using parent report data from the Twin Study of Child and Adolescent Development (TCHAD) about twins born in 1985-1986 (Paper III). Finally, the fourth paper is a randomized controlled trial of the efficacy of a CBT-based self-help treatment for insomnia with co-morbid problems, including allergy (Paper IV).

4.1 PAPER I

Methods: Forty-one undergraduate students, 22 with allergy of whom 16 had asthma, and 19 healthy controls, were studied in a regular study period and in association with a large exam. Of the atopic participants, 14 were female and eight male, with a median age of 25 years (range 21-31 years). Non-atopic participants had a median age of 24 years (range 21-27 years), 10 were female and nine male.

Material: Subjects completed questionnaires on stress and health behaviors, underwent lung function tests, bronchial methacholine challenge, measurements of exhaled nitric oxide and urine cortisol. Blood cells were phenotyped, and cytokines from mononuclear blood cells were analysed.

Statistical analyses: Wilcoxon's signed-rank test, or a paired t-test for normally distributed data, was used for within subject comparisons. Mann–Whitney U-test, or an unpaired t-test for normally distributed data, was used for comparisons between subjects. Levels of cytokines were logarithmically transformed to normalize distributions. A p-value of less than .05 was considered significant. Gender distribution between the atopy and the control group was tested by a X^2 test. Statistical analyses were performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA).

4.2 PAPER II

Methods: = paper I

Materials: Students completed questionnaires on stress, anxiety, depression, tension, stress behaviors (type A-behavior pattern) and self-rated health (SRH), and during one week during each period they filled out daily diaries on sleep and allergic symptoms.

Statistical analyses: Analyses of variance (ANOVAs) were used to test for main effects of group (atopic vs. non-atopic participants), condition (non-examination vs. examination period), and interactions, for all variables except allergic symptoms. Allergic symptom item scores were Z-transformed before being aggregated since different scales were used. Exploratory factor analyses were performed on allergic symptom variables to reaffirm the division of items into categories of asthma, eczema, and rhinitis. Some items were excluded during this process due to zero variability or because they did not fall into the categories. Finally, for allergic symptoms, t-tests were performed to compare low-stress and stress allergic symptoms scores within the atopy group. (Allergic symptom scores were not calculated for the non-atopic group.) Effect sizes are given as η_p^2, which is interpreted as

explained variance in the sample. All analyses were made with SPSS 15.0 for Windows (SPSS. Rel 15.0. Chicago: SPSS Inc.; 2006), and the alpha level was set to .05.

4.3   PAPER III

Methods: This prospective epidemiological study was based on data from the TCHAD study. The target sample consisted of all the 1480 twin pairs born in Sweden between May 1985 and December 1986 who were alive and lived in Sweden.

Material: We used parent-report questionnaires of asthma, rhinitis, eczema and sleep problems at wave 1 (in 1994) and wave 2 (in 1999), from which 1101 (75%) and 1063 (72%) of the parents responded, respectively.

Statistical analyses: Logistic regression models were developed to investigate the effect of (1) sleep problems at age 8 on asthma, rhinitis and eczema at age 13; and (2) asthma, rhinitis and eczema at age 8 on sleep problems at age 13. Increasingly complex models from bi-variate analyses to multivariate analyses were fitted to account for the confounding influences of a) pre-existing associations between the two assessments (i.e. the correlation between allergy and sleep problems at age 8-9) and stability in the outcome measure – e.g., the correlation between allergy assessed at age 8-9 and 13-14 (Model 2) and b) well established covariates, including birth weight and socioeconomic status (Model 3). Odds ratios (OR) with 95% confidence intervals (CI) were estimated using generalized estimating equations models, which allow us to account for the dependent nature of the twin observations. All the statistical analyses were performed in the SAS 9.1.3 using the GENMOD procedure (SAS Institute, Inc., Cary, NC).

4.4   PAPER IV

Methods: This randomized controlled trial included 133 media recruited participants with insomnia (109 women). Participants had a mean age of 47.9 (standard deviation (SD) 13.9) years, a long history of sleep difficulties (11.8 (SD 12.0) years), and most (92.5%) had co-morbid problems (e.g. allergy, pain, or depression) including a large proportion with allergies (57.9%). Participants were randomized into three groups; self-help treatment only (n=45), self-help treatment with telephone support (n=44), and wait-list control group (n=44), and underwent assessment at base-line, after six weeks and after five months (i.e. follow-up assessment). The two treatment groups underwent the self-help treatment for insomnia during the first six weeks after base-line assessments, during which time the treatment group with telephone support also received one 15 minute telephone call per week from a therapist. The self-help book was based on established cognitive behavioral methods for insomnia, including sleep restriction, stimulus control, and cognitive restructuring.

Material: Participants were interviewed and completed on-line, self-report questionnaires on sleep, psychological distress, and perceived stress, as well as one week of daily diaries on sleep and allergic symptoms at each assessment.

Statistical analyses: One way ANOVAs and X^2 associations were used to compare groups on background variables. Outcome data was analyzed for interactions between group and time-point, with Multivariate Analyses of Variance (MANOVAs) for the measures of 1) sleep timing, 2) subjective sleep variables, and 3) day-time functioning and psychological distress. If interactions were significant for the MANOVAs, 2-way ANOVAs were used as follow-up tests for each variable for two groups at two time-points at a time, and analyzed for interactions – i.e. if and how groups differed in their response. η_p^2, interpreted as amount of explained variance in the sample, was included as an effect size measure for interactions.

Change-values within each group were calculated with 95% Confidence Intervals, and for within group effect sizes Cohen's d was used. ANOVAs were also calculated with the different co-morbid problems (e.g. allergy) as between-subjects variable instead of treatment group, and Insomnia Severity score. For all analyses of variance, Huynh-Feldt corrections were applied when sphericity could not be assumed based on Mauchly's Test of Sphericity. Thirteen outliers were found in sleep diary data, and following the recommendations by Tabachnick & Fidell [161], score alteration was performed in order not to lose valuable data, but lessen the impact of outliers. Treatment response and remission rates were calculated; as suggested by Morin et al. [162], participants were considered treatment responders if their Insomnia Severity Index (ISI) score changed with 8 points or more compared to pre-assessment, and as treatment remitters if their absolute ISI score was less than 8. Except correlations and ANOVAS analyzing effects of co-morbidity, all outcome analyses are computed for intent-to-treat data with last observation carried forward, based on 133 pre-assessment questionnaires and 132 pre-assessment sleep diaries (one lost due to poor data quality). Analyses were conducted using PASW statistics 17 and 18 (SPSS Inc. Chicaco, Illinois).

4.5 ASSESSMENTS

4.5.1 Stress

Stress can be measured at different stages of the stress process. In this thesis, two measure of stress appraisal, or perceived stress, have been used. However focus has been on the stress response and allostatic load. Stress response measures include emotional and behavioral aspects of stress, as well as measures of endocrine and immunological aspects.

4.5.1.1 Stress perception

Paper I: Current, or acute, stress level was measured with a one-item Stress - Visual Analogue Scale (VAS). Students were asked to rate their overall level of stress on the VAS, a straight horizontal line measuring 100 mm, with anchors placed at both poles from "no stress" to "worst possible stress". Participants were given written instructions: "Rate the level of stress you're experiencing at this moment. Mark with an X on the line." Despite its simplicity, the reliability and validity of VAS ratings has been demonstrated [163].

