ADHD and stress

Diurnal cortisol levels, early psychosocial adversity and perceived stress

JOHAN ISAKSSON

The Hypothalamus-Pituitary-Adrenal axis (HPA-axis) with its end product cortisol mediates the physiological response to stress thereby promoting mobilization of energy. The cortisol levels follow a diurnal rhythm with a distinct awakening response. Regulation of the HPA-axis differs among persons with certain psychiatric disorders when compared with controls. Some reports concern Attention-Deficit/Hyperactivity Disorder (ADHD) but findings are inconclusive. The main aim of the present thesis was to investigate diurnal levels of saliva cortisol in school aged children with ADHD and age matched non-affected comparisons, also taking early adversity, perceived stress and ADHD-medication into consideration.

Children with ADHD had lower cortisol levels at awakening, 30 minutes later and before going to bed than comparisons. When the study group was split into three different age groups similar results were found only for children above 10 years of age. Within the ADHD group, subtype of ADHD or co-occurring symptoms did not affect the cortisol levels. Furthermore, children in the ADHD group had to a higher degree been exposed to foetal and childhood psychosocial adversity than comparisons.

Since exposure to early adversity has been associated with both ADHD and HPA-axis functioning, such exposures could theoretically explain the low cortisol levels in ADHD via early programming of the HPA-axis. However, no relation was found between exposures to psychosocial adversity and diurnal cortisol levels. Neither did continuous medication with stimulants or atomoxetine explain the low cortisol levels. Possibly, medication may rather increase the levels.

Finally, children with ADHD scored higher on perceived stress, measured by the Pressure-Activation-Stress (PAS) scale, than the comparison group. Female sex was also associated with higher stress in both groups, as well as increasing age in the comparison group. As with psychosocial adversity, no association was found between the higher PAS-scores and the lower cortisol levels, indicating the complexity of the stress regulating system.

The results indicate a down-regulated or displaced HPA-axis with lower cortisol levels in children with ADHD. Stress related fragility – with more exposure to early stressors, higher perceived stress and lower diurnal cortisol levels – seem to accompany ADHD during childhood.

Keywords: ADHD, HPA-axis, cortisol, hypocortisolism, diurnal rhythm, trauma, adversity, medication, perceived stress, gender differences

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ISSN 1651-6206
urn:nbn:se:uu:diva-211808 (http://urn.kb.se/resolve?urn=nbn:se:uu:diva-211808)
To my children
Tilda, Vilgot & Klara
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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**Abbreviations**

ACTH  Adrenocorticotropic Hormone  
ADHD  Attention-Deficit/Hyperactivity Disorder  
ADHD-C  ADHD, combined type  
ADHD-HI  ADHD, predominantly hyperactive/impulsive type  
ADHD-I  ADHD, predominantly inattentive type  
ANS  Autonomic Nervous System  
AVP  Argine Vasopressin  
BAS  Behavioural Approach System  
BIS  Behavioural Inhibition System  
CD  Conduct Disorder  
CRH  Corticotropin Releasing Hormone  
CRHR1/2  Corticotropin-releasing hormone receptor  
DNA  Deoxyribonucleic Acid  
EEG  Electroencephalography  
ETI-SR  The Early Trauma Inventory - Self Report  
FTF  Five to Fifteen  
GR  Glucocorticoid Receptor  
HPA-axis  Hypothalamus-Pituitary-Adrenal axis  
MC2-R  Melanocortin Receptor 2  
MC4R  Melanocortin Receptor 4  
MR  Mineralocorticoid Receptor  
ODD  Oppositional Defiant Disorder  
PAS-scale  The Pressure-Activation-Stress scale  
PNS  Parasympathetic Nervous System  
POMC  Proopiomelanocortin  
PTSD  Posttraumatic stress disorder  
RNA  Ribonucleic acid  
SCN  Suprachiasmatic nucleus  
SNAP-IV  Swanson, Nolan and Pelham ADHD symptom rating scale  
SNP  Single Nucleotide Polymorphism  
SNS  Sympathetic Nervous System  
SSRI  Selective Serotonin Re-uptake Inhibitors
Introduction

The Hypothalamus-Pituitary-Adrenal axis (HPA-axis) with its end product cortisol plays an important role in helping the individual to adapt to psychosocial, physiological and chemical challenges. One of its main functions is to promote mobilization of energy. The secretion of cortisol also follows a diurnal rhythm with a distinct awakening response. Regulation of the HPA-axis differs among persons with certain psychiatric disorders when compared with controls (Tsigos and Chrousos, 2002). For instance, depression is associated with high levels of cortisol, possibly as an expression of impaired glucocorticoid-mediated feedback inhibition (Pariante and Lightman, 2008). A hyperactivation of the HPA-axis has also been reported for obsessive-compulsive disorder (Gustafsson et al., 2008), panic disorder (Wedekind et al., 2000; Abelson et al., 2007) and anorexia nervosa (Lo sauro et al., 2008). A decreased functioning of the HPA-axis has been associated with atypical depression (Tsigos and Chrousos, 2002), chronic fatigue syndrome (Roberts et al., 2004; Papadopulus and Cleare, 2011; Tak et al., 2011), posttraumatic stress disorder (PTSD) (Yehuda, 1998; Heim et al., 2000) and fibromyalgia (Griep et al., 1998; Riva et al., 2010). Several reports concern Attention-Deficit/Hyperactivity Disorder (ADHD). However, the results are not conclusive; both hyper- and hypo-functioning have been reported as well as no differences in comparisons with children without ADHD-symptoms. Hypothetically, a down-regulated HPA-axis in children with ADHD fits with theories that regard ADHD as a consequence of under-arousal (Fairchild, 2010). A different regulated HPA-axis could be attributed to aspects like genetic variations (Wüst et al., 2004), early adversities (Lupien et al., 2009), perceived stress (Juster et al., 2010) or medication (Janowsky et al., 1983).

ADHD

ADHD is a neurobehavioral developmental disorder with three subgroups (table 1) characterized by predominantly symptoms of inattention (ADHD-I), hyperactivity/impulsiveness (ADHD-HI) or both types, “combined” (ADHD-C). The prevalence has in a comprehensive meta-analysis been estimated to 5.9–7.1% in children/adolescents and more common among boys (Willcutt, E.G., 2012). Furthermore, the prevalence is estimated to be similar across countries and regions of the world (Willcutt, E.G., 2012).
Table 1. Diagnostic Criteria for the Attention-Deficit/Hyperactivity Disorder according to DSM-IV:

A. “Persistent pattern of inattention and/or hyperactivity-impulsivity that is more frequently displayed and is more severe than is typically observed in individuals at comparable level of development.” Individual must meet criteria for either (1) or (2):

(1) Six (or more) of the following symptoms of inattention have persisted for at least six months to a degree that is maladaptive and inconsistent with developmental level:

Inattention
(a) often fails to give close attention to details or makes careless mistakes in schoolwork, work or other activities
(b) often has difficulty sustaining attention in tasks or play activity
(c) often does not seem to listen when spoken to directly
(d) often does not follow through on instructions and fails to finish schoolwork, chores or duties in the workplace (not due to oppositional behaviour or failure to understand instructions)
(e) often has difficulty organizing tasks and activities
(f) often avoids, dislikes, or is reluctant to engage in tasks that require sustained mental effort (such as schoolwork or homework)
(g) often loses things necessary for tasks or activities (e.g., toys, school assignments, pencils, books or tools)
(h) is often easily distracted by extraneous stimuli
(i) is often forgetful in daily activities

(2) Six (or more) of the following symptoms of hyperactivity-impulsivity have persisted for at least six months to a degree that is maladaptive and inconsistent with developmental level:

Hyperactivity
(a) often fidgets with hands or feet or squirms in seat
(b) often leaves seat in classroom or in other situations in which remaining seated is expected
(c) often runs about or climbs excessively in situations in which it is inappropriate (in adolescents or adults, may be limited to subjective feelings of restlessness)
(d) often has difficulty playing or engaging in leisure activities quietly
(e) is often “on the go” or often acts as if “driven by a motor”
(f) often talks excessively

Impulsivity
(g) often blurts out answers before questions have been completed
(h) often has difficulty awaiting turn
(i) often interrupts or intrudes on others (e.g., butts into conversations or games)

B. Some hyperactive-impulsive or inattentive symptoms must have been present before age 7 years.

C. Some impairment from the symptoms is present in at least two settings (e.g., at school [or work] and at home).

D. There must be clear evidence of interference with developmentally appropriate social, academic or occupational functioning.

