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Hypocortisolism in recurrent affective disorders

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To Emma, Moa and Linda

En droppe droppad i Livets älv
har ingen kraft att flyta själv
Det ställs ett krav på varje droppe
Hjälp till att hålla de andra oppe!

Tage Danielsson

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ABSTRACT

Bipolar disorders and recurrent depressions are two common psychiatric disorders with a life time prevalence of approximately 1% and 8%, respectively. Despite treatment these patients suffer from affective symptoms up to 50% of the time, resulting in lower well-being. The average life length is also reduced with 10-15 years, mainly attributable to suicide and cardiovascular disease. Increased stress is one of many factors that have been shown to be linked to an increased risk for developing affective disorders and some comorbid somatic conditions such as metabolic disturbances and cardiovascular disease. An increased stress level is known to cause hyperactivity of the hypothalamic-pituitary-adrenal-axis (HPA-axis) with increased cortisol secretion. Hyperactivity of the HPA-axis (or hypercortisolism) is one of the most replicated neurobiological finding in depression. In other stress related disorders it has however been shown that prolonged stress over long periods of time can lead to a state of low HPA-axis activity, hypocortisolism. Since persons with recurrent affective disorders such as bipolar disorder and recurrent depression are exposed to a high degree of recurrent and chronic stress it could be expected that in addition to hypercortisolism, a state of hypocortisolism could also develop in these disorders, potentially exerting an influence upon the psychological and somatic wellbeing among these patients.

The major aim of this thesis was to evaluate whether hypocortisolism is related to relevant psychiatric and somatic phenotypes in recurrent affective disorders.

In bipolar disorder, individuals with hypocortisolism exhibited a higher degree of depression and low quality of life compared to patients with normal HPA-axis activity. In recurrent depression, individuals with hypocortisolism exhibited shorter leukocyte telomere length than patients with normal or high HPA-axis activity, which is an indication of an accelerated aging process. In a sample of both bipolar and recurrent depression patients, hypocortisolism was associated with an increased proportion of obesity, dyslipidemia and metabolic syndrome compared with patients with normal or high HPA-axis activity. Patients with recurrent depression showed a higher occurrence of hypocortisolism than the control sample representative of the general population. Patients with bipolar disorder showed a similar occurrence of hypocortisolism as the control sample. Among bipolar disorder patients with a low degree of lifetime with lithium prophylaxis, there was an inverse correlation between age and HPA-axis activity. In contrast, among patients with a higher degree of lifetime with lithium prophylaxis as well as among the controls, there was no correlation between age and HPA-axis activity. Accordingly, hypocortisolism was most common among older patients with a low degree of lifetime with lithium prophylaxis.

In conclusion, hypocortisolism in both recurrent depression and bipolar disorder was associated with multiple clinically-relevant phenotypes. Additionally it was shown for bipolar disorder patients that increasing age was a risk factor for hypocortisolism and that prophylactic lithium treatment was a protective factor. It is argued that the protective effect of lithium towards the HPA-axis is attributable to its mood-stabilizing effect, which in turn reduces the chronic stress level. These results provide new insight into the role of hypocortisolism and chronic stress in recurrent affective disorders warranting further studies and hopefully providing clues to improved treatment strategies.

Key words

Affective disorders, Bipolar disorder, Cortisol, Depression, HPA-axis, Hypercortisolism, Hypocortisolism, Lithium, Metabolic syndrome, Obesity, Quality of life, Recurrent depression, Stress, Telomeres

POPULÄRVETENSKAPLIG SAMMANFATTNING

Bipolär sjukdom och recidiverande depressioner tillhör gruppen affektiva sjukdomstillstånd. Risken att drabbas av dessa sjukdomar under sin livstid är 1 % respektive 8 %. Trots behandling så lider de drabbade av depressiva och maniska/hypomaniska symptom cirka 50 % av tiden. Under sjukdomsperioderna är vanligtvis symptomen allvarliga men även mellan episoderna påverkar symptomen individens livssituation. De drabbade upplever också en sänkt livskvalitet och uppvisar kortare medellivslängd jämfört med normalbefolkningen. Den kortare medellivslängden beror framförallt på ökad risk för självmord och hjärt-kärlsjukdom.

Stress är en av flera riskfaktorer för depression och är också en riskfaktor för hjärt-kärlsjukdom. Stress medför hyperaktivitet i hormonsystemet som reglerar utsöndringen av stresshormonet kortisol. Hormonsystemet kallas HPA-axeln efter engelskans hypothalamus-pituitary-adrenal-axis (hypotalamus-hypofysen-binjurebarken). Hyperaktivitet i HPA-axeln med medföljande höga kortisolnivåer (s.k. hyperkortisolism) är en av de mest replikerade biologiska avvikelserna vid depression. I andra stressrelaterade sjukdomar har det emellertid visat sig att hög stress över lång tid kan leda till en sänkt aktivitet i HPA-axeln med låga kortisolnivåer (s.k. hypokortisolism). Personer med bipolär sjukdom eller recidiverande depressioner är utsatta för återkommande och långvarig stress och man kan därför misstänka att hypokortisolism kan förekomma även vid dessa sjukdomar.

I den här avhandlingen frågar vi oss om det är vanligt med hypokortisolism hos personer med bipolär sjukdom eller recidiverande depressioner samt om hypokortisolism är associerat med den psykiska och somatiska hälsan vid dessa sjukdomar. Vi ville också börja undersöka vilka faktorer som innebär ökad risk för att utveckla hypokortisolism.

Avhandlingen består av fyra olika studier. I den första studien visade vi att hypokortisolism var vanligare bland patienter med recidiverande depressioner än i en kontrollgrupp representativ för normalbefolkningen. Vi visade också att längden på telomerer i vita blodkroppar var kortare hos deprimerade jämfört med kontrollgruppen och att patienter som uppvisade hypokortisolism hade kortare telomerer än de med normal eller hög HPA-axel aktivitet. I kontrollgruppen uppvisade både de med hög och de med låg HPA-axel aktivitet kortare telomerer än de med normal HPA-axel aktivitet. Hypokortisolism var i kontroll gruppen också associerat med en högre grad av inflammation. Korta telomerer anses vara ett mått på biologiskt åldrande och kumulativ stress. Således stärker resultaten bilden av att depression är en stressrelaterad sjukdom och att hypokortisolism kan utvecklas vid en alltför hög långvarig stressbelastning.

Den andra studien visade att bland bipolära patienter med hypokortisolism var depression nästan dubbelt så vanligt och låg livskvalitet sex gånger vanligare än bland bipolära patienter med normal HPA-axel aktivitet. Även bland patienterna med hyperkortisolism var det nästan dubbelt så vanligt med depression och mer än fyra gånger vanligare med låg livskvalitet, jämfört med de som uppvisade normal HPA-axel aktivitet. Förekomsten av både hypokortisolism och hyperkortisolism var lika hög bland bipolära patienter som i kontrollgruppen.

I den tredje studien visade vi att det fanns ett samband mellan högre ålder och lägre HPA-axel aktivitet bland bipolära patienter. Sambandet var starkast bland de patienter som varit utan förebyggande litiumbehandling under stora delar av sitt liv. Bland de patienter som fått profylaktisk behandling med litium under en större del av livet fanns det däremot inget samband mellan ålder och HPA-axel aktivitet. I kontrollgruppen såg vi inte heller detta samband. Hypokortisolism visade sig också vara vanligast bland äldre patienter (>47 år) med tidigare låg användning av litium. Vi föreslår att stress genererad av de ofta förekommande depressiva och maniska symptomen kan bidra till utvecklingen av hypokortisolism och att litium, genom att minska dessa symptom, och den ackumulerade stressen, kan skydda individer med bipolär sjukdom från att utveckla hypocortisolism.

Den fjärde studien utfördes på en patientgrupp bestående av både bipolära patienter och patienter med recidiverande depressioner. Studien visade att fetma, övervikt, höga blodfetter och metabolt syndrom förekom i betydligt högre omfattning bland patienter med hypokortisolism jämfört med de som hade en normal eller förhöjd HPA-axel aktivitet. Detta samband sågs också i kontrollgruppen. Ansamlingen av metabola avvikelser hos patienter med hypokortisolism antyder att det skulle kunna vara framförallt denna grupp som har förhöjd risk för hjärt-kärlsjukdom.

Sammanfattningsvis visar resultaten att hypokortisolism är förknippat med både försämrad psykisk och fysisk hälsa vid bipolär sjukdom och med försämrad fysisk hälsa vid recidiverande depressioner. Vid bipolär sjukdom var högre ålder en riskfaktor för hypokortisolism medan profylaktisk litium behandling var en skyddande faktor. Resultaten bidrar till nya insikter om betydelsen av kronisk stress och hypokortisolism vid recidiverande affektiva sjukdomar och kan förhoppningsvis ge viktiga ledtrådar till framtida studier ämnade att förbättra behandlingsstrategierna vid dessa sjukdomar.

ORIGINAL PAPERS

This thesis is based on the following papers which will be referred to throughout the text using Roman numerals (I-IV).