In paper II, acute stress was measured with a question about tension**,** phrased: "How do you feel right now" and to be rated on a 9 step scale, anchored at 1: feel very relaxed and calm, 5: neither [relaxed] nor [tense], and 9: feel very tense and pressed, on the verge of what I can handle.

Paper IV: Stress appraisal was measured with The Perceived Stress Scale (PSS-10) which measures perceived stress in daily life [164, 165]. The PSS-10 has 10 items with response alternatives 0 (never) to 4 (very often). Total score ranges from 0-40, and higher scores reflect higher levels of perceived stress.

4.5.1.2 Stress response

Psychological Distress, Anxiety & Depression
In study II, trait anxiety was measured with the State-Trait Anxiety Inventory (STAI-T; [166]. State anxiety was measured with the Hospital Anxiety and Depression Scale (HADS) [167], which also measures depression.

Study IV: The Clinical Outcomes in Routine Evaluation - Outcome Measure (CORE-OM) evaluates general psychological distress [168]. This 34 item scale has response alternatives 0 (often) to 4 (almost all the time), yielding a total score between 0 and 112, and higher scores reflect more psychological distress.

Stress behaviors
Study II: Stress behaviors (so called Type A behaviors) were measured with The Everyday Life Stress scale [169]; 20 statements referring to stress behaviors in everyday life situations, with four response alternatives (coded as 0–3): 0, almost never; 1, sometimes; 2, often; 3, almost always. Total score ranges from 0 to 60, and it is commonly said that above 30 points indicates an elevated level of stress behaviors, and over 40 points a very high level.

Immune changes
Study I: Complete blood count with leukocyte differentials was performed. Cells were analyzed by flow cytometry using a FACS-Calibur flow cytometer (BD, San Jose, CA, USA). In flow cytometry, cells are incubated with different antibodies that attach to cells with the right surface antigen or antibody receptor. The different antibodies are coupled to a fluorescent dye. Cells with glowing antibodies then pass through a thin tube in the flow cytometer, where they are automatically counted. Flow cytometry can give number and/or proportions of different types of cells.

Study I Cytokines: Enzyme-linked immunosorbent assay (ELISA) (OptEIATM Human sets, Pharmingen, BD Biosciences, San Jose, CA, USA) was used to determine levels of IL-2, IFN-g, IL-4 and IL-5 in supernatants from peripheral blood mononuclear cells. As with flow cytometry, ELISAs also use antibodies, but to detect proteins rather than cells. After several steps of preparation, antibodies and enzymes attach to the sought after protein, a substance is added that the enzyme can convert to a detectable signal, often a fluorescent color. The intensity, or magnitude, of the fluorescent light is read by a machine. This magnitude is then interpreted as an amount of the sought after protein, using a standard curve constructed with known concentrations.

Cortisol
Study I: Urine cortisol concentrations were determined by standard laboratory methods (time-resolved fluorometry - Auto Delfia Cortisol Kit, Wallac OY, PerkinElmer, Wellesley, MA, USA). Urine was collected from 10 PM the evening before until the first morning urine on both assessments days. This type of assay is similar to the ELISA procedure; several steps of preparation with antibodies and the sought after molecule precede the reading of fluorescence in a machine, and the magnitude is interpreted as the amount of the molecule.

4.5.2 Sleep

Although disturbed sleep may be seen as part of the stress response, sleep can also be seen as a moderating factor. Sleep can be measured in many different ways, with both subjective and objective measures that obviously differ in the information gained. In this thesis, only self-report measures have been used. Self-report measures are practical and economical tools to evaluate sleep. In addition, no objective measures, but only subjective reports of sleep complaints are central for an insomnia diagnosis [65, 66]. Sleep diaries represent a core assessment component in insomnia research [170], although patients with insomnia generally over-estimate wake-time [171]. Nonetheless, sleep diaries have been shown to correlate well with objective measures [172], and self reported sleep quality is strongly related to total sleep time and amount of SWS [173].

4.5.2.1 Sleep diaries

In study II, sleep related diary questions were selected from the Karolinska sleep diary [174]. They included bed-time and rise-time, sleep latency ("Amount of time it took you to fall asleep (after lights were turned off)"), sleep quality (rated on a five-point Likert-type scale, 1="very bad" to 5="very good"), whether participants felt refreshed after (1="not at all refreshed", 5="fully refreshed"), whether they slept enough (1="no, definitely insufficient", 5="yes, definitely sufficient").

In study IV, a full sleep diary [68] was used. It was recorded during one week at each of three assessment points. The sleep diary includes registration of bed time, time of falling asleep, length of night time awakenings, time of waking up and time of getting out of bed, as well as subjective sleep quality, positive and negative day-time evaluations, day-time activities, stress at bed time, and medications. From these data, sleep timing measures (i.e. sleep onset latency, wake after sleep onset, total sleep time and sleep efficiency (calculated as total sleep time/total time in bed)) may be calculated, and subjective diary measures used (i.e. sleep quality, day-time fatigue (i.e. "On a scale 0-5, where 0 is 'not at all', and 5 'very much so', rate how tired you have felt today"), and positive day-time ratings (i.e. "On a scale 0-5, where 0 is 'not at all', and 5 'very much so', rate how alert/well functioning/happy you have felt today"). All sleep diary measures were computed as the mean of the daily ratings over the week. Sleep diaries are the most widely used outcome measures in insomnia research [175].

4.5.2.2 Insomnia Severity

Study IV: The Insomnia Severity Index (ISI) [68] is a 7-item patient-reported outcome assessing the severity of initial insomnia (difficulties falling asleep), middle insomnia (difficulties sleeping through the night) and late insomnia (waking up too early in the morning); sleep satisfaction; interference of insomnia with daytime functioning; noticeability of sleep problems by others; and distress about sleep difficulties. A 5-point scale was used to rate each item, yielding a total score ranging from 0 to 28. Higher score indicates more severe insomnia within 4 severity categories: absence of insomnia (score of 0-7); sub threshold insomnia (8-14); moderate insomnia (15-21) and severe insomnia (22-28). The ISI has adequate psychometric properties and is sensitive to measuring treatment response [176].

4.5.2.3   Measures of sleep interfering behaviors

Study IV: A safety behavior is a strategy employed in order to prevent a feared outcome from occurring. Safety behaviors have been associated with impairment in sleep and daytime functioning in individuals with insomnia [177]. Therefore the Sleep Related Behaviors Questionnaire (SRBQ) was used to assess counter productive safety behaviors in insomnia [177]. The scale has 32 items which are scored between 1 (almost never) to 5 (almost always), yielding a total score range of 32-160. Higher scores reflect higher levels of safety behaviors.

Study IV: Rigidly held attitudes and beliefs about sleep play an important role in sustaining insomnia problems [178]. Dysfunctional Beliefs and Attitudes about Sleep (DBAS) [179] is a 30-item self-report measure identifying sleep disruptive cognitions (e.g. beliefs about the immediate and long-term negative consequences of insomnia, and beliefs about the need to control insomnia). Although developed as a visual analogue scale, it was transformed into a Likert-type scale with responses 0-10 for the use on a web-site. Scores range is 0-300, with higher scores indicating more maladaptive beliefs regarding sleep.