E. The disturbance does not occur exclusively during the course of a Pervasive Developmental Disorder, Schizophrenia, or other Psychotic Disorders and is not better accounted for by another mental disorder (e.g., Mood Disorder, Anxiety Disorder, Dissociative Disorder, or a Personality Disorder).
ADHD affects several important domains in a child’s life. Children with ADHD experience more social dysfunction (Gaub & Carlson, 1997), have fewer friends (Hoza et al., 2005), get more easily into conflicts with adults and peers (Nijmeijer et al., 2008), have a higher incident of learning problems (Loe et al., 2007) and academic underachievement since the school situation is not adapted to their special needs (Ek et al., 2011). These impairments persist into adulthood for 50%, although treatment improves the long-term outcomes (Shaw et al., 2012). ADHD is also associated with other disorders that may affect the child’s function. For instance, two out of three children with an ADHD diagnosis have one or more coexisting psychiatric diagnoses like developmental coordination disorder, oppositional defiant disorder (ODD), conduct disorder (CD), depression, anxiety disorder, bipolar disorder, tic disorders, obsessive compulsive disorder, and autism spectrum disorder (Cormier, 2008).

Treatment with stimulants like methylphenidate and amphetamine are the first-line pharmacological choice, although non-stimulant agents like atomoxetine are also approved and have shown good effects for treatment of ADHD in children (Wigal, 2009). The combination of medication and behavioural intervention (a multimodal approach) is often held as a preferred treatment and appears to be linked to the best outcome (Murray et al., 2008). Behavioural interventions often include parental training programs, school consultation and psycho-education. Parental training programs have been recommended as a first intervention for children with ADHD (Pelham & Fabiano, 2008). Furthermore, free fatty acid supplementation and artificial food colour exclusion seems to reduce ADHD symptoms while the effects of neuro-feedback and working memory training still remain unclear (Sonuga-Barke et al., 2013).

Psychological theories about ADHD

Executive function

Executive functions refer to the higher-order cognitive processes that control and organize behaviour, thereby involving planning, sequencing, reasoning, holding attention, working memory, inhibition and selection of appropriate behaviours (Johnson et al., 2009). ADHD is seen as a dysfunction involving fronto-striatal neural circuits including dopaminergic and noradrenergic systems (Johnson et al., 2009; Sonuga-Barke et al., 2010). Especially problems with response inhibition, planning and working memory are associated with ADHD (Nigg, 2005). It has been argued that response inhibition (underpinning symptoms of impulsivity) is the critical deficit in ADHD, resulting in the other executive dysfunctions (Johnson et al., 2009). However, the theory of executive functions has been criticized for not corresponding to the com-
plexity of symptoms in ADHD. Furthermore, the deficits are not found in all children with ADHD (Johnson et al., 2009). Further, the theory does not explain the high intra-individual variability of cognitive performance in children with ADHD (Skirrow et al., 2009).

Theories on arousal, activation and motivation

An alternative set of theories emphasize a more dynamic approach where the cognitive deficits in ADHD are seen as secondary to motivational or energetic systems. This is in contrast to the theory on executive functions were the deficits are seen as core cognitive deficits that are independent of context and state (Sonuga-Barke et al., 2010). Instead, the energetic state – and as a consequence, the cognitive performance – can be manipulated through e.g. using incentives and altering the speed in which the test is presented (Skirrow et al., 2009). The term energetic often refers to “arousal”, as a state of being awake and reactive to stimuli. As operationalized by Pfaff et al (2007) a higher generalized arousal involves greater responsiveness to stimuli, more voluntary motor activity and more emotional reactivity. The resulting arousal includes cerebral cortical activation, hormonal changes, autonomic alerts and activation of behaviours (Pfaff et al., 2007).

According to the state regulation theory – which is based on the cognitive energetic model of information processing efficiency by Sanders – ADHD reflects an inability to mobilize an optimal energy for a task. Although task efficiency is based on elementary cognitive stages like stimulus encoding, memory search, decision making and motor preparation, these cognitive stages are regulated by the arousal and activation processes. Arousal is defined as a phasic physiological response to a stimulus and is most relevant in an early stage of information processing, e.g. stimulus encoding (Sonuga-Barke et al., 2010). Activation is defined as a tonic long lasting readiness for action that affects motor preparation (Sonuga-Barke et al., 2010). Activation is related to the concept of sustained attention and response speed. Effort is needed to meet task demands and compensate for sub-optimal energetic states of arousal and activation. This effort allocation is strongly related to motivation and can compensate for suboptimal arousal or activation (Sonuga-Barke et al., 2010). It is hypothesized that the deficits of ADHD are foremost associated with under-activation in combination with a dysfunctional effort allocation (Sergeant, 2000; Johnson et al., 2009).

In the optimal stimulation theory (Zentall, 2005) manifestations of ADHD-symptoms are seen as expressions of increased activity which aims at promoting arousal in the individual (e.g. through shifts in attention, talking, seeking stimulation, risk-taking, aggressive behaviour, impulsiveness or novelty seeking). Even though all people need additional stimuli in certain familiar settings and when faced with boring and repetitive task, individuals with ADHD who may have a sub-optimal regulation of arousal have a stronger need of stimulation (Zentall, 2005). This reasoning is in line with
another context-dependent theory: The delay aversion theory. According to this theory individuals with ADHD seek a shorter delay and symptoms of inattention and hyperactivity are seen as attempts to reduce the subjective experience of delay (Johnson et al., 2009).

Another theory associated with ADHD is the reinforcement sensitivity theory (Bijttebier et al, 2009) which postulates two major motivational systems: The Behavioral Inhibition System (BIS) with aversive motivational functions (avoidance, extinction) in response to stimuli associated with punishment, novelty, high intensity and fear and the Behavioural Approach System (BAS) which organizes behaviour in response to appetitive stimuli like reward or relief from punishments. Later the theory was revised to include a third system: The Fight/Flight/Freeze system. ADHD has been associated with an imbalance in the motivational system with an under-active BIS as well as an over-active BAS, leading to a failure to inhibit behaviours and to increase arousal and attention (Beauchaine, 2001; Bijttebier et al, 2009). Psychophysiological research suggests that both these motivational systems are affected through the sympathetic branch of the autonomous system (Beauchaine, 2001) and it has been hypothesized that an under-active BIS may be associated with reduced cortisol levels since an active BIS increases attention and increases the arousal in order to promote inhibitory activations like avoidance and extinction of behaviours (Fairchild, 2010).

A similar theoretical construct that also emphasizes under-arousal is the theory of behaviour disinhibition (Hirshfeld-Becker et al., 2003). Behaviour disinhibition refers to a tendency to react to novelty with boldness and spontaneity, as opposite to behavioural inhibition which refers to restraint, reticence, avoidance or distress (Hirshfeld-Becker et al., 2003). Children with behaviour disinhibition are hypothesized to have a high threshold to arousal (sympathetic and limbic), which they may compensate for by novelty seeking and impulsiveness (Hirshfeld-Becker et al., 2003). ADHD has been associated with behaviour disinhibition (Hirshfeld-Becker et al., 2003; King et al., 1998). Furthermore disinhibition has been related to cortisol decrease, whereas behaviour inhibition has been associated with increased cortisol levels (Blair et al., 2004).

**Stress**

“Stress” is a complex concept often used in different ways with several connotations. One of the most widespread stress theories is the cognitive activation theory of stress. As formalized by Ursin and Eriksen (2004) it covers several aspects: the stimuli (stressors), subjective reports of an experience, a general non-specific increase in arousal and finally the feedback to the brain from the stress response. The authors argue that it is not relevant to measure the stressor since it is evaluated differently depending on the individual’s
appraisal of the situation, which is based on previous experiences of the stimulus, and expectations of the outcome. The latter may be positive (coping) or negative (the response does not make any difference [helplessness] or lead to a negative result [hopelessness]). The experience of the stress, measured by questionnaires, may be more relevant in stress research. The non-specific arousal that follows stress stimuli is regarded as uncomfortable and motivates the individual to find a solution (Ursin and Eriksen, 2004). The increase in arousal is often measured by observing neuroendocrinological activation through salivary cortisol measurements (Kristenson et al., 2012), but other measurements of physiological activation like levels of catecholamine’s, cardiovascular responses or immunological reactions may also be applied (Vanaelst et al., 2012). Finally, as a feedback to the brain, the experience of the stress response – which is measured by questionnaires like anxiety scales – may add to the feeling of being stressed (Ursin and Eriksen, 2004).