I. Mikael Wikgren, Martin Maripuu, Thomas Karlsson, Katarina Nordfjäll, Jan Bergdahl, Johan Hultdin, Jurgen Del-Favero, Göran Roos, Lars-Göran Nilsson, Rolf Adolfsson, and Karl-Fredrik Norrback. Short telomeres in depression and the general population are associated with a hypocortisolemic state. Biol. Psychiatry. 2012,71, 294-300

II. Martin Maripuu, Mikael Wikgren, Pontus Karling, Rolf Adolfsson and Karl-Fredrik Norrback. Relative hypo- and hypercortisolism are both associated with depression and lower quality of life in bipolar disorder: A cross-sectional study. PLoS One. 2014, 16;9(6):e98682

III. Martin Maripuu, Mikael Wikgren, Pontus Karling, Rolf Adolfsson and Karl-Fredrik Norrback. Bipolar patients at risk for developing hypocortisolism - Long-term lithium treatment is preventive. Submitted manuscript.

IV. Martin Maripuu, Mikael Wikgren, Pontus Karling, Rolf Adolfsson, and Karl-Fredrik Norrback. Relative hypocortisolism is associated with obesity and the metabolic syndrome in recurrent affective disorders. Submitted manuscript.

LIST OF COMMONLY USED ABBREVIATIONS

ACTH	adrenocorticotropic hormone
AHA	American Heart Association
bp	base pairs
CAR	cortisol awakening response
CRH	corticotropin releasing hormone
CVD	cardiovascular disease
DSM	Diagnostic and Statistical Manual of Mental Disorders
DST	dexamethasone suppression test
HPA	hypothalamic-pituitary-adrenal
ICD	International Classification of Diseases
IDF	International Diabetes Federation
NHLBI	National Heart, Lung, and Blood Institute
PNS	parasympathetic nervous system
SNS	sympathetic nervous system

1. INTRODUCTION

The work in this thesis sought to explore whether hypocortisolism, a condition characterized by low cortisol levels and believed to develop after long-term chronic stress, is associated with the psychiatric and somatic health of patients with bipolar disorder and recurrent depression. This work could be important for future efforts aimed at improving life conditions for persons suffering from affective disorders.

1.1 Affective disorders

The patient laugh, sings, dances… he bites himself… sometimes is wicked and kills… sometimes he is anxious and seized by terror or hate… sometimes he is abulic… (it is) an intermittent disease… repeated once a year or more often melancholia occurs in autumn whereas mania in summer… mania occurs in young people and melancholia in adults … the melancholic is sad, afraid; he isolates himself and cries; he thinks… about death… he exaggerates his evils and his faults… and his illness; he thinks himself a terrible sinner… he is desperate.

(Posidonius Greco-Syrian scholar, 135-51 BCE)[1]

The ancient scholar Posidonius describes the characteristics of a patient with mood swings that we, today, would probably refer to as bipolar disorder.

1.1.1 What is an affective disorder?

Affective disorders are characterized by recurring episodes of disturbances in mood, thought, and activity regulation, leading to clinical syndromes of depression, hypomania/mania, or mixed forms. Depression is characterized by low mood, loss of energy, and loss of interest or pleasure in nearly all activities. Mania/hypomania represents the opposite state, with an elevated or irritable mood, increased psychomotor activity, and racing thoughts. (Table 1, 2)[2-5].

The affective disorders are classified as depressive or bipolar based on the occurrence of a manic or hypomanic episode. Depressive disorders are characterized by depressive episodes, whereas bipolar disorders are defined by alternating manic/hypomanic and depressive episodes. The main subtypes of bipolar disorders are: bipolar type I, bipolar type II, cyclothymic disorder, and bipolar disorder not otherwise specified. Bipolar type I is the most classic form of bipolar disorder (manic-depressive) with at least one manic episode, whereas bipolar type II is characterized by hypomanic episodes alternating with episodes of depression[2, 5].

Table 1. Symptoms of major depressive episode according to DSM-IV[2].

• Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure.
1. Depressed mood or irritable most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad or empty) or observation made by others (e.g., appears tearful).
2. Decreased interest or pleasure in most activities, most of each day
3. Significant weight change (5%) or change in appetite
4. Change in sleep: Insomnia or hypersomnia
5. Change in activity: Psychomotor agitation or retardation
6. Fatigue or loss of energy
7. Guilt/worthlessness: Feelings of worthlessness or excessive or inappropriate guilt
8. Concentration: diminished ability to think or concentrate, or more indecisiveness
9. Suicidality: Thoughts of death or suicide, or has suicide plan

Table 2. Symptoms of Manic episode according to DSM-IV[2].

• A distinct period of abnormally and persistently elevated, expansive, or irritable mood, lasting at least 1 week (or any duration if hospitalization is necessary).
• During the period of mood disturbance, three (or more) of the following symptoms have persisted (four if the mood is only irritable) and have been present to a significant degree.
1. Inflated self-esteem or grandiosity
2. Decreased need for sleep (e.g., feels rested after only 3 hours of sleep)
3. More talkative than usual or pressure to keep talking
4. Flight of ideas or subjective experience that thoughts are racing
5. Distractibility (i.e., attention too easily drawn to unimportant or irrelevant external stimuli)
6. Increase in goal-directed activity (either socially, at work or school, or sexually) or psychomotor agitation
7. Excessive involvement in pleasurable activities that have a high potential for painful consequences (e.g., engaging in unrestrained buying sprees, sexual indiscretions, or foolish business investments

1.1.2 Disease burden

The point prevalence for depression is estimated at approximately 5%[6], and lifetime risk at about 15%[7, 8], although a lifetime prevalence as high as 50% has been reported[9]. Depression is almost twice as common among women than men[8]. The risk for a new depressive episode after the first episode is 50% and increases to 80% after the second episode[10]. Thus the lifetime risk of recurrent depressive episodes is relatively high, approximately 7-8%. The prevalence of bipolar disorder is close to 1%[3, 11], of which type I comprise roughly 0.6% and type II, approximately 0.4%[12]. However, a prevalence of up to 5% has been reported when applying less strict criteria for the manic/hypomanic phases[13].

Although the classic view of affective disorders constitutes of alternating affective episodes and symptom free intervals, there is a growing awareness of inter-episodic symptoms[14-18]. In a series of longitudinal studies of patients with recurrent depression and bipolar type I and type II disorders over an average of 9-13 years, inter-episodic syndromal and subsyndromal symptoms were frequent. Patients demonstrated affective symptoms approximately 50% of the time during follow-up (recurrent depression, 59%; bipolar type I, 47%; bipolar type II, 54%), and depressive symptoms were dominant in all three patient groups[19-21]. Psychiatric comorbidity with anxiety symptoms and substance abuse is also common in both depression and bipolar disorder[6, 7, 22, 23]. In addition to the suffering during affective episodes, these disorders pervasively affect several other aspects of life, including relationships, education, and work[4, 8, 24, 25]. The often chronic course of these disorders has highlighted the importance of in addition to acknowledging the affective symptoms also address factors such as global functioning and quality of life[4, 8, 26-30].

The expected life span of patients with severe mental disorders such as major depression and bipolar disorders is about 10-15 years shorter than that of the general population[31-33]. The main reasons for this disparity is the high prevalence of suicide and cardiovascular disorders (CVD)[31, 34, 35]. Compared to the general population, risk of suicide is 10-20 times higher in individuals with affective disorders[33, 34]. The cardiovascular mortality rate in bipolar patients is twice as high as in the general population, and bipolar patients die approximately 10 years earlier due to CVD compared with the general population[35]. CVD is also common among individuals with depression[34, 36]. Moreover, risk factors for CVD, such as obesity, dyslipidemia, and metabolic syndrome, are more prevalent in affective disorders than in the general population[37-40].

1.1.3 Treatment

In general, for severe depression antidepressants and electroconvulsive therapy are the most effective treatments. In mild and in moderate depression also psychotherapy solely or in combination with antidepressants are recommended treatments. The goal with all treatment is to achieve a complete remission, and treatment after the first episode is recommended for an additional 6-9 months post-remission[41]. Long-term, often lifelong treatment, is recommended in the case of recurrent episodes. For bipolar disorders, mood-stabilizing agents are the golden standard for reducing the number and severity of mood episodes. Lithium is the oldest, most widely used, and generally the most efficient mood stabilizer[42-44], although anti-epileptics and second generation antipsychotics also have prophylactic mood-stabilizing effects[42, 45, 46]. Psychoeducation and different forms of psychotherapy are other means of preventing and treating bipolar affective episodes[47].

In summary, recurrent depression and bipolar disorders are relatively common psychiatric disorders with frequent affective episodes, low quality of life, low global functioning, high suicide rates, and accompanying serious somatic comorbidity. There is thus a large need for research aimed at understanding, preventing, and improving treatment of these disorders.

1.2 Mechanisms of depression

Although depression is a common and devastating disorder that has plagued mankind for as long as we know, its biological underpinnings are only to some extent known[4]. Hence, clinical and research criteria for depression are largely based on pre-determined clinical signs and symptoms, without consideration of potential etiological factors[2, 5]. However, it is a plausible assumption that depression is a biologically heterogeneous disorder, supported by e.g. inconsistent responses to treatment and highly variable courses and outcome[4].