4.5.2.4   Parent reports of impaired sleep

In Study III, sleep was assessed by 4 items from the Child Behavior Checklist for Ages 6-18 (CBCL/6-18) [180], which is a standardized questionnaire for parents to rate the frequency and intensity of behavioral and emotional problems exhibited by their children in the past six months. The CBCL/6-18 consists of 111 statements, and the parents rate each item on a 3-point scale (0 = not true; 1= sometimes true; 2 = often true). The sleep items used in the present study were: "Has nightmares", "Overtired", "Sleeps less than other kids", and "Sleep problems". For each of the 4 sleep items, dichotomized variables were created to compare participants for whom parents had responded 1 (sometimes true) or 2 (often true) with those who had not been given a score of 1 or 2.

4.5.3 Allergy

4.5.3.1   Measures of lung function

Study I: Spirometry was performed, including Peak Expiratory Flow (PEF) which is a measure of the maximum expiratory flow measured as liters/minute; Forced Expiratory Volume 1 second (FEV_1) which is the volume expired during the first second; Vital Capacity (VC), the total volume that can be measured (excluding the Residual Volume (RV) which remains in the lungs); Forced Vital Capacity (FVC) which is when FEV_1 and VC are measured during the same maximum expiration. PEF rate, VC and FVC were determined using the MasterScope direct reading spirometer (Erich Jaeger GmbH, Hoechberg, Germany), software version 2.53.2. Percentage predicted FEV_1 was calculated as measured FEV_1/predicted FEV_1, and percentage predicted PEF was calculated as measured PEF/predicted PEF.

Study I: Nitric Oxide (NO) was determined in oral single breath exhalations with a NIOX*s*, using standardized procedures [181] (Aerocrine AB, Solna, Sweden).

Study I: Metacholin challenge test was performed using a dosimeter-controlled jet nebulizer Spira Electro 2 (Respiratory Care Center, Hämeenlinna, Finland). After recording the

baseline FEV1, the subject first inhaled saline followed by a methacholine solution. With the nose clipped, methacholine was inhaled for a specified number of breaths, in concentrations from 14.2 to a maximum cumulative dose of 7256 mg, with dose increments around every third minute. FEV1 was measured 2.5 min after each dose of methacholine and the test stopped when FEV1 had decreased by at least 20% from its post-saline value. The logarithmic metacholine doses were plotted against the percentage of the post-saline FEV1. PD20 values were calculated from the cumulative dose–response curves by linear interpolation and expressed as log values.

4.5.3.2 *Allergic symptom diaries*

Study II and IV: The diary included two types of questions concerning subjective symptoms of allergy phrased to cover asthma, rhinitis, and eczema respectively; e.g. "How often did you experience asthma [/eczema] symptoms [/from the eye and nose] today?" with response alternatives 0: "not at all" – 6: "all the time"; and "Have you been troubled by wheezing [/itching eczema/symptoms from eyes and nose/] during the night or morning?" with response alternatives 0: "not at all troubled" – 3: "seriously troubled". The latter questions were also phrased to cover symptoms in the morning and evening, respectively. Individual questions were combined to represent symptoms of asthma, eczema and rhinitis, respectively.

4.5.3.3 *Parent reports*

Study III: To evaluate presence of asthma, rhinitis and eczema, we used questions derived from the International Study of Asthma and Allergy in Childhood (ISAAC) [182]: "Has your child ever had asthma" (yes/no) and "Has your child ever had itchy eyes and runny nose" (allergic rhinitis, yes/no) along with questions on recurrent itchy rash on arms and legs, face and abdomen (eczema, yes/no).

5 RESULTS

5.1 PAPER I - CHANGES IN IMMUNE REGULATION IN RESPONSE TO EXAMINATION STRESS IN ATOPIC AND HEALTHY INDIVIDUALS.

The aim of paper I was to increase the understanding of the effects of a brief naturalistic stressor particularly on allergy-relevant immune-parameters, and outcomes associated with regulation of the allergic inflammation (numbers of blood cells such as regulatory T-cells and NK-cells) and allergic aggravation (as measured by exhaled NO and lung function). We hypothesized that stress would be accompanied by a decrease in the Th1/Th2 balance and that this effect would be larger in atopic subjects.

The hypothesis that the Th1/Th2 balance would decrease in response to stress, particularly in atopic participants, was confirmed; the IFN-γ/IL-4, IL-2/IL-4 and IL-2/IL-5 ratios were significantly reduced in atopic students during stress.

The proportion of NK cells was significantly lower in atopics compared with controls at the low-stress phase. Interestingly, the proportion of NK cells further decreased in atopics, but not in controls, during the stress phase. Both atopic and non-atopic participants also had increases in regulatory T cells at the time of the exam.

Atopic individuals with asthma exhaled significantly more NO than control subjects, and control subjects only responded with a small but significant reduction in exhaled NO in response to stress. Also, during stress the FEV_1 value increased slightly but significantly in the group of controls, but not in the group of atopic participants with asthma.

5.2 PAPER II - EFFECTS OF EXAMINATION STRESS ON PSYCHOLOGICAL RESPONSES, SLEEP AND ALLERGIC SYMPTOMS IN ATOPIC AND NON-ATOPIC STUDENTS

The aims of this paper was to compare responses between atopic and non-atopic individuals to a major examination, and to characterize which changes in sleep, affect, self-rated health (SRH) and allergic symptoms are elicited by this brief naturalistic stressor.

For both atopic and non-atopic students, tension, anxiety, and depression deteriorated in response to examination, as did sleep latency and sleep quality. Overall, atopics were more tense, had more anxiety, longer sleep latencies, and were less well rested than non-atopics. Non-atopic students rose from bed later during the examination period. In response to examination, atopic students reported increased frequency of stress behaviors (e.g. eating fast), while decreased stress behaviors were reported by non-atopic students. Allergic symptoms were not affected.

5.2.1 Additional analyses

5.2.1.1 Self rated health

Lately, there has been a growing interest in subjective health perception (i.e. SRH), as it is a strong and independent predictor of health and mortality [183, 184]. Little is known about changes in perceived health in a response to stress. The Swedish council on technology assessment in health care has concluded that SRH should be included in studies of allergic diseases, such as asthma[185]. In a recent thesis, it was shown that pro-inflammatory cytokines are related to poorer self-rated health [186].

Self-rated health in this study was measured with SRH-5 [187] i.e. the question "How would you rate your general health?", rated on a five-point scale ranging from 1 – 'very good', to 5 – 'very bad'.

An ANOVA showed that SRH (see table 5.2) did not differ between the groups but was negatively affected by stress in both atopic and non-atopic participants ($F_{(1, 36)}$=13.412, p<.001, η_p^2=0.271).

Table 5.2. Mean SRH rating according to atopic status and condition.

		Study period Mean (SD)	Examination period Mean (SD)
Self rated health	Atopic participants	1.7 (0.7)	2.0 (0.7)
	Non-atopic participants	1.4 (0.5)	1.7 (0.6)

5.3 PAPER III - DEVELOPMENT OF ALLERGIES AND SLEEP DISTURBANCES IN CHILDHOOD AND ADOLESCENCE

The aim of paper III was to investigate whether aspects of disturbed sleep in childhood (age 8-9) predicts development of allergy in adolescence (age 13-14), and vice versa; if presence of allergy in childhood increases the risk of developing sleep problems in adolescence.