In general, the stress response is regarded as an alarm that occurs to novel situations, in situations where there is something missing, when there is a homeostatic imbalance, or where there is a threat to the organism (Levine and Ursin, 1991). Homeostasis refers to a complex dynamic equilibrium in the organism that is challenged by stressors, from within or without (Tsigos & Chrousos, 2002). Arousal and attention are stimulated during stress; cardiac output, respiration and catabolism are increased and blood flow redirected to provide fuel to the brain, heart and muscles in order to help the organism handle the stressor (Tsigos & Chrousos, 2002). These physiological reactions constitute an effective and conserved set of systems shared with other mammals and involve two main systems: The HPA-axis that is part of the endocrine system and acts by releasing cortisol (see below) and the more rapid regulating autonomic nervous system (ANS) with its two parts, the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). The SNS mobilize and accelerates the activating systems. The PNS decelerates the same systems. Actually, withdrawal of the parasympathetic activity proceeds sympathetic activation which follows a few seconds later (Porges, 2001). These immediate responses are commonly called the fight or flight response. The HPA-axis and ANS are in continuous interplay with each other. The central components of the stress systems are located in the hypothalamus, the limbic system and the brainstem (Ulrich-Lai and Herman, 2009). Information concerning physical stressors like blood loss or infection is received by the brainstem and triggers reflexive response whereas psycho-social stressors are processed in a more top-down regulation trough limbic forebrain structures like amygdala, hippocampus and prefrontal cortex (getting additional information from higher-order sensory processing and memory). Both these types of stressors activate the HPA-axis and the ANS (Ulrich-Lai and Herman, 2009).
Another term often used in stress research is “allostasis” which refers to the physiological changes that occur in response to changes in the environment and refers to the process to achieve homeostasis and promoting the organism to adapt (Karatsoreos & McEwen, 2011). An appropriate responsiveness is regarded as crucial for a sense of well being, adequate task performance and positive social interactions whereas an inappropriate responsiveness may have a negative impact on growth and development as well as on endocrine, metabolic, immunological system and may increase the risk of developing psychiatric disorders (Charmandari et al., 2005). Chronic stress may lead to a cumulative strain (allostatic load) on neuroendocrine, immune, metabolic and cardiovascular systems leading to unbalanced systems and pathophysiology (Juster et al., 2010).

Cortisol

The HPA-axis (see figure 1) with its end product cortisol is an important part of the neuroendocrine system and is foremost regarded as a stress regulating system, mediating the response to environmental changes with a peak plasma cortisol level tens of minutes after initiation (Ulrich-Lai and Herman, 2009). As a consequence, cortisol (high or low levels) is regarded as a biomarker for assessing chronic stress or allostatic load (Juster et al., 2010; Karatsoreos & McEwen, 2011). The central station of the stress system is located in the paraventricular nucleus in Hypothalamus where the corticotropin releasing hormone (CRH) and argine vasopressin (AVP) are secreted stimulating Adrenocorticotropic hormone (ACTH) from the pituitary. Circulating ACTH is the key regulator of glucocorticoid (cortisol) secretion by the adrenal cortex (Tsigos and Chronus, 2002). Cortisol then interacts with the glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) in multiple tissues. It also interacts with receptors regulating the HPA-axis, through receptors at the hypothalamus and the pituitary, where they are responsible for a feedback inhibition of the system by decreasing further stimulation of cortisol release (Pariante and Lightman, 2008). Except for the variation of cortisol levels related to stressors and feedback processes, cortisol levels also change in a circadian, pulsatile fashion, showing diurnal variation characterized by high levels of awakening, a further increase during the morning and a gradual decrease over the day until midnight (Tsigos and Chronus, 2002). The activated HPA-axis has a number of peripheral effects, like mobilizing energy stores, potentiating the release of epinephrine, increasing cardiovascular tone and inhibiting the immune system (Teicher et al., 2002; Tsigos and Chronus, 2002). It affects gonadal function and the secretion of growth hormone (Teicher et al., 2002; Tsigos and Chronus, 2002). To summarise: When the organism is challenged, the HPA-axis contributes to the adaptation by giving higher priority to the mobilization of energy and lower priority to
e.g. digestion, growth, reproduction and immune/inflammatory processes. Cortisol is also involved in certain advanced cerebral functions like acquisition of new memories and the emotional appraisal of events (Pariante and Lightman, 2008). On a more basic level it regulates neuronal survival and neurogenesis (Pariante and Lightman, 2008).

Figure 1. A simplified schematic representation of the HPA-axis and its main peripheral functions.

Cortisol is commonly measured in saliva. Saliva levels reflect the concentration of unbound free cortisol, and are highly correlated ($r = 0.89$) with serum levels (Umeda et al., 1981). Cortisol levels are often measured in association to a stressor in order to measure stress-reactivity, after administration of dexamethasone (a synthetic steroid with capacity of suppressing the secretion of ACTH) or at several sampling points in order to capture the diurnal rhythm and the deviations/slopes between sampling points, including the cortisol awakening response (Kristenson et al., 2012) which is regarded as sensitive to ongoing stressors (Vanaelst et al., 2012). Although there is a significant day-to-day variance, the variation between individuals and the within-the-day variance represent a larger portion of the total variance of cortisol levels (Van Hulle et al., 2012).

The circadian rhythm is regulated from the Suprachiasmic nucleus (SCN) located within the hypothalamus. The SCN is thought to regulate circadian rhythms through both the HPA-axis and the ANS, affecting many physiological (including the sleep/wake cycle, core body temperature, heart rate, hormone secretion) and psychological (cognitive performance, personality
and behaviour) processes (Hofstra and de Weerd, 2008; Imeraj et al., 2012). Interestingly, ADHD is associated with a disrupted circadian rhythm with optimal arousal later in the day, later sleep times, difficulties with morning awakening and delayed melatonin onset (Imeraj et al., 2012).

Hyper-/hypocortisolism

Cortisol dysregulation may in its most extreme form lead to diseases like Cushing’s syndrome with to high cortisol levels and Addison disease with to low cortisol levels. Cushing’s syndrome is associated with weight gain, depression, subjective muscle weakness, headache, osteoporosis, diabetes and hypertension (Prague et al., 2013). The incidence is estimated to 2–2.5 cases per million people a year (Lindholm et al., 2001). Addison disease is caused by adrenal cortex hypofunction with subsequent lower levels of glucocorticoids, mineralocorticoids and androgens, as well as higher levels of ACTH (Betterle and Morlin, 2011). The prevalence is estimated to 110 – 144 cases per million individuals (Betterle and Morlin, 2011). The clinical signs are fatigue, lack of energy, general malaise, joint pain, dizziness, postural hypotension etc. (Betterle and Morlin, 2011). A mild form of hypocortisolism has been associated with chronic fatigue syndrome, fibromyalgia and PTSD, where the hypocortisolism is hypothesized to increase pain perception, stress sensitivity and fatigue (Fries et al., 2005; Tak et al., 2011).

Other biomarkers for stress (and ADHD)

Catecholamine’s are involved in hormonal and SNS-related activation and are as such important biomarkers for stress (Tsigos and Chronus, 2002; Juster et al., 2010). Epinephrine increases heart rate and glucose levels as well as decreases digestive and immune functions; norepinephrine increases blood pressure, constricts blood vessels and modulates brain activity; dopamine is a neurotransmitter associated with motivation, movement, cognition as well as increasing blood pressure and heart rate. Lower levels of norepinephrine and dopamine have been associated with ADHD (Prince, 2008). Levels of cortisol is often regarded as a better biomarker for stress since catecholamine’s is more immediate and transient, more sensitive to exercise and more expensive to analyze (Vanaelst et al., 2012).

It is also common to focus on more peripheral changes of the stress response as the cardiovascular and respiratory responses (e.g. heart rate, heart rate variability, skin conductance and blood pressure). Metabolic and anthropometric markers (waist-to-hip ratio and body mass index) may also be relevant just as measures of the immune system (e.g. interleukin-6) (Juster et al., 2010). In line with theories of under-arousal, skin conductance (as a measurement of activation of sympathetic activation) seems to be reduced in children with ADHD (Barry et al., 2009). Cardiac vagal control (as a meas-
urement of the PNS) also seems to be reduced in children with non-medicated ADHD, whereas heart rate seems to be elevated (Rash et al., 2012).

Also, measurement of electroencephalography (EEG), especially an elevated Theta/Beta ratio, has been discussed as a physiological marker of ADHD. A higher Theta and a lower Beta would fit with theories of hypo-arousal in children with ADHD (Barry et al., 2003). However, it has been argued that this does not constitute a reliable diagnostic measurement of ADHD although substantial subgroups of ADHD patients had an excess Theta and Theta/Beta ratio (Arns et al., 2013). Recently The U.S. Food and Drug Administration allowed marketing of the first medical device based on brain function, investigating the theta/beta wave ratio, to help assess ADHD in children and adolescents.