There are several theories about the biological systems that could be involved in depression. Some of the biological disturbances that have been studied include: the monoamine-deficiency hypothesis, which postulates a deficiency of norepinephrine or serotonin neurotransmission in the brain; hypothalamic-pituitary-adrenal axis (HPA-axis) dysregulation; altered glutamatergic neurotransmission; reduced GABAergic neurotransmission; abnormal circadian rhythms; deficient neurosteroid synthesis; monoamine-acetylcholine imbalance; inflammation; and vitamin-D deficiency[4, 48]. Depressive symptoms are also often observed in endocrine disorders such as: hypothyroidism[49, 50], hyperthyroidism[49-51], hyperparathyroidism[52], Addison's disease[53-56] and Cushing's syndrome[55-58]. Melancholic and atypical subtypes of depression further support the assumption that there are several biological causes of depression. Although these subtypes meet the diagnostic criteria for depression, they are characterized by symptoms that to some extent are contrary to each other and melancholic and atypical depression have also been associated with specific biological dysfunctions[59, 60]. The challenge to understanding the biological underpinnings of depression is further complicated by the possibility of heterogeneity in individuals' sensitivity to biological dysregulations. Moreover, biological dysregulation, and/or an individual's sensitivity to it, may vary with the stage of the disorder. Stress is one risk factor that has repeatedly been linked to depression, and to several of the biological systems associated with depression[61].

1.3 Stress, health and the hypothalamic pituitary adrenal axis (HPA-axis)

We are all beset by disturbing forces that upset our balance

(Hippocrates)[62]

Like the affective disorders, stress in relation to human health has been contemplated for a few thousand years[63, 64].

1.3.1 What is stress?

Hans Selyes' work in the 1930s, in which he recognized the paradoxical effects of hormonal systems which can both protect and harm the body during stressful situations, forms the basis of stress research in the modern era[65, 66]. Selyes expanded on previous work by Claude Bernard and Walter Cannon on the milieu interieur and homeostasis[63, 64, 67]. Today, homeostasis is seen as a complex dynamic equilibrium that is constantly challenged by internal or external adverse effects[68]. A frequently-used definition of stress is: a state in which homeostasis is actually threatened or perceived to be so[63, 68]. Any external or internal stimulus that is a threat or is perceived as a threat to homeostasis is called a stressor[63, 68]. The body is constantly responding to various stressors. This ability to achieve stability through change is critical to survival and has been called allostasis[69].

Acute and chronic hypofunction and hyperfunction in hormonal systems are believed to have negative health consequences. The long-term negative health consequences of stress are believed to develop from excessive challenge accumulation or inadequate function of the stress systems such that they remain active when they are no longer needed or inactive when needed[70]. The cumulative wear and tear on the brain and body resulting from chronic overactivity or underactivity of the systems responsible for maintaining homeostasis is called the allostatic load[70]. Thus the processes involved in protection from the challenges of daily life can have the contradictory effect of causing damage.

1.3.2 Stress response

The autonomic nervous system is central for maintaining homeostasis and is traditionally divided into the parasympathetic and sympathetic nervous system. The functions of these systems are mostly antagonistic. The parasympathetic nervous system (PNS) is active during resting conditions and is often called the "rest and digest" or "feed and breed" system. The PNS promotes digestion, growth, and reproduction. The sympathetic nervous system (SNS) prepares the body for a "fight or flight" response in acute stress situations. In addition, it also has a lower basal activity that maintains functions such as blood pressure and cardiac output stability. The brain is crucial for determining whether a stimulus is threatening and is therefore a key organ in the stress response. Stimuli from brain regions such as the hippocampus, subgenual prefrontal cortex, and amygdala are also processed by the hypothalamus[62, 71].

Diversity in individual stress responses is substantial, probably because of genetic variation and differences in early life experiences[71]. A perceived threat can elicit several immediate stress responses. The locus ceruleus norepinephrine system increases arousal, and the SNS facilitates immediate physical reactions (within seconds) through the sympathomedullary system by releasing the catecholamine hormones (i.e. epinephrine and norepinephrine) from the adrenal medulla. Epinephrine and norepinephrine increase heart rate, mobilizes nutrients (fat and

glucose) for muscle activity, dilate bronchioles in the lungs and blood vessels in skeletal muscles, dilate pupils, and constrict blood vessels in the gastrointestinal tract[62]. Another somewhat slower (within minutes) stress response system, the hypothalamic-pituitary-adrenal axis (HPA-axis), is simultaneously activated. The HPA-axis maintains the acute stress response initiated by the SNS. The hypothalamus (more specifically, the paraventricular nucleus) releases corticotropin releasing hormone (CRH). CRH is transported by the hypophyseal portal circulatory system from the hypothalamus to the anterior lobe of the pituitary gland. The pituitary gland secretes adrenocorticotrophic hormone (ACTH) into the peripheral blood stream, stimulating the adrenal cortex with subsequent release of corticosteroids[62, 72].

The main corticosteroid in stress regulation is the glucocorticoid cortisol. After release from the adrenal cortex, cortisol spreads throughout the whole body and maintains the physiological reactions associated with acute stress[73]. Cortisol effects include: gluconeogenesis, glycogenolysis, lipolysis, increased hepatic glucose secretion, enhanced catecholamine effects, and dampened immunological reactions. HPA-axis activity is also controlled by a negative feedback loop in which cortisol inhibits the release of CRH from the hypothalamus and ACTH from the pituitary gland which dampens and eventually terminates the stress response (Figure 1). The negative feedback affects both mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs). MRs have a stronger affinity to cortisol and dominate during basal conditions, whereas GRs dominate during stressful situations when cortisol levels are higher[73]. It should be noted that CRH in addition to stimulating the HPA axis also activates the locus ceruleus and the sympathomedullary system[62].

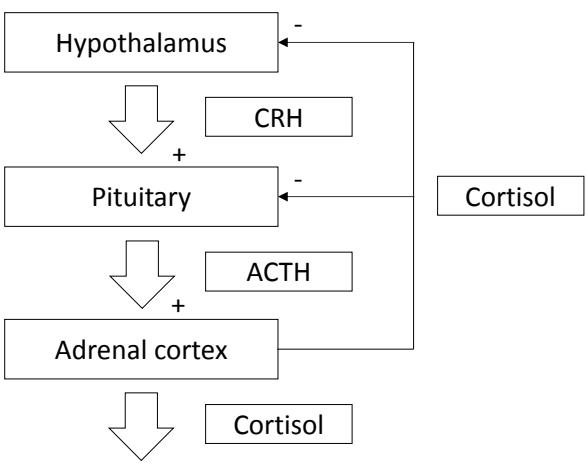

Figure 1. Schematic description of the Hypothalamic pituitary adrenal-axis (HPA-axis). Abbreviations: Adrenocorticotropic hormone (ACTH), Corticotropin releasing hormone (CRH).

Cortisol levels are influenced by large diurnal variation. Factors that influence this diurnal variation include: daylight/darkness, sleep, food, and physical activity. Cortisol values are normally at their lowest at the middle of the night, and at their highest shortly after awakening[72].

1.3.3 Measures of HPA-axis activity

There are several ways to evaluate HPA-axis activity. Most often, cortisol levels are measured. Cortisol can be measured in saliva, blood, urine, or hair. Cortisol concentrations in saliva and blood reflect HPA-axis activity over the last 10-60 minutes[74]. Urine is often collected over a period of 24 hours and reflects the cortisol excretion rate over that time. Cortisol concentration in hair reflects the last months of HPA-axis activity. Cortisol concentrations in single blood or saliva samples are highly dependent on the time of day due to diurnal variation. Thus, blood and saliva samples are often collected several times throughout the day. Several methods for evaluating diurnal variation exist. Common measures include: morning cortisol, evening cortisol, total area under the curve, decline throughout the day, and the cortisol awakening response (CAR). Cortisol in saliva is free and biologically active, whereas in blood a large part of the cortisol is bound to proteins.

Psychological stress tests such as the Trier social stress test are also used to evaluate HPA-axis activity[75]. Hormonal challenge tests are other common measures. These tests are designed to capture the function at different points along the HPA-axis and are believed to be less affected by transient stressors, thus they provide a more comprehensive portrayal of HPA-axis activity. Such tests include the dexamethasone suppression test (DST) that assesses negative feedback sensitivity at the pituitary level, and the DEX/CRH test that inhibits ACTH release with dexamethasone before stimulating the pituitary gland with CRH, primarily evaluating sensitivity to positive CRH stimulus at the pituitary gland. ACTH stimulation assesses adrenal gland sensitivity to positive stimuli and its cortisol-releasing capacity. Other measures of HPA-axis activity are direct evaluation of ACTH or CRH concentrations.