Being overtired at age 8-9 was associated with an increased risk [OR; 95% CI (2.59; 1.31-5.11)] to develop rhinitis at age 13-14, even when controlling for sex, previous rhinitis, socioeconomic status (SES), birth weight, and other sleep problems at age 8-9. Likewise, asthma at age 8-9 was an independent risk-factor for being overtired at age 13-14 [OR; 95% CI (2.64; 1.44-4.84)], controlling for sex, previous overtiredness, SES, birth weight, and asthma at age 8-9. Associations were also found for being overtired at 8-9 with asthma at 13-14 [OR; 95% CI (3.21; 1.84-5.60)], asthma at 8-9 with short sleep at 13-14 [OR; 95% CI (2.63; 1.34-5.18)], asthma at 8-9 with trouble sleeping at 13-14 [OR; 95% CI (2.33; 1.01-5.42)], rhinitis at 8-9 with trouble sleeping at 13-14 [OR; 95% CI (2.52; 1.26-5.03)], and eczema at 8-9 with short sleep at 13-14 [OR; 95% CI (1.85; 1.18-2.91)]. However, these associations disappeared after controlling for other factors, indicating associative predictions, rather than possible causal relationships.

5.4 STUDY IV - EFFICACY OF A BEHAVIORAL SELF-HELP TREATMENT WITH OR WITHOUT THERAPIST GUIDANCE FOR INSOMNIA WITH CO-MORBID PROBLEMS

The aim of this study was to evaluate the efficacy of a cognitive behaviorally based self-help book to treat insomnia in individuals with co-morbid problems, including allergies, and the effect of adding brief therapist telephone support.

The hypothesis that the self-help intervention would improve patients' sleep was largely confirmed. Intention-to-treat analyses showed both self-help groups improving significantly from pre to post treatment in sleep timing, subjective sleep measures and daytime functioning, compared to waiting list. For example, bibliotherapy with and without support gave shorter sleep onset latency (improvement, minutes [95% CI]), 35.4 [24.2 to 46.6], and 20.6 [10.6 to 30.6] respectively, and support gave a higher remission rate (61.4%,

p<.001), than bibliotherapy alone (24.4%). In the two groups receiving bibliotherapeutic treatment, the group receiving telephone support improved more on sleep related measures than the group that did not. Improvements were not seen in the control group (sleep onset latency 4.6 minutes [-1.5 to 10.7] shorter, and remission rate 2.3%). Improved sleep in the treatment groups was generally maintained at follow-up, although some differences between the treatment groups had diminished. Larger number of co-morbid problems was negatively correlated with outcome post treatment, but not at follow up. Only nightmares affected outcome negatively, whereas other co-morbid problems, including allergies, did not.

5.4.1 Additional analyses

Additional analyses were performed to investigate how allergy affected sleep, and how sleep affected allergic symptoms.

First: analyses showed that allergic participants made up a substantial proportion of the sample; a total of 77 participants (57.9%) were diagnosed as having one or more allergic problems. Allergic participants were distributed evenly between the groups (see table 5.4).

Table 5.4. Number of allergic participants according to study group.

	Bibliotherapy with support (n=44)	Bibliotherapy (n=45)	Waiting list control (n=44)	Total (N=133)	*P* Value[a]
Allergies n (%)	29 (65.9%)	24 (53.3%)	24 (54.5%)	77 (57.9%)	.42

Second: The groups did not differ in insomnia severity at pre treatment assessment, nor at post treatment or three month follow up assessment. Comparing the effect of treatment for allergic participants and non-allergic participants (including participants with other co-morbid problems), there was no interaction effect ($F_{(1.879,148.414)}=1.175$, $p=.310$, $\eta_p^2=.015$), i.e. the effect of treatment did not differ between these two groups. (See figure 5.4. 1)

Third: As seen in figure 5.4.2., numeric decreases (i.e. improvements) in allergic symptoms were observed at the three-month follow-up assessment (fu3), compared to the pre-treatment assessment (pre), and post-treatment assessment (post). However, when ANOVAs were conducted to find out if allergic symptom scores differed between the three time points, a main effect was found only for rhinitis symptoms ($F_{(2, 134)}= 6.379$, $p=.002$, $\eta_p^2 = 0.087$). Pairwise comparisons revealed no difference between pre and post assessment ($p=.333$), but there were significant differences between pre and fu3 ($p=.008$), and a trend between post and fu3 ($p=.065$). I.e. significantly less rhinitis symptoms were reported at fu3.
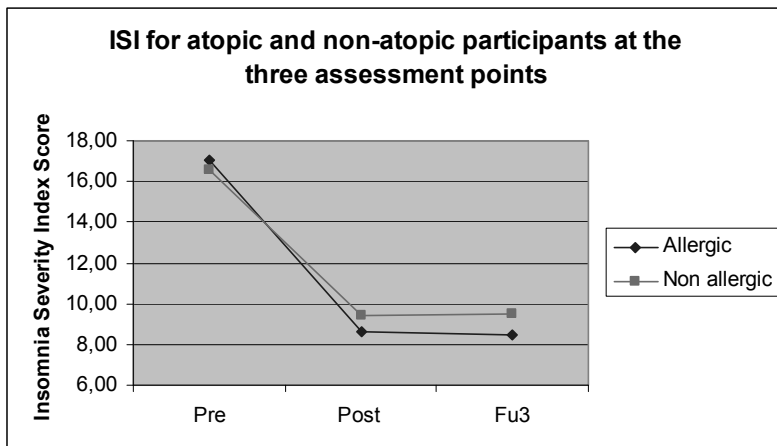
Figure 5.4.1. Mean scores for insomnia severity at pre treatment assessment (Pre), post treatment assessment (Post), and three-month follow-up assessment (Fu3) for treated participants, divided into group according to allergic status.
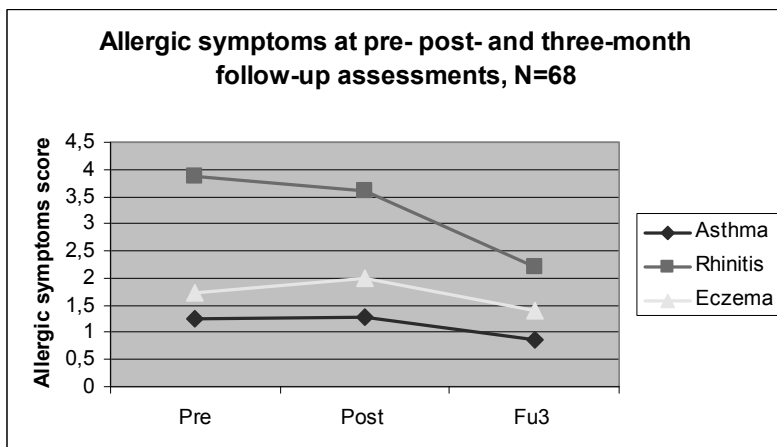

Figure 5.4.2. Mean scores for allergic symptoms at pre treatment assessment (Pre), post treatment assessment (Post), and three-month follow-up assessment (Fu3), for allergic particpants only.

6 GENERAL DISCUSSION

In this thesis, the relations between stress and allergy, and sleep and allergy have been investigated. The findings are discussed, particularly with respect to the allostasis model and allergic disease.

6.1 INITIAL QUESTIONS AND NEW FINDINGS PUT IN CONTEXT

I set out on this project since I was puzzled by the observation by many clinicians and allergic patients that stress aggravates allergic symptoms, and the concurrent scientific findings of a relationship between psychosocial stress and asthma [188] and atopic dermatitis [112]. I was curious about which mechanisms could perhaps explain such observations; In what ways may stress be involved in the expression of atopic disease? What psychobiological mechanisms might be involved? Could it be that parts of the stress process might differ between atopic and non-atopic individuals?