Factors potentially associated with ADHD and HPA-axis functioning

Genetic variation

The genetic background of the majority of relevant DSM-IV psychiatric disorders is regarded as complex, i.e. several genes are presumed to interact in a complex manner with environmental factors to create the phenotype (Freitag et al, 2012). The genes however seem to play an important role in many psychiatric disorders with a heritability typically exceeding 50% where ADHD has one of the highest heritabilities with estimates between 60–80% (Freitag et al., 2012). Genetic factors also seem to be influential for cortisol levels, especially the morning levels (Wust et al., 2000; Bartels et al., 2003; Kupper et al., 2005) with an estimated heritability of 40–60% (Bartels et al., 2003). In a recent twin study the heritability was estimated to 31% for the morning level (30 minutes after awakening) but 0% for the evening level. Instead, shared environment accounted for 71% of the evening level (Van Hulle et al., 2012).

Variation in the genome concerning one single nucleotide (nucleotides are the components constituting deoxyribonucleic acid [DNA] and ribonucleic acid [RNA]) is called SNP (Single Nucleotide Polymorphism). It is the most common form of genetic variation (Frazer et al., 2009). Some SNP:s are parts of coding genes, which means that they may induce changes of the structure of the proteins/peptides that are controlled by the gene. This may in turn lead to altered prerequisites for physiological processes, for instance stress regulation. Studies of SNP variations have turned out to be a useful tool for studying genetic variation in different populations, also in research on ADHD (Lasky-Su et al, 2007). However, even if there is considerable evidence that genetic factors are important in the aetiology of ADHD, stud-
ies on specific genes and gene regions up to now only explain a small amount of the risk for ADHD (Freitag et al., 2012). Foremost dopamine system genes, serotonin genes and genes coding for synaptosomal associated protein have been associated with ADHD (Gizer et al., 2009). Regarding the HPA-axis, cortisol levels have been associated with polymorphisms in MR (DeRijk et al., 2011), GR, CRH, Corticotropin-releasing hormone receptor (CRHR1, CRHR2), Proopiomelanocortin (POMC), Melanocortin receptor 4 (MC4R) and Melanocortin 2 receptor (MC2-R) (Wüst et al., 2004).

To our knowledge, only two studies (Fortier et al., 2013, Kortmann et al., 2013) have explicitly investigated the association between polymorphism regulating the HPA-axis and ADHD. Kortmann et al (2013) found that carriers of the val allele of MR-I180V had higher scores of ADHD-symptoms and impairment. Contrarily, Fortier et al. (2012) found no association between the MR gene polymorphism and ADHD-symptoms, behavioural or cognitive measures in a study on children with ADHD but without a control group. However in the GR gene, ER22/23EK (G allele) was associated with social and attention problems while BcL1 K (G allele) and A3669G (G allele) were associated with errors in cognitive test.

Psychosocial adversity

Foetal psychosocial adversity (often defined as maternal exposure to negative life events or maternal psychopathology) and childhood psychosocial adversity (often defined as unfavourable family characteristics, socioeconomic disadvantage and parental psychopathology) have in a number of studies been associated with ADHD (Van den Bergh & Marcoen, 2004; Biederman & Faraone, 2005; Rodriguez & Bohlin, 2005; Talge et al., 2007; Hjern et al., 2010). Exposure to prenatal as well as early childhood psychosocial adversity has also been associated with HPA-axis hyper-functioning in children (Lupien et al., 2000; Cicchetti & Rogosch, 2001; O’Connor et al., 2005). However, different kinds of exposure to maltreatment seem to be associated with different cortisol regulation patterns (Cicchetti & Rogosch, 2001; Gunnar et al., 2009) and a down regulated HPA-axis with low diurnal cortisol levels has also been observed in association with early psychosocial adversity (Carlson & Earls, 1997; Gunnar & Vazquez, 2001; Yehuda et al., 2005; Bruce et al., 2009; Gunnar et al., 2009).

The “programming theory” (Teicher et al., 2002; Talge et al., 2007; Lupien et al., 2009) is one of the most commonly proposed mechanisms that may explain how adverse exposures develop into behavioural symptoms. This theory means that exposure to a substantial amount and/or intensity of stressors during sensitive periods of development may lead to alterations in biological systems that may in turn underlie deviating behaviour later in life. Particularly the brain regions involved in the regulation of the HPA-axis seem to be susceptible to such environmental challenges, both prenatally via
maternal glucocorticoids passing through the placenta and postnatally during childhood (Egliston et al., 2007; Lupien et al., 2009).

**Perceived stress**

Increased arousal is regarded as a response to perceived stress and salivary cortisol has routinely been used as a biomarker of psychological stress (Hellhammer et al., 2009). There are a number of studies on adults showing that ADHD-symptoms are associated with higher levels of perceived stress (Bernardi et al., 2012; Combs et al., 2012; Hirvikoski et al., 2009). Impaired stress intolerance has also been proposed to be characteristic of adult ADHD (Wender et al., 2001). Although there are no studies, to our knowledge, on perceived stress in children with ADHD, it seems probable that children with ADHD have a higher degree of exposures to stressors and thus a higher degree of perceived stress. Hypothetically, a different perception of stress may be associated with differences in cortisol levels. Most stress scales focus on adults. However, one recent developed scale, the Pressure-Activation-Stress (PAS) scale (Lindblad et al., 2008), is intended to be used foremost in children. It has a focus on the perception of stress, both in the sense of the perception of external demands and in the sense of the perception of a postulated physiological arousal.

**Medication**

Medication may affect cortisol levels as well as ADHD-symptoms. In addition to glucocorticoid treatment, medication with selective serotonin re-uptake inhibitors (SSRI) (Hinkelmann et al., 2012) and psychotropic substances (Papadopoulos & Cleare, 2011) has been associated with altered cortisol levels. Also other chemical substances, like marijuana (Ranganathan et al., 2009), cocaine (Mello, 2010), nicotine (Mello, 2010), caffeine (Lovallo et al., 2005), have been associated with altered cortisol levels. Findings on effects of ADHD-medication on cortisol levels in children with ADHD are inconclusive with reports of elevated levels with either methylphenidate or atomoxetine (Chen et al., 2012), an increase after one month of treatment with methylphenidate but then a gradual decrease towards initial levels (Wang et al., 2012) or no effect at all of methylphenidate on cortisol levels (Maayan et al., 2003; Lee et al., 2008).

**Confounders**

Age, sex, puberty and season of sampling are commonly seen as possible confounders in studies on cortisol and psychopathology (Törnhage, 2002; Rosmalen et al., 2005). Age and sex are also important to take into account in studies on ADHD since more boys than girls are diagnosed and the diagnosis varies with age (Willcutt, 2012).
Aim and scope of this thesis

The general aim was to investigate diurnal levels of saliva cortisol in school aged children (6–17 years of age) with ADHD and age matched healthy comparisons also taking early psychosocial adversity, perceived stress and ADHD-medication into consideration.

Four main research questions, one for each paper, were investigated:
- Do children with ADHD have different cortisol levels than comparisons?
- Does degree of early psychosocial adversity differ between children with ADHD and comparisons? In addition, can degree of adversity explain differences in cortisol levels?
- Can current ADHD-medication explain differences in cortisol levels?
- Does degree of rated perceived stress, as measured with the PAS-scale, differ between children with ADHD and comparisons and do PAS-scores correlate with diurnal cortisol levels?
Methods

Subjects and procedure

Children/adolescents (6–17 years of age) of the ADHD group were recruited from four child psychiatry outpatient units in three Swedish counties. The ADHD-diagnosis refers to the last assessment by the clinically responsible child psychiatrist before inclusion in the study. The ADHD-diagnoses had been based on a clinical interview, a previous physical/neurological assessment and neuropsychological testing by a clinical psychologist (choice of tests adapted for the individual needs of the child and the degree of uncertainty about the diagnosis) as well as parental and teacher symptoms ratings. Written project information was presented at a regular examination or – in one county – by mail to the parents and to the child (an age-adapted version). After written informed consent from parents (and child when ≥ 15 years of age) questionnaires about psychosocial adversity, current medication (ADHD-medication as well as any other medication) and perceived stress (children ≥ 11 years) were mailed to the family together with tubes for saliva samplings and instructions. Clarifications were given by phone. Information about diagnosis/es and symptom ratings (Swanson, Nolan and Pelham ADHD-symptom rating scale [SNAP-IV] and the Five to Fifteen [FTF] parental questionnaire, see below) was collected from the medical record. All data were re-coded and all identifying information was destroyed, thereby implementing total anonymity.