The DST is one of the tests that have received most attention in psychiatry research. It was originally intended for diagnosing Cushing's disease and was introduced to psychiatry by the work of Carrol et al[76]. In a classic DST, 1 mg or more of the synthetic glucocorticoid dexamethasone is administered in the evening. The next morning, blood cortisol levels are measured. Low morning cortisol is the normal response since dexamethasone inhibits cortisol release through negative feedback, mainly at the pituitary gland. High cortisol concentrations post DST signifies decreased sensitivity to the negative feedback inhibition and is associated with HPA-axis hyperactivity. The classic DST is suitable for identifying HPA-axis hyperactivity but cannot distinguish individuals with increased negative feedback sensitivity from those with normal negative feedback sensitivity. The reason for this is that a high dexamethasone dose results in a large inhibition that "hides" hypersuppressors among normal responders. However, the DST has also been performed with a low dexamethasone dose (0.25-0.50 mg). The normal response to administration of low-

dose DST is a partial inhibition of cortisol release. Thus, it is also possible to identify individuals with a high sensitivity to negative feedback resulting in very low post-DST cortisol concentrations. When a low-dose DST is administered, body size differences might affect the dexamethasone concentrations in blood. Therefore, a weight-adjusted very-low-dose DST has also been used when evaluating HPA-axis activity in study participants with large variability in body size, such as in obesity research[77, 78].

1.3.4 Stress and affective disorders

Affective episodes are often preceded by stressful life events, especially in early episodes[61, 79, 80]. Based on these observations, Post constructed the sensitization/ kindling hypotheses, which postulate that earlier mood episodes leave biological scars that predispose individuals for later episodes[81]. Akin to this is the notion that severe stress may cause depression in early stages whereas in later stages only minor stress is necessary[82]. It has also been proposed that early life stress can be critical in predisposing individuals to increased stress sensitivity in adulthood[83]. Despite the well-established overarching finding that stress often precedes depression, the roles of various stressors and of different sensitivities to stress at different time points during the course of the illness are still under debate.

The idea that stress precedes depression and the fact that stress increases cortisol release has contributed to the hypothesis that depression may be associated with HPA-axis hyperactivity. In fact, Reiss et al. described disturbed HPA-axis regulation in mental disorders 66 years ago[84]. Carrol et al., other pioneers in this field, described a blunted negative feedback of depressed individuals as captured by the DST[76]. HPA-axis hyperactivity in depression and bipolar disorders were later reported by several studies according to reviews and meta-analyses[85-88] and has been referred to as one of the most consistent biological findings in depression[87]. The association between depression and HPA-axis activity has been most clear in inpatients and patients with melancholic or psychotic depression[88]. HPA-axis hyperactivity has also been identified between the affective episodes[89, 90], in mania[91], and in persons at high risk of depression or bipolar disorder[92, 93]. Moreover, HPA-axis hyperactivity has also been associated with a higher risk of depression recurrence[94]. Whether HPA-axis hyperactivity is a state or trait of these disorders is currently being disputed.

1.3.5 Cardiovascular disease, affective disorders, and stress

Cardiovascular disease (CVD) is one of the most significant factors contributing to the shorter life expectancy associated with affective disorders[31, 34, 35]. Although lifestyle factors such as diet, reduced physical activity, smoking, and medications are believed to account partially for the increased cardiovascular morbidity, there is a need for more research that examines fundamental biological links between affective disorders and cardiovascular disease[95]. Stress has in the general population been estimated to account for a 1.5-fold increase in CVD risk[96]. One explanation for this association is that stress promotes the development of obesity and other metabolic

disturbances[96]. Patients with recurrent affective disorders are exposed to an increased degree of chronic stress. Obesity, dyslipidemia, and the metabolic syndrome are overrepresented among individuals with depression and bipolar disorder[37-39, 95].

Cortisol is central to stress regulation and a key regulator of energy metabolism. Cortisol increases levels of glucose and fatty acids in the circulation and promotes insulin resistance and centralization of body fat[73]. Cushing's syndrome with excess cortisol due to cortisone treatment or hypophyseal or adrenal tumours is associated with increased risk of centripetal obesity, dyslipidemia, and metabolic syndrome[97, 98]. However, in the general population the association between HPA-axis activity and obesity or metabolic syndrome are far from consistent and both high and low HPA-axis activity have been associated with obesity as well as with features of the metabolic syndrome[99-102]. One study addressing obesity in relation to HPA-axis activity even reported a U-shaped association between diurnal cortisol slope and obesity[103]. Reviews conclude that despite strong interest in the field, no general conclusions can be made regarding the correlation between obesity and HPA-axis activity[104-107]. The heterogeneous results have been suggested to be associated with various background factors, such as chronic stress, or that the HPA-axis disturbances associated with obesity is very subtle[104-106].

1.4 Depression as a stressor

Stress can activate the HPA-axis which increases cortisol secretion. Prolonged exposure to high cortisol levels may cause depression. However, it is noteworthy that suffering from depression can also act as an "internal" stressor[61]. A depressive episode is associated with increased prevalence of stressful life events, and during depression, events may be perceived as even more stressful[108]. The depressive or manic symptoms themselves can also act as significant stressors. Thus, HPA-axis hyperactivity may cause depression, but may also be the consequence of depression. Furthermore, stress due to depression can increase or maintain an already elevated HPA-axis activity. Bipolar disorder and recurrent depression, as mentioned earlier, exhibit syndromal or subsyndromal affective symptoms approximately 50% of the time[19-21]. When affective symptoms are acknowledged as stressors, the lifetime accumulated stress becomes substantial. If health is affected by the wear and tear of life, i.e., the allostatic load, then cumulative stress exposure is likely to be of great importance for the physical and mental health of individuals with recurrent affective disorders[109-111].

1.5 Telomere length as an index of accumulated stress exposure

Telomeres are the outermost parts of the chromosomes and are believed to be essential in maintaining genomic stability[112]. They are constructed of non-coding repetitive DNA sequences (TTAGGG) between 0.5 and 15 kilo base pairs (bp) in length[113]. Telomeres are shortened with each cell division (50-200 bp) partly because the DNA replication enzymes cannot duplicate the DNA to the very ends of the chromosomes. The rate of telomere shortening is however not only affected by cell division but is also influenced by factors such as oxidative stress that can accelerate the telomere attrition rate. When telomeres become critically short, the cell goes into apoptosis or cellular senescence[114, 115]. Telomerase is an enzymatic complex involved in repair and elongation of telomeres. Telomerase activity is absent in most cell-types but present in some cell types, such as stem cells and lymphocytes. The telomerase activity in such cell types does not however seem to be able to fully counteract the telomere erosion upon cell division but rather decrease the attrition rate except for in adult male germ cells[116, 117].

Telomere shortening is believed to be a lifelong process that has been linked to psychological as well as physical stress[118, 119]. Stress has both been associated with increased telomere attrition and impaired telomerase activity. Although there is still much to learn about telomere dynamics, it is a promising index of biological ageing and cumulative stress[118, 119]. Three articles published before 2011 supported an association between depression and shortened telomeres[120-122], thus corroborating the idea that depression is associated with chronic stress. One study did not demonstrate the presence of shorter telomeres among depressed patients, but found that lifetime duration of illness was inversely associated with telomere length[123].

1.6 Stress-induced hypocortisolism

As previously described, stress activates the HPA-axis resulting in HPA-axis hyper activity. HPA-axis hyperactivity has also repeatedly been observed in depression and bipolar disorder. However, in several other stress-related disorders, evidence for the contrary has emerged. HPA-axis hypoactivity has been identified in conditions such as: posttraumatic stress disorder (PTSD)[124, 125], burnout[126, 127], chronic fatigue syndrome[128-130], chronic pain[131], irritable bowel syndrome[132], and fibromyalgia[133, 134]. Hellhammer and Wade proposed that these seemingly contradictory findings could be explained by the possibility that early stress would be associated with HPA-axis hyperactivity, which with time could develop into hypoactivity[135].

Two reviews reported on HPA-axis hypoactivity in stress related disorders often associated with clinical symptoms of stress sensitivity, fatigue, and pain. The term ‘hypocortisolism’ was used to describe the proposed phenomenon[136, 137]. These reviews concluded that hypocortisolism refers to a deficiency of cortisol that could be

caused by dysregulation at several points along the HPA-axis. They also established some core characteristics of hypocortisolism: reduced adrenocortical secretion (at least temporarily during the circadian cycle), reduced adrenocortical reactivity, and enhanced sensitivity to feedback inhibition of the HPA-axis[137]. It was also proposed that a low dose DST is the most sensitive measure of hypocortisolism[136]. Further, Miller et al. concluded, in a meta-review, that hormonal activity is increased at stress onset but as time passes an initial state of HPA-axis hyperactivity evolves into a state of HPA-axis hypoactivity[74].