6.1.1.1 The impact of stress on allergic symptoms

In the first study, results showed that acute stress perception and stress responses – changes in allergy relevant immune parameters as well as emotional and behavioral responses including sleep – did differ between atopic and non-atopic individuals. The immune changes seen in atopic participants could increase expression of allergic symptoms. The emotional and behavioral response in both atopic and non-atopic participants could be conceptualized as increases in allostatic load. Interestingly, allergic symptoms did not increase, which raised new questions about the specific circumstances during stress that lead to increases in allergic symptoms.

Around the time data was collected for the first study, a series of studies were published, showing that a brief naturalistic stressful condition produced greater allergy related immune response to challenge [103], and that different types of acute stressors, such as computer stress and mobile telephone ringing, enhanced skin wheal responses in atopic individuals [104, 107]. However, there were also studies, which like our own showed allergy relevant changes in immune function, but not in symptom expression such as lung function [116]. A few years later, Chen & Miller presented a model proposing that immune changes during stress may not cause asthma symptoms, but rather set the stage for increased symptoms if there is concomitant exposure to allergens [101]. Support for this model has also come later with studies similar to those of Kimata (above), but with a more conventional stress inducing task (the Trier Social Stress Test) [189]. Since our participants were not exposed to allergens during the course of the study, this could explain the lack of changes in allergic symptoms. That being said, it should be kept in mind that in animals, and possibly also in humans, allergic or allergy relevant immunological processes can be behaviorally conditioned to and subsequently elicited by exposure to a neutral stimulus such as a taste [190-192].

6.1.1.2 Acute and chronic stress in allergy

Also the type of stressor under study could perhaps account for some differences in allergic symptom expression. From a general allostasis perspective, short term stress is seen as an adaptive response which enhances many functions central to survival, including immune function [45]. Chronic stress, on the other hand, has been suggested to suppress immune

function [13]. However, it is also noted that enhanced immune function may be detrimental under certain conditions, for instance in autoimmune diseases or allergies, whereas suppression of at least some aspects of immune function may be beneficial in these circumstances [193]. In line, a recent study showed that experiencing a very powerful stressor (loss of a child) actually decreased the risk of developing amyotrophic lateral sclerosis (ALS) [194], something that could possibly be explained as following a stress-induced tilt in immunological balance.

Although there are studies that have shown improvements in some allergic symptoms in response to acute stressors [e.g. asthma 195], acute stress does not seem to necessarily be beneficial in the case of allergy. For instance, Sandberg et al. [114] showed that acute life stress significantly increased the risk of asthma exacerbations, and that this risk was further increased, and onset of exacerbations hastened, if the acute stressor occurred on top of chronic stress [114]. Chronic stress as such entailed no increased risk of exacerbations. In line, a recent study by Marin et al. compared asthmatic children with high or low chronic family stress. Children with high chronic family stress had increased production of Th2-cytokines (IL-4, IL-5), and Th1-cytokines (IFN-γ) (from peripheral blood mononuclear cells) in response to an acute stressor resulting in a Th2 dominance. The combination of acute and chronic stress was also associated with increased asthma symptoms. In contrast to the Sandberg study, there was no relationship between acute events and cytokine production in the absence of chronic stress [196].

In an animal model of allergic asthma, airway cytokine responses to acute and chronic stress differed between asthmatic and non-asthmatic animals. Kang et al. shoved that acute stress produced a Th2 predominance in allergic animals (by increasing IL-4 and decreasing IFN-γ levels), but a Th1 predominance in non-allergic ones (by decreasing IL-4 levels). Chronic stress on the other hand, produced a Th1 predominance (by decreasing cytokine levels overall) in both asthmatic and non-asthmatic animals. Kang et al. conclude that "These findings suggest that a significant shift toward Th2 predominance in asthmatic mice under acute stress may be a mechanism underlying exacerbation of asthma" [197].

There may be a problem with the terminology here; acute stress for the mice in the Kang study was one hour of exposure to bright light in an open box combined with mild rotation, and chronic stress was the same stressor but one hour a day for four days. Acute stress for the children in the Sandberg and Marin studies was "specific events with discrete onset and offset" [196]. However, most were major life events such as loss of important social relations; a relative passing away, a close friend moving to another city, or a parent being laid off. Chronic stress in the Sandberg study was ongoing social problems such as drug abuse, child abuse or chronic illness of a close family member, and in the Marin study "the quality of interpersonal relationships among family members". I do not see that the label *acute time limited stressor* as used in the Segerstrom meta analysis would be the best to describe the acute onset events used in either the Marin or the Sandberg studies. From a child's experience, are these events really discrete and short-term. Since loss is major stressor, I would be inclined to suggest that they would perhaps be better described as *stressful event sequences*, i.e. one major event, such as the loss of a spouse or a major natural disaster, gives rise to subsequent related challenges.

Although the nature of stressors varied across studies, the effect is quite consistent and in line with other studies of both acute laboratory stressors [e.g. 102], brief naturalistic stressors [e.g. 103] and chronic stressors, such as low socio-economic status [e.g. 198, 199]. Thus, many types of stress do seem to promote a Th2 shift and (likely in case of exposure to allergen) may increase allergic symptoms in allergic individuals.

Can a Th2-shift then be seen as a sign of allostatic load? Perhaps that could be the case in allergic individuals. A Th2-shift may not necessarily be the "normal" effect of stress, although some studies have seen such a shift also in non-allergic individuals in response to stress [200] and disturbed sleep [142]. However, other studies find that a Th1/Th2 skew in response to stress is typical of allergic disease, and not a general response to stress [196].

6.1.1.3   Allergy as an expression of allostatic load

Based on the original conception of allostatic load, several biological markers have been suggested, and combined into a 10-item allostatic load index (ALI) [201]. This "was a cumulative index that took into account various possible stress responses involving blood pressure, glucose metabolism (and consequent obesity), inflammation markers, and hormonal responses. It measured ''primary'' biological parameters (including the hormone and inflammation markers) and secondary health outcomes (the cardiovascular and metabolic risk factors). The index consisted of systolic blood pressure (SBP), diastolicblood pressure (DBP), waist to hip ratio (WHR), total cholesterol (TC), glycosylated haemoglobin (HbAlc), high density lipoprotein (HDL) cholesterol, dehydroepiandrosterone (sodium) sulphate (DHEA-S), urinary cortisol, urinary norepinephrine (NE), and urinary epinephrine (EPIN)". [202] There is obviously a very clear connection from these measures to Cardiovascular disease (CVD), but the relation to allergic disease is more obscure.

However, from another angle, in study I (paper II), we found that atopic individuals reported more stress (or Type A) behaviors during the stressed period, whereas the non-atopic students reported less such behaviors [203], an aspect we are not aware has been studied before in relation to allergy. Type A behavior pattern has previously been strongly linked to CVD [204]. Interestingly, a relation between allergies and CVD has long been hypothesized [205], but has been explained as side effects by drugs used to treat asthma. However, recent findings suggest that asthma and cardiovascular risk could be linked by obesity and negative affect [206] as well as by pro-inflammatory mechanisms [207-209]. Recall from above that pro-inflammatory cytokines were also seen in response to chronic stress in studies of both mice [197] and men [196]. In addition, they are involved in chronic allergic inflammation [210]. A similar hypothesis has been put forward which argues that repeated acute or chronic psychologically stressful states may cause inflammatory processes that lead to CVD and type 2 diabetes [211].