For the comparison group, children of the same ages and from schools in the same areas as the study persons were invited by mail or parental meetings, depending on the decision of the principal. In the written information we clarified that children with verified or suspected ADHD should not participate. When the informed consent from parents (and child when ≥ 15 years of age) was returned, the following material was mailed to the family: questionnaires about psychosocial adversity, current medication, perceived stress (children ≥ 11 years), SNAP-IV, tubes for saliva sampling and sampling instructions. Clarifications were given by phone. The data were made anonymous as described above. Figure 2 outlines the inclusion and exclusion of participants with respect to the different manuscripts.

Children in the non-medicated pre-clinical group were recruited from community teams – specialized at early identification of children with neuropsychiatric disorders – which they had been referred to for child neuropsy-
Psychiatric assessment due to suspicions of ADHD. After written informed consent from parents (and child when ≥ 15 years of age) questionnaires about psychosocial adversity and current medication (ADHD-medication as well as any other medication) were mailed to the family together with tubes for saliva samplings and instructions. SNAP-IV was collected from the community teams. Clarifications were given by phone and the data were made anonymous as described above.

Figure 2. Chart illustrating the inclusion of participants in the sub-studies.

<table>
<thead>
<tr>
<th>ADHD group</th>
<th>Pre-clinical group</th>
<th>Comparison group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recruited from four child psychiatry outpatient units</td>
<td>Recruited from community teams – specialized at early identification of ADHD</td>
<td>Recruited from schools</td>
</tr>
<tr>
<td>Participation rate: 30%; n = 209</td>
<td>Participation rate: 36%; n = 28</td>
<td>Participation rate: 27%; n = 231</td>
</tr>
</tbody>
</table>

Paper 1. ADHD & cortisol
- 8 children excluded due to saliva sampling on a non-school day. Included n = 201

Paper 2. ADHD & adversity
- 4 children excluded since adversity Questionnaires were not returned. Included n = 197
- Included n = 221

Paper 3. ADHD-medication & Cortisol
- Included 147 children on methylphenidate and 21 children on atomoxetine
- Not on ADHD-medication. Included n = 20
- PAS-scores were available in 102/130 children ≥ 11 years of age

Paper 4. ADHD & perceived stress
- PAS-scores were available in 146/150 children ≥ 11 years of age
- The recruitment of participants in the ADHD group was started before the PAS-questionnaire was included in the study design.
Questionnaires

Symptom questionnaires

Parental ratings on SNAP-IV were used for estimating the severity of ADHD-symptoms and for excluding comparisons with high ADHD-ratings (Swanson et al., 2001). Since the clinical diagnoses were ICD-based, the SNAP-IV ratings were also used for identifying DSM-related subtypes of ADHD (ADHD-I, ADHD-HI or ADHD-C). The version applied has 30 items (9 for inattention, 9 for hyperactivity/impulsivity, 8 for ODD and 4 control questions), scored by parents on a 4-point scale: 0 for “not at all”, 1 for “just a little”, 2 for “quite a bit“ and 3 for ”very much“.

Parental ratings on the FTF questionnaire were applied for identifying co-occurring symptoms in the ADHD group only. This instrument has been developed in the Nordic countries and covers concomitant problems of ADHD (Kadesjö et al., 2004). It comprises 181 three-graded statements, arranged into domains and sub-domains. In paper 1, we selected data from sub-domains reflecting symptoms related to psychiatric disorders with an association to HPA-axis dysfunction: social skills (autism spectrum, 27 items), obsessive-compulsive symptoms (8 items), internalizing emotional problems (depression/anxiety, 12 items) and externalizing emotional problems (ODD/CD, 13 items).

Psychosocial adversity

The core of the items of the maternal questionnaire about foetal psychosocial adversity were the same as in a prospective study by Rosengren et al (1993) – reformulated to be used retrospectively – but the response alternatives (yes/no) were from a later adapted version of the same scale (Rodriguez and Waldenstrom, 2008). The 11 questions concern experiences during pregnancy of separation/divorce; relocation; change of employment; unemployment; insecurity at work; severe financial problems; legal sanctions; severe illness or accident (related to oneself or someone close); worries about someone close; death of someone close.

The parental questionnaire about childhood psychosocial adversity during the first six years of life was based on general trauma items of The Early Trauma Inventory - Self Report (ETI-SR) Short Form (Bremner et al., 2007). This questionnaire has been confirmed to have good validity and reliability in another study on assessing childhood trauma in post partum women (Plaza et al., 2011). The choice of using yes/no-items was made according to the author’s experiences with this scale (Plaza et al., 2011). Our original plan was to recruit patients during the – sometimes – critical referral phase (this approach was later changed due to practical obstacles). In order to avoid any negative influence on the referral process we reduced number of items to
nine concerning accidents; natural disasters; own, parental, siblings’ or friends’ severe injury/illness; parental psychiatric illness; substance abuse in the home environment; having witnessed violence.

Self-perceived stress.

For measurement of perceived stress we used the PAS-scale (Lindblad et al., 2008). The PAS-scale focuses on two stress dimensions, “pressure” (too many things at the same time, not time enough, feel under pressure from school demands / demands at home / inner demands, feel helpless, never feel really free) and “activation” (rush, eat rapidly, keep a high speed all the day, difficult to relax). It has a condensed format (11 items) on a 5-point scale: 0 for “never”, 1 for “rarely”, 2 for “sometimes”, 3 for “often” and 4 for “always”. It is based on a theoretically specific stress concept and is formulated to be as age and culture independent as possible. It has been created from a larger battery that was tested in a representative sample of 1124 Swedish adolescents, 11–16 years of age. The PAS-scale has been psychometrically tested in two studies on Swedish samples (Lindblad et al., 2008, Wiklund et al., 2012).

Cortisol analyses

Cortisol was analyzed in saliva and expressed in nmol/L. Sampling was performed at home during one ordinary weekday (a school-day) when waking up, 30 minutes after waking up, at 4 PM or when coming home from school and when going to bed. Information was given that the sampling should be done before brushing teeth, at least 30 minutes after eating or drinking and at least one hour after sport activity. The families filled in the sampling times immediately after completing saliva sampling. Samples were collected by swabs (Salivette; Sarstedt Inc., Rommelsdorf, Germany), which the participants kept in their mouth 1–2 minutes until soaked with saliva. The swabs were placed in plastic sampling tubes. The tubes were centrifuged and stored at minus 70 centigrade’s until analyzed with radioimmunoassay technique using the Spectria Cortisol (125I) kit from Orion Diagnostica, Espoo, Finland (Hansen et al., 2003).

Statistics

All analyses were performed with the statistical package for the social sciences (SPSS). As saliva cortisol showed a skewed distribution we used non-parametric statistical analysis, as well as parametric analysis of log-transformation of the saliva cortisol values, for calculation of group differ-
ences. We used linear regression models in order to investigate the relation between ADHD and cortisol (log. transformed), adjusting for the factors: age, sex, sampling time, sampling season, perceived stress, psychosocial adversity and ODD-symptoms. We also used logistic regression to calculate the odds ratio of belonging to the ADHD group based on exposure to psychosocial adversity, sex, age (years) and awakening cortisol levels. Spearman $R$ was used for correlation calculations, except in paper 2 where Pearson $R$ was used with log transformed cortisol levels. In paper 1, we split the groups into different age categories (7–10, 11–13 and 14–17 years of age) in order to investigate a presumed influence of age on cortisol levels. In paper 2, we used $\chi^2$ to calculate group differences for exposure to adversity. As a consequence of the low internal consistency of the psychosocial adversity questionnaires and with the intention of facilitating the interpretation of the results, exposures to adversity was transformed into categorical variables by severity. Interaction effects between sex and group belongingness on exposures to adversity was calculated with a general linear model. In paper 3, we compared differences in cortisol levels between the groups with a general linear model where the ADHD/Non-Med group constituted reference. Since the groups sizes were unequal in paper 3, we also performed a Sheffé Post-Hoc test. In paper 4, we used a linear regression model in order to investigate the relation between ADHD and PAS-score, adjusting for age, sex and ADHD-medication. We also calculated a ratio by dividing cortisol levels for each sampling occasion with PAS-scores. Two tailed tests with $p$ values < .05 were considered significant.