1.7 Hypocortisolism in affective disorders

When accounting for the high stress exposure in recurrent affective disorders, one might suspect that, with time, HPA-axis hyperactivity could develop into HPA-axis hypoactivity in these disorders. Accordingly, some studies, most often performed with older cohorts, reported HPA-axis hypoactivity in depression[138-141]. Higher number of previous depressive episodes was found to be associated with lower urinary cortisol levels[140]. However, there are also studies that report on HPA-axis hypoactivity in other subpopulations with depression, such as young persons with chronic symptoms[142], treatment-resistant depression with fatigue among adults[143], atypical depression[59, 144], depression among individuals with abdominal obesity[145] and job stress-induced depression[146]. Two studies in older populations described a U-shaped association between HPA-axis activity and depression, where both high and low activity were associated with depression[138, 141]. Further support for a U-shaped relationship between HPA-axis activity and depression can be observed in Cushing's syndrome and Addison's disease[53-57]. Depression is common in both Cushing's syndrome, characterized by high cortisol exposure due to endogenously increased cortisol production or cortisone medication, as well as in Addison's disease, characterized by cortisol deficiency. Further, depressive symptoms in both disorders often subside shortly after correction of the cortisol levels. Similarly, withdrawal from cortisol medication, which is often associated with a relative cortisol deficiency after long-term treatment, has also been associated with depression[56, 147].

In summary, there is evidence that stress may cause depression, but also that affective symptoms can act as stressors. Hypocortisolism is believed to develop after long-term stress exposure. The recurrent affective disorders are exposed to recurring, prolonged stress. Stress is also a risk factor for metabolic disorders. Moreover, both hypoactivity and hyperactivity of the HPA axis have been associated with depression. However, very few studies have identified the role of hypocortisolism in recurrent affective disorders.

2. AIMS

The major aim of this thesis was to evaluate whether hypocortisolism is related to relevant psychiatric and somatic phenotypes in recurrent affective disorders. A secondary aim was to begin investigating the potential etiology of hypocortisolism in these disorders.

Specific aims:

1. To evaluate leukocyte telomere length as well as hypocortisolism and hypercortisolism in recurrent depression, and to study the relationships between leukocyte telomere length and both hypocortisolism and hypercortisolism.
2. To evaluate hypocortisolism and hypercortisolism in bipolar disorder and their associations with depression, anxiety, quality of life, and global functioning.
3. To evaluate the relationships between HPA-axis activity, age and long-term treatment with mood stabilizers in bipolar disorder.
4. To evaluate the relationships between hypocortisolism and obesity, dyslipidemia, hypertension, blood glucose, and metabolic syndrome in recurrent affective disorders.

3. MATERIALS AND METHODS

3.1 Study cohorts

The studies in this thesis were conducted with three study cohorts: the Umeå affective study, bipolar cohort[148], the Umeå affective study, recurrent depression cohort[149]; and the Betula cohort, which is a general population sample[150, 151]. The number of participants from each study sample in Paper I-IV is shown in Table 3.

3.1.1 The Umeå Bipolar Cohort

The bipolar study cohort was recruited from a specialized outpatient unit at Umeå University Hospital between 1998 and 2007. Patients with bipolar disorders type I and II were considered for participation. All were outpatients without mania or hypomania but with varying degrees of depressive symptoms. Patients on oral corticosteroid medication were excluded. In total, 159 bipolar patients, of which 97 (61%) were of type I and 61 (39%) of type II, met the inclusion and exclusion criteria and consented to participation (Paper III). One of these patients did not participate in the study protocol required for Paper IV and was excluded in that study, rendering 158 patients. For Paper II, patients on any corticosteroid regimen, including asthma spray, nose spray, and eye drops were excluded from analysis resulting in 145 bipolar patients completing that study.

3.1.2 The Umeå Recurrent depression Cohort

The recurrent depression sample was recruited from the same specialized outpatient unit as the bipolar cohort during the years 1998 to 2001. Outpatients with at least two severe depression episodes requiring inpatient care who fulfilled the inclusion and exclusion criteria (Paper I) were considered for participation. In Paper I all patients with leukocyte telomere length measurements were included rendering in 91 patients. In Paper IV, the 87 patients who were without oral corticosteroid medication and for whom we had DST data were included.

3.1.3 The Betula Cohort

Betula is a longitudinal study with an aim to study memory, health, and aging[150, 151]. The Betula cohort comprised of individuals randomly selected from the general population in the Umeå region. The Betula controls in this thesis were representative of the general population in the northern region of Sweden[152]. For Paper I, controls with telomere length measurements were included (n=451). For Paper III and IV, 258 controls from a subsample of the Betula cohort with a specific stress focus were included. In Paper II, 145 out of the 258 controls from the stress focus subsample were matched (including age, sex and corticosteroid medication) with the bipolar sample.

Table 3. Basic overview of number of study participants in Paper I-IV.

Study sample	Paper I	Paper II	Paper III	Paper IV
Umeå bipolar cohort		145	159	158
Umeå recurrent depression cohort	91			87
Betula cohort	451	145	258	258

3.2 Questionnaires

The current state of depressive and anxiety symptoms, quality of life, and global functioning, as well as perceived stress were evaluated with self-assessment scales. The following self-assessment scales were used to evaluate depressive and anxiety symptoms as well as perceived stress in Paper I: Beck Depression Inventory (BDI)[153], Beck Anxiety Inventory (BAI)[154], Center for Epidemiologic Studies - Depression (CES-D)[155], and the Perceived Stress Questionnaire (PSQ)[156]. In Paper II depressive and anxiety symptoms were evaluated with two separate sets of scales for each entity. Montgomery Åsberg Depression Rating Scale Self-assessment (MADRS-S)[157, 158] and BDI were used to evaluate depressive symptoms. The Brief Scale for Anxiety Self-assessment (BSA-S)[158, 159] and BAI were used for the evaluation of anxiety symptoms. Quality of life was evaluated with the World Health Organization Quality of Life Assessment-100 (WHOQOL-100)[160], and global functioning with the self-assessment version of the Global Assessment of Functioning (GAF) scale[161]. More details about the questionnaires are provided in Papers I and II. In Paper III, BDI was used to evaluate depressive symptoms.

3.3 Treatment with mood-stabilizing prophylaxis

Medication histories were retrieved from the medical records of all bipolar patients to determine the cumulative time with exposure to mood-stabilizing prophylaxis. Lithium, anti-epileptics, and second generation antipsychotics were classified as mood stabilizers[43-46]. The total number of days on each medication was recorded for each subject. Only medication periods of one year or longer were acknowledged since shorter periods raised uncertainty about whether the prescribed medication was actually used, and about duration of its use. Shorter medication periods were also expected to have a smaller impact on long-term health. Lithium is the golden standard of mood stabilizers and has been, historically, the most frequently used medication[43, 44]. Therefore time on mood stabilizer was evaluated for lithium separately as well as a general mood-stabilizing effect, where all mood stabilizers were evaluated together.

3.4 Measures of HPA-axis activity

The main measure of HPA-axis activity was a very-low-dose weight-adjusted DST. A very-low-dose DST variant was chosen since, compared to the classic DST (1 mg dexamethasone or more), it has the potential to disclose both high and low HPA-axis activity from a normal HPA-axis activity. A weight-adjusted DST, instead of a fixed very-low-dose DST, was selected based on the theoretical consideration that a larger body will likely have a greater distribution volume and a potentially higher rate of metabolism. These factors could potentially reduce the dexamethasone concentration in the body.

The weight-adjusted very-low-dose DST applied has previously been used in obesity research[77, 78]. The weight adjustment (3.5 µg of dexamethasone phosphate per kilogram of bodyweight) results in an interval of individualized dexamethasone dosages centering around the dose of the fixed very-low-dose DST of 0.25 mg, i.e. 0.175 mg and 0.280 mg for a person weighing 50 and 80 kg, respectively. Dexamethasone was administered at 11 p.m. and a blood sample for cortisol measures was collected between 8 a.m. and 10 a.m. the following morning.

To provide a basal measure of HPA-axis activity and to evaluate compliance of the DST, a basal morning serum cortisol (drawn between 8:00 - 10:00 a.m.) and post-DST dexamethasone serum concentration were measured in subsamples of study participants (n = 300 and n = 195, respectively). Cortisol was measured with an electrochemiluminescence immunoassay and dexamethasone was determined with a radio-immunoassay technique.

3.5 Post-DST cortisol as an index of relative hypocortisolism

Papers I and II discuss how results from the DST could be used to identify hypocortisolism and examine the relevance of the test as an indicator of hypocortisolism. There are no generally-established cortisol cut-off values to define high and low HPA-axis activity when a very-low-dose weight-adjusted DST is used. We opted to use the 25th and 75th percentile of the post-DST cortisol values in the control population to define hypoactivity, normal activity, and hyperactivity in both the control and the affective study samples. In Paper I, 253 individuals were included in the control sample and the cut-off values for low and high HPA-axis activity were set to 220 nmol/l and 390 nmol/l, respectively. In Paper II, the cut-offs were defined by 145 control subjects that were age- and sex-matched with the bipolar sample; the low and high cut-offs were 221.76 nmol/l and 408.75 nmol/l, respectively. The latter cut-offs were also used in Papers III and IV. In Papers II-IV, we adopted the terms 'relative hypocortisolism and relative hypercortisolism'. Relative hypocortisolism and hypercortisolism were designated as the more proper terminology, since the exhibition of hypocortisolism or hypercortisolism is denoted in relation to the

controls. Moreover, with the use of quartiles, 50% of controls are also regarded as exhibiting either HPA-axis hypoactivity or hyperactivity. Throughout this thesis, I will interchangeably use the terms hypocortisolism and relative hypocortisolism as well as hypercortisolism and relative hypercortisolism.