Now, although the place of Th2 dominance is unclear, it may seem like a logical step to link all these findings and suggest that the connections between stress and allergies may also be mediated by an inflammatory pathway, rather than by the classical allostatic load measures (see above). Indeed, Priftis et al. [88] recently suggested precisely this; that allergy is to be conceptualized as a form of allostatic (or as they would prefer, cacostatic) load, where elevated pro-inflammatory cytokines, such as TNF-α, IL-1, and IL-6 are the principal conduits, which may induce a state of hyporesponsiveness of the HPA axis, whereby attenuated cortisol secretion and exacerbation of allergic airway inflammation ensue.

6.1.1.4   Atopy as a stressor – increasing allostatic load

Although perceived stress in relation to an experimental or quasi-experimental stressor has often not been shown to differ between allergic and non-allergic participants [102, 120], in study I, we found stress levels to be elevated in atopic students as compared to non-atopic students as a response to a brief naturalistic stressor [212].

Atopic participants in study I had some signs of higher allostatic load than the non-atopic ones; they had higher levels of anxiety and depressive symptoms, and altered sleep patterns. In line, Liu et al. [103] also found students' anxiety and depression scores to be significantly higher during an examination period.

Lower cortisol levels were seen in atopic participants. This could be seen as an indication of a lower level of allostatic load. However, an attenuated activity and/or responsiveness of the HPA axis is consistently shown in allergic patients [213], and has been shown to be related to the severity of allergic inflammation [127]. Indeed, Angelica Buske-Kirschbaum [214] finds that hyporesponsiveness is a key feature of atopic disease (with previous hyperresponsiveness in infancy).

In addition, Hellhammer et al. [215] suggest that there may be two major subtypes of the HPA-axis response to stress, where one is hypocortisolemy. They found that the hypocortisolemic subjects had a lower allostatic load (as measured with an AL-index like the one described above) but that they scored higher on measures of depression, perceived stress, and physical complaints, which is in line with the findings among our atopic participants.

It is also possible that the more negative emotional states could be an effect of underlying endocrine and immunological deviations, including inflammation, adding to the allostatic burden. Thus, one possible interpretation of available data is that allergic disease – even when not actively expressed in allergic symptoms (for instance most of the atopic participants in study I had few allergic symptoms during the course of the study) – equals increased allostatic load. It is possible that a negative circle could ensue, where inflammation and subsequent negative mental states would constitute an additional burden for atopics compared to non-atopics in the face of stressors, adding to allostatic load causing more negative feelings adding to the negative effects of a future stressor, resulting in higher allostatic load and eventually allostatic overload, for instance expression of (allergic) disease or psychopathology such as depression [31]. In line, connections between allergy and increased levels of psychological distress have been reported repeatedly [e.g. 117, 122, 216, 217], and recently also a connection between allergy and suicide [124, 218].

6.1.1.5 Allergy and sleep

In study I, both atopic and non-atopic students experienced worse sleep quality during the examination period, which is in line with other studies on the effect of stress on sleep [e.g. 219]. Further, sleep latency and feelings of being well rested in the morning differed between atopic and non-atopic participants; atopic students took longer to fall asleep, and felt less well rested, although time in bed did not differ between the groups. The prolonged sleep latency during stress is in line with the fact that behavioral responses to stressors includes increased arousal and wakening [220], however, the fact that it only occurred in atopic students could be seen as support for the hypothesis that allergy increases allostatic load.

The connection between allergy and impaired sleep seen in this thesis can be seen as a partial explanation for the previously mentioned differences in acute stress levels between atopics and non-atopics. Disturbed sleep is part of the stress response, and contributes to increased levels of stress and discomfort, which could be expected to lower the threshold for experiencing stress, or increase stress perception reactivity. If the effects of stress are cumulative, as the allostasis model suggests, then an event might be experienced as more stressful if you already have a stress load to begin with (the straw that brakes the camel's

back...). Thus, non-restorative sleep could add to the negative circle described above, with impaired recovery and increased allostatic load in atopic patients. In fact, a very recent review puts forward the hypothesis that allergic rhinitis leads to mood and anxiety disorders and an increased risk of suicide, via sleep impairment [221].

6.1.1.6 Impact of sleep on the development of allergy

Study I then showed increased levels of disturbed sleep in our participants with atopy [203]. This has also been seen in several other studies [e.g. 135, 136-140]. In addition, several recent finding also support a connection between stress and disturbed sleep [e.g. 57, 58].

With recent studies indicating an etiological connection between stress in infancy and childhood, and later development of atopic disease [77, 132], new questions arose; specifically about the role of stress in the development of atopic disease. Since sleep is important for maintaining homeostasis, disturbed sleep can be considered a stressor in and of itself [30], which, as we have seen above, can sometimes lead to exacerbations of allergic symptoms. In addition, disturbed sleep has been associated with a Th2 cytokine shift [142].

We therefore set out to find an epidemiological material where we could study whether impaired sleep could be related to later development of allergy, and vice versa. In paper III, we found that an indicator of impaired sleep (fatigue) was related to later development of rhinitis. However, when controlling for confounding factors such as gender, birth weight, and socioeconomic status, other sleep measures could not predict the development of allergic disease, even though cross sectional relations were found. This could be a problem of sufficient power in the study, since for instance only 60 children developed asthma between the ages of 8-9 and 13-14.

Another explanation could be that parents are better at noticing high levels of fatigue (a main characteristic of disturbed sleep) rather than disturbed sleep itself, particularly since parents are more likely to be around their children while they are awake compared to when they are sleeping. I.e. the reliability of this variable may be better than that of the other variables. However, as fatigue is also part of the sickness response seen in inflammation [141], it could also be a "true" effect of fatigue – i.e. fatigue is not necessarily a result of impaired sleep.

6.1.1.7 Impact of allergy on development of sleep problems

We also found that asthma was related to more fatigue at a later age. In line with the reasoning above, it is possible that asthma – well known to disturb sleep acutely – could result in both worse sleep and fatigue across time. The connection could be mediated partly through the effects of annoying symptoms; e.g. trouble breathing prevents you from dozing off. However, it could also be an effect of alterations in the endocrine and the immune systems seen in atopic individuals. For instance, several of the Th2 cytokines which are over expressed in atopy are also known to inhibit sleep [54].

Many allergic individuals continue to have sleep problems despite adequate treatment for their allergies [148]. One explanation of this could be if treatment reduces symptoms, but does not clear the underlying physiological dysfunction. The person could then for instance be left with a low grade inflammation, similar to what is seen in depression [206], which may have negative consequences for sleep. An alternative explanation for the remaining sleep problems in these individuals could be that the acute sleep problems resulting from the symptoms, have developed into insomnia, through the traditional pathways suggested in the CBT model for insomnia; heightened arousal, and cognitive

changes such as more focus on sleep and the consequences of impaired sleep, and dysfunctional behavioral strategies to handle fatigue and night-time waking [68].