**Ethics**

The study was approved by the Regional Ethical Review Board in Uppsala, no. 2009/034 and was performed in accordance with the latest version of the Declaration of Helsinki. Informed consent of all participants was obtained after the nature of the procedures had been fully explained. All data were re-coded and all identifying information was destroyed, thereby implementing total anonymity.
Results

Paper I. ADHD and cortisol levels

Children with ADHD had significantly lower saliva cortisol levels at awakening ($p < .001$), 30 minutes later ($p < .001$) and before going to bed ($p = .015$) than non-affected comparisons (figure 3). Sex had no influence on cortisol levels. Age however had an influence on cortisol levels; when splitting the group into different age categories only children above 10 years of age had lower levels than healthy comparisons (figure 4). There was a trend that the morning increase was lower in the ADHD group ($p = .058$). In a multivariate linear regression model (adjusted for sex, age, season and sampling time in relation to cortisol log scale data) belonging to the ADHD group predicted lower cortisol levels at waking up ($b = -.245, p < .001$) and 30 minutes later ($b = -.197, p = .001$). Within the ADHD group, subtype of ADHD or co-occurring symptoms did not affect the cortisol levels. The degree of severity of ADHD-symptoms was not associated with cortisol levels in the ADHD group, except for a weak negative correlation between the afternoon sample and hyperactivity symptoms.

Figure 3. Medians of cortisol levels (nmol/L) on the four sampling occasions in the ADHD group (201 children, 6–17 years of age, with ADHD-symptoms) and non-affected comparisons of the same ages (221 children).
Figure 4. Median of cortisol levels (nmol/L) on the four sampling occasions (morning, 30 minutes later, afternoon, bedtime) split into age categories: 7–10 (at most 68 individuals in the ADHD group and 67 in non-affected comparisons), 11–13 (at most 63 in the ADHD group and 61 in non-affected comparisons) and 14–17 (at most 64 in the ADHD group and 79 in non-affected comparisons).

Paper 2. ADHD and early psychosocial adversity

Children with ADHD (n = 197) had to a higher degree been exposed to foetal (55% and 44%, respectively; \( p = .041 \)) and childhood (50% and 33%, respectively; \( p < .001 \)) psychosocial adversity than comparisons (n = 221). Reports of at least one foetal or childhood psychosocial adversity did not differ between subgroups of ADHD. More than one exposure of foetal and/or childhood psychosocial adversity, as well as lower awakening cortisol levels and male sex, were predictors of belonging to the ADHD group (table 2).

Girls in the ADHD group had higher reported rates than female comparisons of at least one psychosocial adversity during foetal period and childhood, whereas boys in the ADHD group had higher reported rates of at least one adversity during childhood – but not during foetal period – compared with male comparisons. There was a significant interaction effect between sex and group belongingness for foetal adversity (\( f = 5.21; p = .023 \)) but not for childhood adversity.
Since exposure to foetal and childhood psychosocial adversity has been associated with both ADHD and HPA-axis functioning, such exposures would theoretically explain these low cortisol levels in ADHD via early programming of the HPA-axis. However, in a multivariate linear regression, the association between low morning cortisol levels and ADHD-symptoms remained when adjusted for foetal and childhood psychosocial adversity as well as for sex, age, sampling time and ODD-symptoms. No relation was found between exposures to psychosocial adversity and diurnal cortisol levels on any sampling point, but there was a positive correlation between childhood adversity and the cortisol morning increase in children with ADHD.

Table 2. Logistic regression of the association between foetal/childhood psychosocial adversity (no adversity constitutes reference), age, awakening cortisol level and sex (female constitutes reference) in relation to belongingness to the ADHD group, with odds ratios, p-values and Nagelkerke R square.

<table>
<thead>
<tr>
<th></th>
<th>OR (CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Foetal adversity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Little (one)</td>
<td>0.9 (.46–1.77)</td>
<td>ns</td>
</tr>
<tr>
<td>Several (&gt; 1)</td>
<td>2.03 (1.04–3.99)</td>
<td>.039</td>
</tr>
<tr>
<td><strong>Childhood adversity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Little (one)</td>
<td>0.99 (.52–1.86)</td>
<td>ns</td>
</tr>
<tr>
<td>Several (&gt; 1)</td>
<td>3.77 (1.57–9.05)</td>
<td>.003</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>1.01 (.91–1.13)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Cortisol</strong></td>
<td>5.60 (2.30–13.64)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5.70 (3.24–10.06)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td><strong>Model R²</strong></td>
<td></td>
<td>.32</td>
</tr>
</tbody>
</table>

Paper 3. ADHD-medication and cortisol levels

Medians of cortisol levels in the three groups are presented in figure 5. Children with atomoxetine medication had – in a general linear model adjusted for age, sex, sampling time and symptom scores – higher cortisol levels at bedtime than non-medicated ADHD-comparisons \((b = 0.473; p = 0.031)\). This association remained significant in a Scheffe Post-Hoc analysis \((p = \)
There was also a trend towards higher cortisol levels 30 minutes after awakening in children on stimulants ($b = 0.129; p = 0.074$) compared to non-medicated.

Figure 5. Medians of cortisol levels (nmol/L) on the four sampling occasions, in children with ADHD not on medication (ADHD/Non-Med; n = 20), on stimulant medication (ADHD/Stim; n = 147) and on atomoxetine (ADHD/Atox; n = 21).

**Paper 4. ADHD and perceived stress**

Children with ADHD scored higher ($p < .001$) on the global PAS-scale and on the sub-dimensions of perceived pressure ($p < .001$) and activation ($p < .001$) than the comparison group (figure 6). The PAS-scores were similar over ages in the ADHD group while they increased with age in the healthy group. Female sex was associated with higher stress in both groups. There was no difference on PAS-scores between subgroups of ADHD.

No association was found between PAS-scores and cortisol levels on any sampling occasion in neither group. Children in the ADHD group had a lower ratio of cortisol levels/perceived stress than the school sample on all sampling occasions. This lower ratio was built up both by the higher PAS-scores and the lower cortisol levels in children with ADHD.
Figure 6. Mean scores (never = 0, rarely = 1, sometimes = 2, often = 3, always = 4) of perceived everyday stress in the ADHD group (n = 102) and comparison group (n = 146), also split in the two stress dimensions. Differences marked (Mann-Whitney: ***$P < .001$). Confidence intervals.
Discussion

The main aim of the present thesis was to investigate diurnal levels of saliva cortisol in school aged children with ADHD and age matched healthy comparisons also taking early adversity, perceived stress and ADHD-medication into consideration. The results suggest a down-regulated HPA-axis with lower cortisol levels in children with ADHD, especially the morning levels. These lower levels could not be explained by the higher exposure to psychosocial adversities, current ADHD-medication or the higher degree of perceived stress in children with ADHD. The main findings and implication are discussed in more detail below.

Paper 1. ADHD and cortisol levels

Hypothetically, three different explanations to the low cortisol levels in the children with ADHD can be identified. First, they may indicate a displaced diurnal curve, meaning that maximum levels either have been passed or not reached at the time of the morning peak of the comparisons. A delayed morning peak would explain the low morning levels in our study but not the low evening levels. The hypothesis of a displacement of the diurnal curve needs future consideration. Second, the low levels may be genetically influenced. There is considerable evidence that genetic factors are important in the aetiology of ADHD (Freitag et al., 2012) and as already stated the morning cortisol levels seem to be more strongly influenced by genetic factors than afternoon and evening levels (Wust et al., 2000; Bartels et al., 2003; Kupper et al., 2005). Third, given the association between psychosocial adversity and ADHD (Biederman et al., 1995; Biederman and Faraone, 2005), one may alternatively – or complementarily – hypothesize an altered programming of the HPA-axis which is characterized by increased plasticity prenatally and during childhood (Maccari and Morley-Fletcher, 2007).

Another aspect to discuss is the role and consequence of these lower levels. Theoretically, the low cortisol levels fit with theories that regard ADHD as secondary to deficits of under-arousal, under-activation and aspects of motivation; according to the state regulation theory, ADHD reflects an inability to mobilize an optimal energy for a task (Sonuga-Barke et al., 2010). Symptoms of ADHD – like fluctuation in cognitive performance, slower response time and difficulties with sustained attention – might be a conse-
sequence of a low and sun-optimal arousal, with an altered ability to manage and respond to stimuli. Theories on under-arousal could also contribute to other findings associated with ADHD. For instance, the beneficial effects of incentives on performance (Rosch & Hawk, 2013) indicate that children with ADHD are in need of more motivational incentives to maintain an optimal energetic state. In healthy individuals, fast conditions induce over-arousal whereas slow conditions induce under-arousal. Children with ADHD are more sensitive to the speed in which the task is presented, especially when the task is presented at a slow speed (Sergeant, 2000). Probably, children with ADHD are more easily manipulated into under-arousal due to their lower state regulation. According to the optimal stimulation theory (Zentall, 2005) and the delay aversion theory (Johnson et al., 2009) symptoms of ADHD, like hyperactivity and being easily distracted, are regarded as expressions of attempts to increase arousal and reduce subjective experience of delay.