Reviews that focus on the subject of hypocortisolism propose a low-dose DST as the most sensitive measure of this condition[136, 137]. Further, two of the core characteristics of hypocortisolism was suggested to be low basal cortisol levels and increased negative feedback sensitivity of the HPA-axis[137]. One possible explanation for the low-dose DST being a sensitive indicator of hypocortisolism is that the test, with its subtle negative feedback of the HPA-axis, potentially taps both the basal cortisol level and the negative feedback sensitivity.

Papers I and II support the notion that the post DST cortisol taps both the basal cortisol level, measured as pre-DST morning cortisol and the negative feedback sensitivity, measured as difference in pre-DST and post-DST morning cortisol. The tables below (Table 4A and B) show the basal morning cortisol levels and the degree of cortisol suppression in relation to relative hypocortisolism and relative hypercortisolism. The tables include all study participants with a basal morning cortisol value and the post DST cortisol cut offs are the same as in Paper II.

Table 4A. Basal morning cortisol levels and cortisol suppression in hypocortisolism compared to non-hypocortisolism (eucortisolism + hypercortisolism).

Study sample	Basal morning cortisol			Cortisol suppression		
	Hypocortisolism Mean (std) nmol/l	Non-hypocortisolism Mean (std) nmol/l	*p*	Hypocortisolism Mean (std) nmol/l	Non-hypocortisolism Mean (std) nmol/l	*p*
Recurrent depression cohort (*n* = 87)	401 (127) *n* = 28	490 (138) *n* = 59	0.005	301 (127) *n* = 28	93 (150) *n* = 59	<0.001
Bipolar cohort (*n* = 77)	357 (146) *n* = 20	480 (186) *n* = 57	0.009	217 (135) *n* = 20	34 (146) *n* = 57	<0.001
Betula cohort (*n* = 136)	349 (111) *n* = 42	422 (103) *n* = 94	<0.001	198 (136) *n* = 42	60 (98) *n* = 94	<0.001

Table 4B. Basal morning cortisol levels and cortisol suppression in hypercortisolism compared to non-hypercortisolism (eucortisolism + hypocortisolism).

Study sample	Basal morning cortisol			Cortisol suppression		
	Hypercortisolism Mean (std) nmol/l	Non-hypercortisolism Mean (std) nmol/l	*p*	Hypercortisolism Mean (std) nmol/l	Non-hypercortisolism Mean (std) nmol/l	*p*
Recurrent depression cohort (*n* = 87)	559 (153) *n* = 25	421 (114) *n* = 62	<0.001	47 (183) *n* = 25	206 (146) *n* = 62	<0.001
Bipolar cohort (*n* = 77)	574 (189) *n* = 28	376 (136) *n* = 49	<0.001	-12 (143) *n* = 28	136 (151) *n* = 49	<0.001
Betula cohort (*n* = 136)	497 (99) *n* = 26	377 (100) *n* = 110	<0.001	2 (102) *n* = 26	127 (122) *n* = 110	<0.001

In summary, low post-DST cortisol values were associated with low basal cortisol values and increased cortisol suppression. In line with this, a high-post DST cortisol was associated with high basal cortisol and reduced cortisol suppression. This suggests that low post-DST cortisol levels taps two of the core characteristics of hypocortisolism. Along with previous literature, this observation provides a rationale for describing low post-DST cortisol levels as relative hypocortisolism and that high post-DST cortisol levels reflect relative hypercortisolism.

3.6 Metabolic disorders, telomere length, and inflammation

There are several definitions of the metabolic syndrome. Paper IV applies a consensus definition from 2009 which incorporates definitions by the International Diabetes Federation (IDF) and the American Heart Association (AHA)/National Heart, Lung, and Blood Institute (NHLBI)[162]. The diagnostic criteria for metabolic syndrome are fulfilled when any three of five risk factors are present: increased waist circumference, elevated triglycerides, reduced high-density lipoprotein (HDL) cholesterol, hypertension, and elevated fasting glucose. Metabolic disorders and CVD risk factors for parameters not included in the definition above were also assessed. These risk factors were: obesity, overweight, high total cholesterol, high low-density lipoprotein (LDL) cholesterol, high LDL/HDL ratio, and high total cholesterol to HDL (TC/HDL) ratio. The methods section of Paper IV provides further descriptions of these risk factors. All blood samples were fasting morning samples and were analyzed in a routine clinical biochemistry battery. Blood pressure was measured in a sitting position after 15 minutes of rest. Two blood pressure measurements, with a 5-minute interval between them, were performed and the mean value was calculated.

Leukocyte telomere lengths were measured using a quantitative polymerase chain reaction (PCR)[163]. The PCR amplifies telomere repeats and a single copy of a housekeeping gene for each DNA sample. The relative telomere length is then reflected by the ratio of these measures. Ratios are converted to telomere restriction fragment lengths (measured in base pairs) using correlation data based on samples that were analyzed with both quantitative real time PCR and Southern blot.

C-reactive protein (CRP), an acute phase protein, was measured to assess the degree of low grade inflammation (Paper I). Since a high CRP level usually is due to an infection, participants with a CRP level above 10 mg/l were excluded. Analyses were performed in plasma with the Immunolite 2000 High Sensitivity CRP kit.

3.7 Ethics

All studies were approved by the Regional Ethical Review Board in Umeå, and all study participants signed a written informed consent.

3.8 Statistical methods

Statistical analyses were performed with SPSS (PASW 18, 19 and SPSS 22). Hypotheses were always 2-sided and probability values below 0.05 were considered significant. The Student's t-test and ANCOVA were used when testing for differences between two means. Pearson's chi-square test or Fishers exact test was used when testing for differences in distribution of categorical data. Pearson's correlation coefficient was used to analyze linear dependencies when using interval scales, and Spearman's rank correlation coefficient was used when evaluating correlations with psychometric scale scores. Significant differences in psychometric scale scores between patients and controls were determined using the Mann-Whitney U test in Paper I. Multiple logistic regressions and linear regressions were used for multivariate statistics.

4. RESULTS AND DISCUSSION

4.1 Relative hypocortisolism and hypercortisolism in recurrent depression and bipolar disorders (Papers I, II)

Previous research has focused mostly on HPA-axis hyperactivity in episodes of depression and mania. HPA-axis hyperactivity has also been found in euthymic phases of the disorders[89, 90]. Although it is less common, HPA-axis hypoactivity has also been reported in depression[138, 140, 141] and in bipolar disorder[164]. We address two questions: 1) How prevalent are relative hypocortisolism and hypercortisolism in recurrent depression and bipolar disorder? 2) Are hypocortisolism and hypercortisolism associated with the current symptomatology of bipolar disorder?

4.1.1 Proportion of relative hypocortisolism and hypercortisolism in recurrent depression and bipolar disorders (Papers I, II)

In the patient samples, the proportions of relative hypocortisolism and hypercortisolism were similar to or higher than in the control sample. The recurrent depression sample exhibited a significantly greater proportion of hypocortisolism than the control sample (35% vs. 25%; $p = 0.041$) (Paper I). Patients in the recurrent depression cohort with hypocortisolism demonstrated significantly lower post-DST cortisol levels than those with hypocortisolism in the control sample (100 vs. 155 nmol/l; $p = 0.001$). The prevalence of hypercortisolism was not significantly different between recurrent depression patients and control subjects (30% vs. 25%).

In Paper II, bipolar disorder patients exhibited a similar proportion of hypocortisolism as the control sample (26% and 25%, respectively). The magnitude of hypocortisolism was also similar between the two groups (152 nmol/l vs. 148 nmol/l, $p = 0.770$). The proportion of patients with hypercortisolism was somewhat higher in the bipolar sample (33%) than in the control sample (24%), but the difference was not significant ($p = 0.119$). The patient group did, however, express a more pronounced hypercortisolism than controls (581 nmol/l vs. 516 nmol/l, $p = 0.012$).

Most measures of HPA-axis activity have no well-defined cut-offs for classifying activity as high or low. Mean basal cortisol or post DST cortisol values in patients with depression are often compared with those of a control sample. Although patients with current depression often exhibit high HPA-axis activity, the values largely overlap with ranges seen in the general population[88]. We used estimations based on two meta-analyses to roughly judge the prevalence of high and, indirectly, low HPA-axis activity in previous studies[86, 88].

The largest meta-analysis included 361 studies and 18,454 individuals with depression, and the method for measuring HPA-axis activity varied between the studies. In this meta-analysis, the average effect size of HPA-axis hyperactivity in depression, compared to controls, was 0.60 ($p < 0.001$)[88]. This might suggest that approximately 70-75% of depressed patients exhibited cortisol values above the median cortisol value in the control samples[165]. However, when only studies using the highest methodological standard were subjected to meta-analysis, the average effect size was considerably smaller ($d = 0.33$), indicating that roughly 60-65% of depressed patients had higher cortisol levels than the median value among controls. This meta-analysis demonstrates that HPA-axis hyperactivity is common among depressed individuals, but it also indicate that approximately 25-40% of depressed individuals will likely exhibit lower cortisol levels than the median value among controls.