6.1.1.8 Improving sleep in allergic individuals

With all these possible pathways linking allergy and impaired sleep, it seemed like a logical final step of this project to develop and evaluate a treatment for impaired sleep (insomnia), to investigate whether such treatment could improve sleep for individuals with allergy and insomnia. Paper IV presents the general results from a randomized controlled trial of a CBT treatment for insomnia and shows that sleep can be improved in individuals with insomnia, also if they have co-morbid problems (including allergies). Sub-analyses showed that sleep improved as much for allergic participants as it did for participants without allergies. Indeed, additional analyses of the data showed that over half the participants in the study had allergies, and that rhinitis symptoms decreased significantly at three-month follow up. Thus, insomnia severity improved after treatment, and some time later allergic symptoms decreased. In line with the hypothesis that impaired sleep increases allostatic load, one could hypothesize that improved sleep, over the course of some time, had lowered the cumulative allostatic load, leading to less allergic symptoms.

6.2 LIMITATIONS AND STRENGTHS

A general problem for studies on stress is the lack of a comprehensive stress theory to put findings into a broader perspective. In this thesis, one of the "micro models" – the allostasis model – has been used. A micro model is certainly better than no model, but they all still lack the ability to predict aspects of the whole stress process. However, refining micro models can eventually lead to the development of a comprehensive theory that may be able to better define aspects such as what is a stressor (and for whom?), what constitutes a stress response, and not least how and when stress may be advantageous or deleterious. The CATS (see under Models and Theories of Stress) is an attempt to put together findings from different areas, and is worthy of more attention in future research. Another aspect is that even a comprehensive model is not The Final Model, since research and new research methods give rise to new knowledge partly by rejecting previous knowledge and theories. Clearly, to explore the links between stress, sleep and allergy, an interdisciplinary approach is warranted. The present project was conducted in close and fruitful collaboration between researchers trained in e.g. psychology, medicine, physiology, and sleep medicine, and utilized methods stemming from different methodological research traditions.

Some specific limitations of the studies are also present. In the first study (paper I and II), we did include many allergy relevant immune measures. However, we did not include measures of allergy relevant functional measures of the immune cells, but only number of cells and amount of cytokines. The biological significance of these changes is unknown. The participants were not exposed to allergen during the course of the study. In one way, this is a limitation since we cannot say anything about how stress modulates the response to antigen. However, it is also a strength, in that we do show allergy relevant immune changes occurring *despite* the absence of allergen. Another limitation is that the study was done with students with well managed allergy only. The generalizability of the results to other groups, including for instance other age groups and individuals with more severe allergy is limited. In addition, for paper I, we did not use any measure of well-being or positive affect, which has been shown to differ between allergic and non- allergic individuals [222] and account for differences in immune response [223]. In addition, no measure of chronic stress was

used, so we cannot say anything about the possible additional impact of chronic stress in the background of this brief naturalistic stressor. Further, the order of observation was not balanced; i.e. all students were observed first during the non-examination period and second during the examination period. Strengths of the study was the thorough screening of participants including medical evaluations, the inclusion of measures of lung function, airway inflammation, stress hormones, allergic symptoms, and the inclusion of non-atopic individuals as a control group.

In study III no measure of stress was included, only measures of impaired sleep. Although impaired sleep can be seen as an indicator of an ongoing stress response, including a stress measure would have made possible another set of calculations to account for additive or interactive effects of stress and sleep on the development of allergic problems. In addition, the study did not include optimal questions of sleep disturbances. In the same vicinity, at least in Swedish, "overtired" can have a connotation of being unable to wind down. It is thus possible that the connections seen were mediated by other factors than impaired sleep. Also, despite the population approach, the statistical power was not enough to perform interesting sub-analyses, such as the relative importance of genetic and environmental influences using basic twin analyses. Further, our data indicate causal relationships between sleep and allergy, but we cannot exclude the possibility that some third factor is involved and cause both these connections. For instance, there may be underlying alterations in the function of the stress-systems (e.g. HPA-axis and SAM-systems) that are important for both the development of allergies and sleep disturbances. Further, no control was done for other diseases, therefore it cannot be excluded that fatigue is really predictive of all-cause morbidity, rather than specifically for allergic disease (or even more specifically: rhinitis). Another limitation is that we only used parent-report data. Although parents are often the best available informants for young children, the children in this study were, especially at the second assessment, old enough to answer for themselves, and their answers may have been more reliable than their parent's. The presence of allergic disease was also determined using parent reports of allergic symptoms. Although this is a limitation, the questions used were from a large world-wide multi center study of prevalence of allergic diseases (International Study of Asthma and Allergy in Childhood – ISAAC), and can thus be directly compared. Some strengths with the study is its prospective design, which does allow for estimates of prediction, and its inclusion of enough subjects to find new cases of allergy between the ages of 8-9 and 13-14.

Finally, in study IV interviewers were not blinded to treatment condition, and therefore, interview data may be biased. On the other hand, all questionnaires were filled out on the internet, a feature that has been suggested to increase participants' willingness to reveal unpleasant information, such as not improving with treatment, by reducing the impact of social desirability. Another limitation is the timing of measurements. Since pre- and post assessments took place during spring and early summer, whereas the three-month follow-up assessment took place in October, it is not inconceivable that the decrease in allergic symptoms is a function of time of year, rather than an effect of improved sleep. To be able to shed light on this question, it would have been better to include participants and to time measurements with regards to allergen exposure peaks. Ideally, pre-treatment assessments would have been done one spring, after which treatment could be implemented, and then a follow up assessment could take place again next spring. It would then have been possible to see how allergic symptoms may impact sleep improvements or vice versa in the longer term. If improvements that could not be accounted for by lower levels of pollen were seen in allergic symptoms, this would have given stronger support for an impact of sleep via the

inflammatory pathway in allergy. In addition, including measures of self-rated health (SRH) would also have been interesting, to provide a global outcome measure of high predictive validity. In this study, allergic disease was determined using a questionnaire. Although it was screened by an allergy specialist, we cannot exclude misclassification. In addition, no biological measures were included, so the biological correlates of the treatment effects are shrouded in mystery. However, among strengths of the study can be mentioned its randomized and controlled design, and that it included a comparably large number of participants, out of whom a substantial proportion (almost 60%) were allergic and many had other co-morbidities.

6.3   FUTURE CONSIDERATIONS

Although clinical observations of stress related exacerbations of allergic symptoms and epidemiological studies finding relations between psychosocial stress and allergy have been a ground for investigating mechanisms for such relationships, it is unknown what proportion of allergic patients experiences this relation. Systematic studies would be needed to find that out. In addition, it may be that individuals' own experience of this relationship is one factor that could explain differences in stress responses. Testing that hypothesis could be done in experimental studies.

Trying to get a grip of the research on stress, I have been struck by the lack of agreement over many things. Future studies need to clearly specify not just how the word stress is used (if at all since other suggestions have been provided that may be more accurate), and what stage(s) in a stress process are studied. Although many stress researchers are beginning to do so, increased efforts should be made as to clarify whether stressors under study are acute or chronic, or combined. In addition, studies are needed that can clarify whether for instance emotional expressions are a cause for, or an effect of stress (or both). It may be that a distinction between primary and secondary stressors is a fruitful way to go, as suggested by for instance Pearlin [224]. In addition, which individual differences are important in determining responses to stress is a question still far from having a definite answer. There is obviously still much work needed in this area.

I was also surprised by the lack of agreement of the concept of "disturbed sleep". It seems to include at least three different kinds of "disturbed sleep". 1) Unnecessary or involuntary sleep deprivation; one is kept awake, or sleep is repeatedly interrupted, even though sleep pressure is high. This would include sleep fragmentation from sleep apnea or noise (heavy traffic, airplanes and so forth), but also being kept awake as part of a sleep deprivation experiment. 2) "Necessary" sleep deprivation; there is a stressor or changed circumstance that is a "reasonable" reason one does not sleep; for instance important work deadlines, difficult decisions that need to be made, or little children that need tending. 3) Insomnia; one gets stressed from being awake at night and from feeling tired during the day.