Paper 2. ADHD and early psychosocial adversity

The high exposure of adversities in the ADHD group is in line with previous studies (Biederman and Faraone, 2005; Talge et al., 2007) and may hypothetically reflect a causal link. If so, an underlying mechanism could be early programming of the HPA-axis (Teicher et al., 2002; Talge et al., 2007; Lupien et al., 2009). However, the hypothesis that early psychosocial adversity (prenatal or postnatal) may have a programming effect on the HPA-axis, leading to a hyper- or hypocortisolism preceding psychiatric symptoms like ADHD, was not supported by our findings since there was no relation between adversity and cortisol levels. This is in line with previous studies on the relation between cortisol, externalized behaviour and psychosocial adversity (Murray-Close et al, 2008; Hawes et al., 2009) and in a human research program aimed at testing each step of the programming theory (Sarkar et al., 2008; Bergman et al, 2010; O’Connor et al., 2012).

The higher rates of exposures to foetal and childhood psychosocial adversity among children with ADHD may also reflect a common genetic background where some of the adversities may be seen as direct or indirect expressions of parental psychological and psychiatric symptoms. HPA-axis functioning – expressed as cortisol levels – may act as an independent variable, thereby rather suggesting a genetic background. Previous findings from our group (Isaksson et al., 2012) that foremost the morning cortisol levels are lower in the ADHD group are in line with a genetic explanation of the hypocortisolism in children with ADHD. Also, the lack of relation between the ratings on psychosocial adversity and cortisol levels gives support for a genetic explanation.
Paper 3. ADHD-medication and cortisol levels

Continuous medication with stimulants or atomoxetine did not explain the low cortisol levels in children with ADHD. Possibly, medication may rather increase the levels. Other studies have shown that the dopamine release after stimulant administration correlates positively with a simultaneous cortisol increase (Oswald et al., 2005) and that glucocorticoids may enhance the effects of dopamine in the meso-limbic system, thereby contributing to the efficiency of the reward system and facilitating coping (Marinelli and Pizazza, 2002). Such types of interplay between cortisol and neurotransmitters may constitute fine-tuned regulatory systems for adaptive procedures (Van Craenbroeck et al., 2005). One may thus speculate that the low levels of cortisol in children with ADHD may influence such processes. The interplay between the HPA-axis and neurotransmitters may be a fruitful approach for understanding the development of ADHD-symptoms.

Paper 4. ADHD and perceived stress

The PAS-questionnaire showed a capacity to differentiate between children with or without ADHD which adds to the data on the validity of the scale. The higher scores on PAS for girls are in line with previous studies on gender differences in Swedish adolescents (Schraml et al., 2011; Friberg et al., 2012; Wiklund et al., 2012). Although we did not find any interaction between sex and group, it is noteworthy that girls with ADHD reported very high stress scores. Scores on the PAS-questionnaire did not correlate with cortisol levels. The intention to validate a stress questionnaire against biological markers for stress has been discussed and tested in other studies with mixed results (Osika et al., 2007). Studies on cortisol in relation to various psychological conditions often show opposing and ambiguous results (Kristenson et al., 2012). This is probably due to the complexity of the cortisol response. Even though this lack of association may seem surprising it may actually be adaptive since a loose association between systems promotes consistency and adaptability since the ability to respond to different types of challenges is enhanced.

The lack of relation between our measure of self-perceived stress and cortisol levels does not exclude that exposure to stressors may have contributed to the low cortisol levels; the low cortisol levels in ADHD may still reflect an exhaustion of the HPA-axis where repeated or chronic stressors may result in a hypo-activation of the HPA-axis. Interestingly, the typical increase of perceived stress with age observed in most studies in this field was evident in the school sample but not in the ADHD-group. This suggests that children with ADHD perceive high levels of stress from early years which, in turn, gives some support to the hypothesis of an early exhaustion of the
HPA-axis as an explanation of the low cortisol levels. Furthermore, the differences in cortisol levels between the groups increase with age with markedly higher levels among comparisons.

The low cortisol levels in children with ADHD may alternatively – or complementarily – represent an impaired ability (that may be inherited) to respond to changes in the environment by physiological arousal. Given the complexity of the stress concept it seems necessary to expand studies to include several measurements reflecting various facets of the stress model, including a specific stressor and investigating autonomic responses. We believe that the strength of the PAS-scale lies within its specific stress concept, free from references to specific stressors and with no items reflecting psychiatric symptoms, which opens up for more fine-tuned analyses of “stress” in relation to e.g. psychiatric symptoms and disorders.

General limitations and methodological considerations

The response rate to participate in the study was 27–36%. This could hypothetically induce bias. However, the participating rate was similar in all groups. Perhaps it indicates that the procedure with collecting biological samples and completing several questionnaires was considered too demanding for many families. There is also a risk of recall bias in case-control studies where the disease has already occurred when exposure information is obtained, as in the reports of early psychosocial adversities. For instance, parents may be prone to over-report when seeking for an explanation of the disease or when they have an assumption about its underlying cause (Infante-Rivard and Jacques, 2000).

It was not possible to implement uniform diagnostic interviews for the inclusion of study persons. Still we regard the diagnostic procedure as satisfactory, given the fact that the children recruited from child psychiatry had undergone investigations at specialized teams with the exception of the preclinically recruited children in the ADHD/Non-Med-group (n = 21) who had only been evaluated by parental questionnaires. There were more boys than girls in the ADHD group, which is congruent with the results from a vast majority of previous studies concerning ADHD and gender. Based on this imbalance, we included sex (as well as age and sampling time) as a factor in the regressions.

For optimal cortisol measurement it is advisable to collect saliva on more than one day also assessing participant’s adherence (Clow et al., 2010). However, we wanted the sampling to be done on a regular school day since the day of cortisol assessment is crucial in psychoendocrinological stress studies (Schlotz et al., 2004) and we assumed that the procedure with numerous collecting points would be too demanding and might increase attrition. Also with the aim of avoiding a “too demanding” approach we re-
frained from making an evaluation of pubertal development. Instead age was used as a coarse proxy of pubertal stage.

Other questionnaires could have been chosen that might have produced different results. For instance, other types of stressors like exposure to sexual, physical and emotional abuse would have had a measurable impact on the cortisol levels. Our instruments on psychosocial adversities focused on “classical” traumatic experiences like accidents, illness or violence, just as the LITE-questionnaire (Greenwald and Rubin, 1999). Taking potentially stressful situations from other sectors of life into consideration – like the Coddington questionnaire (Coddington, 1972) with its broader and more extensive life event approach – had been an alternative. Instead of the PAS-questionnaire we could have chosen the most commonly used scale to measure perceived stress – the Perceived Stress Scale (Cohen et al., 1983) – with items designed to investigate how unpredictable, uncontrollable, and over-loaded respondents find their lives. The Perceived Stress Scale is however better adapted for adults. Furthermore, it focuses more on adaptive processes. An alternative is parental reporting on the child’s stress but this is considered less reliable (Vanaelst et al., 2012).

It is a delicate matter to choose statistical methods in a study where the outcome measures are on a skewed ordinal or interval scale. The procedure with complementary statistical approaches (using parametric as well as non-parametric methods) can help to overcome shortcomings with the individual statistical methods and help to eliminate scaling artifacts.

Implications and future research

Low morning cortisol levels stand out as a biomarker for ADHD. However, it can’t be considered a reliable diagnostic measure of ADHD – due to the wide span and overlap of cortisol data. Rather, the results contribute to a more comprehensive understanding of the core symptoms of ADHD and generate hypotheses about the pathogenesis, offering support to theories of energetic under-arousal. A dysregulation of the HPA-axis seems to be involved in several psychiatric disorders. This may not seem surprising given that the HPA-axis is a major part of the neuroendocrine system involved in several bodily systems and central to our stress regulation and adaptation to environmental changes. Even if foremost higher cortisol levels are associated with negative health aspects, lower levels are not necessarily positive, especially when taken into consideration the higher exposure to stressors and the higher rated perceived stress in children with ADHD. Rather, the lower levels indicate a stress regulating system that lacks flexibility and resilience. In line with this, lower levels of cortisol may affect the regulation of arousal and attention which are part of the symptoms of ADHD, as well as regulation of bodily functions like growth, metabolism and the immune system.
Our findings may also stimulate new hypotheses on therapeutic intervention. Coping with stressors stands out as a life long struggle for quite a few individuals with ADHD. Especially girls with ADHD reported very high stress scores. The need for psychosocial intervention directed towards adapting the school environment and increasing coping strategies, which already is regarded as an important part of the treatment for ADHD, is confirmed. Hypothetically – from a pharmacological perspective – synthetic corticosteroids may improve ADHD symptoms by replacing the cortisol deficiency. Hydrocortisone treatment has been proposed as a treatment for chronic fatigue syndrome (Cleare et al., 2001), another disease associated with hypocortisolism.