These findings do not reveal the prevalence of hypocortisolism, but they do point to the possibility that a large number of depressed individuals have lower HPA-axis activity than the median value in the general population. The meta-analysis also concluded that the association between HPA-axis hyperactivity and depression was most pronounced with old age, severe depression, psychotic depression, melancholic depression, and among inpatients. Subjects with atypical depression showed lower cortisol levels than those found among subjects with non-atypical depression[88].

The second meta-analysis included 17 studies and 704 individuals with bipolar disorder. The study evaluated morning cortisol levels in bipolar individuals and controls and found an effect size of 0.27[86]. This effect size indicate that approximately 60% of the bipolar patients had higher cortisol values than the median value among controls and that approximately 40% had lower morning cortisol levels than the median value among controls[165].

It should be noted that, in our patient samples, ninety percent (90%) of the recurrent depression sample exhibited melancholic features during their latest depressive episode, that all patients were outpatients, few were severely depressed, none showed psychotic symptoms, and that about 60% were in remission on the day of examination. The depression subtype was not identified for bipolar subjects. The meta-analyses were mainly based on current depression. Hypocortisolism in depression has most frequently been found in older patients and in patients with chronic or recurrent depression. Both patient samples in our studies showed a relatively high mean age (48 years in bipolar disorder and 60 years in recurrent depression) and both samples also demonstrated a high degree of chronicity (mean disease duration was 25 years in bipolar disorder and 28 years in recurrent depression patients).

An interesting observation, when comparing our results with previous studies, is that although hypocortisolism was found among a similar or higher proportion of patients compared to the control samples both average pre-DST and post-DST cortisol levels were significantly higher in the bipolar disorder sample than the control sample. Also, 57% of bipolar patients had higher post-DST cortisol levels than the median value of our control sample. In the recurrent depression sample, morning cortisol levels were significantly higher than those of controls. However, there were no significant differences between the post-DST cortisol levels of the recurrent depression patients and the control sample. Also, 69% of recurrent depression patients had higher morning cortisol levels than the median value of the control sample but only 47% had higher post-DST cortisol.

In summary, we found that the proportions of both relative hypocortisolism and hypercortisolism in our patient samples were similar to or higher than those in the control samples. Compared to estimations based on the previous meta-analyses, the proportion of HPA-axis hyperactivity was somewhat smaller and the proportion of HPA-axis hypoactivity was greater. This difference may partly be explained by chronicity of the disorder and outpatient status of our subjects.

4.1.2 Depressive symptoms and quality of life relative to hypocortisolism and hypercortisolism in bipolar disorder (Paper II)

Compared to patients with eucortisolism, a higher proportion of patients with relative hypocortisolism exhibited depression (54% vs. 28%; $p = 0.017$, based on BDI) and low quality of life (42% vs. 7%; $p < 0.001$) in Paper II (Figure 2 and 3). Patients with relative hypocortisolism also exhibited higher mean depressive symptoms and lower quality of life than patients with eucortisolism, as well as higher odds ratios (ORs) of exhibiting depression and low quality of life. Furthermore, hypocortisolism patients exhibited lower mean GAF scores compared to eucortisolism patients.

The proportion of patients with depression and low quality of life was also significantly higher among patients with relative hypercortisolism compared to patients with eucortisolism, 52% vs. 28% ($p = 0.017$, based on BDI) and 33% vs. 7% ($p < 0.001$), respectively (Figure 2 and 3). Patients with relative hypercortisolism also exhibited higher mean depressive symptoms and lower quality of life than patients with eucortisolism, as well as higher ORs of exhibiting depression (according to MADRAS scores but not BDI scores) and low quality of life. Anxiety symptoms were not significantly associated with hypocortisolism or hypercortisolism.

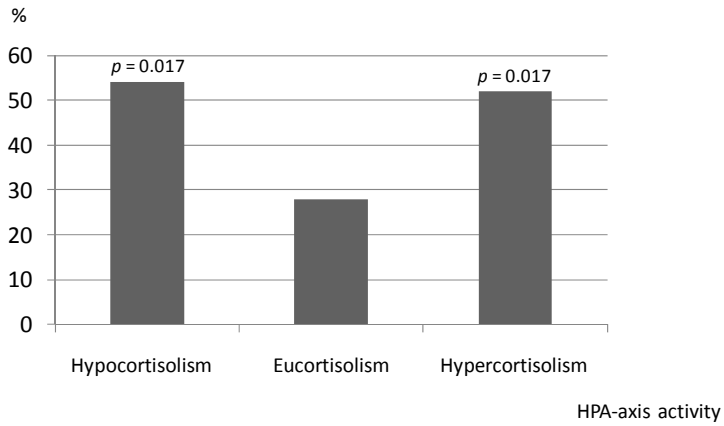

Figure 2. Percentage of patients with depression within the eucortisolism subgroup compared to the subgroups of relative hypocortisolism and relative hypercortisolism, respectively.

Our finding of an association between hypercortisolism and depression in the bipolar disorder sample is consistent with several reports and the general view in bipolar disorder[85, 86]. However, an association between HPA-axis hypoactivity and depression in bipolar disorder has as far as we know not been described previously. Studies in patients with unipolar depression have, on the other hand, showed an association between HPA-axis hypoactivity and depression in older patients and patients with chronic depressive symptoms[138-142]. In summary, our results showed that both hypoactivity and hyperactivity are associated with depression and low quality of life.

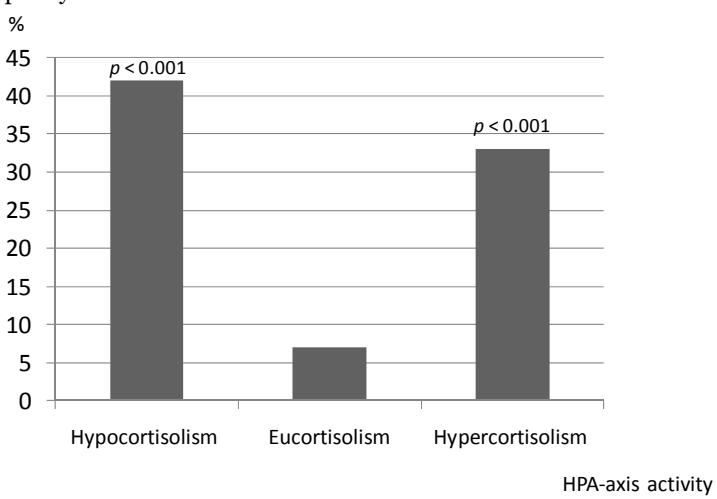

Figure 3. Percentage of patients with low quality of life within the eucortisolism subgroup compared to the subgroups of relative hypocortisolism and relative hypercortisolism, respectively.

Support for a U-shaped association between HPA-axis activity and depression can be found in the abovementioned studies of older cohorts from the general population. These studies reported that both high and low HPA-axis activity were associated with depression[138, 141]. Further support for a U-shaped relationship is evident in Addison's and Cushing's diseases, which are the archetypes of disorders with cortisol deficiency and surplus, respectively. A large proportion of patients newly-diagnosed with either disease exhibit depression, which is often alleviated when cortisol levels are normalized[53-57]. Furthermore, both patients on cortisone medication and those in the phase of cortisone medication withdrawal, the latter often resulting in a temporary cortisol deficiency, have been shown to be at risk for depression[147].

Paper II reports cross-sectional results that cannot explain whether or not the associations it asserts are causal by nature. However, the symptoms of Cushing's and Addison's diseases as well as cortisone medication effects indicate a possible causal relationship between high or low HPA-axis activity and depression. Despite mixed results, longitudinal studies add some support for a causal association. A low mean morning cortisol was predictive of shorter time to recurrence for remitted recurrent depressives over a 5.5 year follow-up period[166]. Another study evaluating both anxiety and depression disorders reported that a low cortisol awakening response (CAR), but not dexamethasone suppression, was associated with an unfavorable or chronic two year course of the disorder[167]. HPA-axis hyperactivity has also been associated with higher risk of recurrence[168]. Moreover, studies aimed at reducing depressive symptoms with glucocorticoid antagonists or glucocorticoids have, in some cases, shown promising results[169-173].

Quality of life is closely associated with depression, but they are considered separate entities[174-176]. Recent reviews have highlighted that quality of life is an important measure of wellbeing and treatment effects in affective disorders[26, 27]. Hypocortisolism and hypercortisolism have to the best of our knowledge not been evaluated relative to quality of life in affective disorders. Based on the associations observed between HPA-axis activity and depressive symptoms, it is not surprising to find a U-shaped association between quality of life and HPA-axis activity as well. This association remained when adjusting for the degree of depressive symptoms (Paper II). Low quality of life among patients with aneurysmal subarachnoid hemorrhage, who often suffer from a relative adrenal insufficiency, has been associated with low basal cortisol. It should be noted, however, that this insufficiency was not believed to be stress-induced[177].