It does not seem reasonable that these kinds of disturbed sleep would have the same effect; not least because they are perhaps topologically alike, but contextually distinct. Although the argument can be made that it is the number of hours or minutes of shortened sleep that is crucial for the effects, I believe the understanding of the effects of "disturbed sleep" would benefit from taking such differences into account.

Moreover, different types of allergies may be affected in partly different ways. A distinction may be needed between the different types of expression (asthma, rhinitis and eczema), since some studies have shown differences in e.g. anxiety levels comparing atopic dermatitis and allergic rhinitis [225]. In addition, atopic and non-atopic background for the

same or similar disease expressions (e.g. atopic vs. non-atopic asthma, rhinitis and dermatitis) could be of relevance. For instance, differences have been seen in allergic and non-allergic rhinitis in the risk of developing sleep apnea [226], and impairment in quality of life has been shown to differ between different types of non-allergic rhinitis [227].

In addition to studying these aspects one at a time, they need to be studied in concert, to improve our understanding of their inter-relations. More efforts are needed to clarify individual differences in the responses to stressors, especially with respect to allergy. Not all persons with atopy have the same amount of Th2-tilt; how can these differences be predicted? Do we need to take more psychologically oriented or other models into account, or can enough of this be explained by differences on a physiological level? I would also like to see more studies investigating the relevance of the allostasis model in allergic disease. I believe the model would need adaptation to fit the allergic diseases better. For instance, can a Th2-shift be considered allostatic load? Perhaps other measures of allostatic load than those commonly used would apply for individuals who are not primarily pre-disposed for the metabolic changes suggested? Finally, although the Chen model of allergic symptoms in relation to stress is very appealing, it is not sufficiently supported for symptoms other than asthma, so more work is needed there as well.

In addition, since sleep is often disturbed in allergy, the present findings suggest a need for treating sleep problems in individuals with allergy, and shows that this can be done. Future studies, however, are needed to confirm treatment effects, and to test if successful treatment of stress and/or sleep may decrease symptom expression. In addition, studies need to test the hypothesis that treatment of sleep disturbances, or psychological distress in children with atopic disposition could diminish the risk for developing allergic disease

6.4   CONCLUSIONS

This thesis presents increased support for, and suggests an expansion of previous findings of stress related immune changes to involve also increases in regulatory T-cells. It provides increased support for the involvement of stress in allergy relevant immune changes in atopic individuals, as well as for increased levels of psychological distress, impaired sleep and increased stress behaviors in atopic individuals as compared to non-atopics, and suggests these changes to be a sign of increased allostatic load in atopics. In addition, the thesis replicates previous findings of cross-sectional relations between impaired sleep and allergic symptoms, but also presents preliminary evidence that aspects of impaired sleep act as risk factors in the development of allergy, and vice versa. Finally, it shows for the first time that individuals with insomnia, despite presence of allergies and other co-morbidities, can be successfully treated using a self-help CBT treatment for insomnia. Interestingly, three months after improvements in insomnia severity, improvements in allergic symptoms were seen. Altogether, the thesis confirms a coupling between stress, sleep, and allergy and suggests future studies to test if successful treatment of stress and sleep may decrease symptom expression or even diminish the risk for developing allergic disease.

7 SAMMANFATTNING PÅ SVENSKA

På senare år har förekomsten av allergiska sjukdomar ökat dramatiskt i västvärlden och omkring 30% av Sveriges befolkning är idag drabbade av allergi. Vad denna ökning beror på är oklart, men beteendefaktorer som stress och sömn tros vara några av orsakerna. Även stress är ett vanligt problem idag, och stress kan förändra bl.a. immunsystemets aktivitet och funktion. Sömn, å andra sidan, är viktig för återhämtning, och störd sömn kan fungera som en stressor i sig. Allergiska patienter uppger ofta att allergiska symptom ökar i samband med stress. Dessutom lider många allergiker av trötthet och störd sömn, särskilt vid exponering för allergen. Det är dock fortfarande oklart hur sambanden mellan stress, sömn och allergi ser ut. Det övergripande målet för denna avhandling var därför att öka kunskapen kring dessa samband.

Avhandlingen baseras på tre studier, varav den första är kvasiexperimentell. Studenter med och utan atopi observerades under en vanlig studieperiod och under en tentamensperiod (artikel I och II). Vid båda tillfällena fick studenterna bl.a. genomgå lungfunktionstestning och lämna blodprov. De fick också fylla i frågeformulär och dagböcker om psykologiska och allergiska symptom samt om sömn. Resultaten visar att både atopiska och icke-atopiska studenter var mer stressade under tentamensperioden, och att de då också kände sig sämre till mods, sov sämre och hade förändringar i immunparametrar, bl.a. en markant ökning av regulatoriska T-celler. Stress gav också specifika förändringar hos de atopiska studenterna, bl.a. en förskjutning mot Th2 dominans, ökad ångest, och mer störd sömn jämfört med de icke-atopiska studenterna. Trots dessa förändringar rapporterades ingen ökning i allergiska symptom.

Artikel III baseras på en prospektiv epidemiologisk studie (Twin Study of Child and Adolescent Development (TCHAD)), och data kommer från frågeformulär ifyllda av föräldrar kring olika aspekter av barns störda sömn och allergi. Studien replikerar tidigare fynd som visar att störd sömn och allergi samvarierar i hög grad. I en mer komplex modell som också kontrollerar för faktorer som kön, födelsevikt, socioekonomisk status och tidigare sömn- och allergiproblem, kunde vi dessutom se att övertrötthet hos barn (8-9 års ålder) kunde förutsäga förekomst av rinit i tidiga tonåren (13-14 års ålder), och att astma hos barn kunde förutsäga förekomst av övertrötthet i tidiga tonåren.

Fynden från studie I-III visar att störd sömn är vanlig hos allergiker. Det behövs alltså behandling av sömnproblem för personer som också har andra komorbida problem, t.ex. allergi. Artikel IV är därför en randomiserad kontrollerad studie för behandling av insomni med komorbida problem, bl.a. allergi. Bedömningar med frågeformulär och sömndagbok gjordes före behandling, direkt efter behandling, och tre månader efter avslutad behandling. Studien visar att deltagare i behandlingsgrupperna får förbättrad sömn, och att allergikers sömn förbättras lika mycket som icke-allergikers, trots allergisymptom.

Sammanfattningsvis visar studierna att stress kan vara en delfaktor som påverkar allergirelevanta immunförändringar hos atopiker, och den kumulativa negativa effekten av stress (s.k. allostatisk belastning) tycks vara förhöjd hos atopiker jämfört med icke-atopiker. Resultaten talar för att stress kan vara en faktor i utvecklingen av allergi och tvärtom, men visar också att störd sömn kan behandlas effektivt med hjälp av en KBT-baserad självhjälpsbehandling, också hos personer med allergi. Avhandlingen styrker ett samband mellan stress, sömn och allergi. Nya studier behövs för att visa om framgångsrik behandling av sömn eller stress kan minska förekomsten av allergiska symptom hos allergiker, eller t.o.m. minska risken att utveckla allergi.

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