Since both ADHD and morning cortisol levels are considered heritable a necessary next step would be to investigate if a combination of ADHD symptoms and cortisol levels constitutes a phenotype that is associated with genetic variation. If so, it could be of great importance for the classification, aetiology and treatment of the disorder.

Given the complexity of the stress regulation it seems necessary to expand studies to also involve a specific stressor and measurements reflecting various facets of the stress model, especially taking into account the other main stress regulating axis – the ANS. By measuring the variation between the heartbeats (heart rate variability) as a measurement of ANS in combination with cortisol levels, a clearer and more comprehensive understanding may emerge.

To even more emphasize the complexity of the stress regulation in general and the HPA-axis in specific, it would be interesting to investigate the sensitivity in cells and target issues for cortisol. For instance, it has been argued that chronic stress results in GR resistance (Cohen et al., 2012). This resistance to cortisol rather than absolute cortisol levels may be the contributing factor to increase health risk in chronic stress. One hypothesis is that due to insensitivity to cortisol in cells and tissues the inflammatory responses are not shut down (Cohen et al., 2012). A dysregulation of the HPA-axis may thus be caused by dysregulation at several levels, e.g. the hypothalamus with secretion of CRH and AVP, at the pituitary with the CRH receptors and the secretion of ACTH, at the adrenal cortex with the ACTH receptor (MC2-R) or production of cortisol, at the corticoid receptors or in the metabolism of cortisol. The dysregulation may also be an indication of a displaced diurnal curve with a delayed morning peak. In line with this, there is some support for a delayed sleep phase syndrome in children with ADHD (Imeraj et al., 2012). The hypothesis of a displacement of the diurnal curve needs further investigation, including measurements of the cortisol awakening response over a longer period of time.

More information is needed on the prognostic value of hypocortisolism in children with ADHD. Theoretically, lower cortisol levels may affect responsiveness to ADHD-medication and the ability to respond to stressors. In this
context studies measuring cortisol levels before and after start of medication seem urgent. To further explore the causality – what comes first, a dysregulated HPA-axis or ADHD – longitudinal research would be informative. It would also be of interest to investigate cortisol levels in adults.

We may also speculate that the lower cortisol levels may be associated with diet. A western-style diet – high in fat, saturated fat, refined sugar and sodium; deficient in omega-3, fatty acids, fiber and folate – has been associated with ADHD (Howard et al., 2011). Cortisol has a metabolic role and is one of the hormones that prevent or correct hypoglycaemia with the main aim of protecting the requirements of the brain. Hypothetically, the low cortisol levels attributed to ADHD may be paralleled by low – or variable – blood glucose levels. These may prompt a diet of high glycaemic index. The lower glucose levels may also influence cognitive function negatively and contribute to the hypothesized under-arousal in ADHD. It would be interesting to incorporate information about diet when studying cortisol levels in children with ADHD.
Conclusions

- Children with ADHD had lower diurnal levels of cortisol than comparisons. This implicates a dysregulation of the HPA-axis, which is involved in several physiological systems and central to stress regulation.
- Even though children with ADHD to a higher degree had been exposed to foetal and childhood psychosocial adversity than comparisons, these exposures could not explain the low cortisol levels via programming effects.
- ADHD-medication may increase cortisol levels and there is no support for the hypothesis that the lower cortisol levels in children with ADHD are due to ADHD-medication.
- Children with ADHD scored higher on the Pressure-Activation-Stress (PAS) scale than comparisons. The higher rates on perceived stress for children with ADHD contrast the lower levels of cortisol, indicating a stress related fragility. Girls with ADHD had the highest PAS-scores.
Sammanfattning på svenska

Bakgrund och frågeställning

Metod
åren, upplevd stress (om barnet ≥ 11 år), aktuell medicinering, ADHD-
symtomskattning (enbart skickat till kontroller; symtomskattningar och dia-
gnos från barn i ADHD gruppen hämtades från barnets journal medan sym-
tomskattningar från den prekliniska gruppen hämtades från VITS-
samordnaren) skickades till familjerna med brev. Vidare skickades fyra rör
med bomullstussar för insamling av saliv under dagen (för vidare analys av
kortisolnivåer) och instruktioner. Alla familjer fick även information per
telefon. Alla forskningsuppgifter anonymiserades.

Resultat

Barn med ADHD hade lägre nivåer av kortisol vid uppvaknandet, 30-
minuter senare och vid läggdags jämftört med en kontrollgrupp. Skillnaden
mellan grupperna förelåg dock enbart hos barn över 10 år. Könstillhörighet
påverkade inte kortisolnivåer. Inte heller subgrupperingar av ADHD eller
förekomst av trotssyndrom påverkade kortisolnivåer inom ADHD-gruppen.
Föräldrar till barn med ADHD skattade fler negativa psykosociala livshän-
delser under graviditeten och de första sex åren än föräldrar till jämförelse-
gruppen. Även om barn med ADHD hade fler livshändelser av dessa slag så
kunde dessa inte förklara de lägre kortisolnivåerna. Inte heller ADHD-
medicinering kunde förklara de lägre nivåerna; Snarare tyder resultaten på
att medicinering med metylfenidat eller atomoxetin kan resultera i höjda
cortisolnivåer.

Barn med ADHD skattade högre än jämförelsegruppen på en skala som
mäter upplevd stress hos skolbarn från 11 år (Pressure-Activation-Stress
scale). En hög grad av upplevd stress förekom tidigt hos barn med ADHD
medan den ökade med åldern hos barn i jämförelsegruppen. Flickor uppgav
högre upplevd stress än pojkar. Det fanns ingen association mellan upplevd
stress och kortisolnivåer. Överlag hade barn med ADHD lägre kortisolnivåer
i förhållande till skattad stress, något som förklaras både av de högre stress-
skattningarna och de lägre kortisolnivåerna.

Slutsatser

Resultaten tyder på en nedreglerad eller förskjuten HPA-axel med lägre kor-
tisolnivåer hos barn med ADHD, framförallt på morgonen. Vidare har barn
med ADHD varit utsatta för fler negativa livshändelser och beskriver en
högre självupplevd stress än en jämnårig kontrollgrupp. De högre skattning-
arna av upplevd stress i kombination med de lägre kortisolnivåerna tyder på
en stressrelaterad känslighet. Detta kan vara uttryck för att HPA-axeln ut-
mattats, alternativt uttryck för en medfödd brist att möta krav/utmaningar på
ett fysiologiskt adaptivt sätt. Att ett så centralt system som HPA-axeln, vilket
påverkar vår förmåga att möta och hantera utmaningar, är påverkat vid ADHD är anmärkningsvärt och öppnar upp för nya frågor och en ökad förståelse av funktionsnedsättningen.
Acknowledgements

The study was carried out at the Department of Neuroscience, child and adolescent psychiatry, Uppsala University. The study was supported by grants from Victoriafonden through the Swedish Brain Foundation, (Hjärnfonden), (no specific grant no.). My PhD position was supported by Uppsala University Hospital Research Fund (ALF), (no specific grant no.).

I am grateful to all children who volunteered and to their parents and schools in Uppsala, Enköping, Gävle and Falun who made it possible for us to recruit the children. I am also grateful to the staff at the child and adolescent psychiatric units in Uppsala, Enköping, Gävle and Falun as well as to the community teams (VITS) of Uppsala County for contributions to the recruitment procedure.

I would like to express my gratitude to all people who have contributed and supported the project. Especially thanks to:

Frank Lindblad, my principal supervisor, for initiating the project, your excellent tutoring and never ending support. I am pleased to announce that thanks to your enthusiasm not only my scientific thinking has improved, but also my speed and endurance as a runner.

Kent Nilsson, my supervisor, for great collaboration, always taking your time to explain the statistics and so enthusiastically sharing your knowledge in research and psychiatry.

Åsa Hogmark, co-author, for introducing me to the recruitment process, for proofreading and being my Wikipedia.

Fred Nyberg, co-author, for contributing to the first paper.

Hans Arinell, statistician, for always taking time to answer my questions and supporting me into doing my own calculations.

Lars Holmberg, for analyzing cortisol in the saliva samples. Thanks for your thorough work.
My wife Pearl for proofreading.

Students (in medicine and psychology) and residents that have been involved in the project: Martin Fridholm, Andreas Grönberg, Onome Eghaga, Josefine Hübinette, Hilke Sievers and Eva Berntsson.

Berit Hård-Wallenqvist, for helping me with the administrative issues.

To my family for your support.
References


A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)