Paper I reported no significant associations between HPA-axis activity and depression or anxiety in the recurrent depression sample. However, a weak tendency to a U-shaped association was observed when the sample was divided into hypocortisolism, eucortisolism, and hypercortisolism based on post-DST cortisol values (mean values: CES-D 11-10-14, BDI 7-6-10, BAI 7-5-11, respectively). This tendency was notably absent in analyses based on basal cortisol or DST suppression (Paper I, Supplemental Information). This lack of significant results could reflect a biological distinction between bipolar disorders and recurrent depression, but

previous studies of depression reported significant associations with both HPA-axis hypoactivity and hyperactivity[138, 141]. Our study results may have been influenced by less depressive symptoms in the recurrent depressive sample compared to the bipolar sample (mean BDI 7 vs. 11), a large proportion of melancholic depression in the latest depressive episode and by the smaller number of participants (91 recurrent depression patients vs. 149 bipolar patients).

A finding of interest regarding the bipolar sample is that the post-DST cortisol levels was, in most cases, not different from the non-depressed controls, yet relative hypocortisolism and hypercortisolism were strongly associated with depressive symptoms and low quality of life. This could provide indirect evidence that bipolar disorder involves an increased sensitivity to HPA-axis dysfunctions. Cushing's syndrome and Addison's disease also point to the possibility that inter-individual differences in sensitivity to cortisol dysregulation is of importance for the development of mood disturbances; only a subset of patients with these diseases expresses depressive symptoms despite the HPA-axis dysfunction[53, 54, 57].

In summary, relative hypocortisolism emerges as an interesting and potentially-treatable biological underpinning of depression and low quality of life in bipolar disorder. Future studies that either reject or support our findings are necessary. To shed more light on the potential causal effects of hypocortisolism and hypercortisolism relative to depression and quality of life in bipolar patients, longitudinal studies would be preferable.

4.2 Hypocortisolism and inflammation (Paper I)

The underlying molecular pathways explaining the associations between HPA-axis hypoactivity, depression, and quality of life are virtually unknown. One potential pathway is inflammation. Inflammation has been suggested as a possible cause of depression[178, 179], and increased levels of inflammatory markers have been reported in both major depression[180] and bipolar disorder[181]. Cortisol is an important immune modulator with an overall dampening effect on inflammatory responses[182, 183]. Thus, low cortisol levels would likely be associated with less inflammatory control and more inflammation. In Paper I, high-sensitivity C-reactive protein (hs-CRP) levels in control subjects with hypocortisolism were higher than in controls with eucortisolism (1.91 vs. 1.36 mg/L, p = 0.040). An association between low HPA-axis activity and inflammation has also been seen in other studies[183, 184]. The subtype of atypical depression is of special interest since it has been shown to exhibit both lower HPA-axis activity and higher levels of inflammatory markers compared to melancholic depression[59, 60, 144]. Altogether, these results indicate a possible connection between hypocortisolism, inflammation, and depression. Support for this hypothesis was found in a study of depression among patients with cardiovascular disease, where depressed patients had higher levels of inflammatory markers and lower cortisol levels than non-depressed patients[185]. If this is further supported in future studies it

could add important knowledge that increase the understanding, identification and specific treatment of depression related to inflammation.

4.3 Depression and leukocyte telomere length (Paper I)

In recent years, several publications have discussed the relationship between depression, cumulative stress exposure, and short leukocyte telomeres[120-123, 186-189]. The results in Paper I showed that leukocyte telomere lengths were significantly shorter in the recurrent depression sample than in the general population sample (277 bp, $p = 0.001$). A recent review of the association between psychiatric disorders and leukocyte telomere length reported that 11 studies (including Paper I) had addressed leukocyte telomere length in major depression[190]. Seven of these studies reported an association between shorter telomeres and depression[120-122, 149, 186, 187, 191]. Three of the studies that reported no significant association were performed on small sample sizes (17 and 18 individuals, respectively)[123, 189], or only in older subjects[188]. One study on depressed individuals reported shorter telomeres in those taking antidepressant medications, but not in un-medicated subjects[192]. An association between depression and short telomeres was also supported by a meta-analysis, although the effect size was small[193].

There are also reports on a dose–response association between duration of depressive symptoms and shorter telomeres[123, 191]. There are however, reports that reject this association, as well as a study that reports short telomeres in individuals with a predisposed, high risk of depression prior to the start of the first depressive episodes (i.e, daughters to depressed mothers)[120, 188, 194]. In Paper I we did not find support for a dose-response association. Leukocyte telomere length in patients was not significantly correlated with disease duration ($p = 0.406$) and although leukocyte telomere length showed the expected negative correlation with age among both patients (17 bp/year, $r = -0.337$, $p = 0.001$) and control subjects (15 bp/year, $r = -0.221$, $p < 0.001$), the difference in telomere attrition rate per year between these two groups was non-significant.

Telomere length in bipolar disorder has shown divergent results and in a meta-analysis no significant association was found between bipolar disorder and telomere length[195]. Lithium-treated bipolar patients exhibited longer leukocyte telomeres than controls representative of the general population[196], whereas shorter mean telomere length and higher percentage of short telomeres were observed in bipolar disorder samples with a lower proportion of patients on lithium prophylaxis[197, 198]. Furthermore, in a rat model of depression, telomerase dysregulation was normalized by lithium[199]. Thus the effects of lithium treatment could be masking an association between bipolar disorder and short telomeres.

One possible explanation to why we found no association between disease duration and leukocyte telomere length in Paper I, could of course be that there is no dose-response association between the accumulated exposure to depression and shorter telomeres. However, methodological aspects could also have masked an association.

The study sample in Paper I had a small variance in disease duration, and possible effects of antidepressant medications were not evaluated. Lithium medication in particular may have confounded results, as 37% of the recurrent depression patients were on lithium treatment.

In summary, our results support an association between shorter telomeres and depression. Although not identified in our patient sample there may be a dose-response association, but this remains to be further investigated. Mixed findings pertaining to a dose-response association could potentially be associated with challenges in evaluating lifetime exposure to depressive symptoms in patients experiencing recurrent episodes.

4.4 Hypocortisolism and leukocyte telomere length (Paper I)

Hypocortisolism and short telomere length are both suggested indicators of increased cumulative stress exposure[74, 119, 137]. Paper I showed that in the recurrent depression sample patients with hypocortisolism compared to patients with eucortisolism or hypercortisolism exhibited significantly shorter leukocyte telomere length (difference of 291 bp, p = 0.037). The control sample demonstrated a similar intra-group association but it did not reach the level of significance (difference of 161 bp, p = 0.200). When hypocortisolism, eucortisolism, and hypercortisolism were instead based on relative DST cortisol suppression (the change in percentage of cortisol between pre-DST and post-DST cortisol), the control groups with either hypocortisolism or hypercortisolism exhibited shorter telomere lengths as compared to the eucortisolism group (difference of 260 bp, p = 0.031 and 251 bp, p = 0.041, respectively). In the patient sample the hypocortisolism, but not the hypercortisolism, group exhibited significantly shorter leukocyte telomere length as compared to the eucortisolism group (difference of 348 bp, p = 0.015 and 184 bp, p = 0.353, respectively).

The association between hypocortisolism and short telomeres could potentially be related both to the processes leading up to hypocortisolism as well as with features of the current state of hypocortisolism. Cortisol has been shown to decrease telomerase activity[200], and an increased cortisol response to an acute stressor has been associated with shorter telomeres[201], which support the theory that short telomeres are a consequence of prolonged hypercortisolism. An already-established state of hypocortisolism was in Paper I associated with increased inflammation levels and increased inflammation is believed to contribute to telomere shortening.

Altogether, the discovery of short telomeres in depression and in hypocortisolism further support depression as a stress disorder, and that hypocortisolism could serve as a marker of increased cumulative exposure to stress. Future studies investigating these hypotheses should preferably have a longitudinal design, following the burden of depressive symptoms, medical treatment, HPA-axis activity, and leukocyte telomere length over time.

4.5 Bipolar patients at risk for developing hypocortisolism (Paper II, III)

Long-term chronic stress is believed to be associated with a shift from initial HPA-axis hyperactivity to a state of low HPA-axis activity, or hypocortisolism. We therefore hypothesized that an increased cumulative stress load throughout life, inherent in bipolar disorder, would result in an abnormal decline in HPA-axis activity over several decades. We also hypothesized that mood-stabilizing treatment, which is known to reduce the time spent suffering from affective symptoms and consequently the accumulated stress load, would exhibit a protective effect against a decline in HPA-axis activity (Paper III).

4.5.1 Post-DST cortisol relative to age and disease duration (Paper II, III)

Disease duration was used as a general proxy for cumulative stress load in Paper II, and patients with hypocortisolism demonstrated longer disease duration than patients with hypercortisolism (27.2 vs. 21.7 years, $p = 0.039$, $n = 85$, Paper II). In Paper III, age was the general proxy for cumulative stress load, and the association between age and post-DST cortisol was investigated. This latter association was significant in the overall bipolar sample (-3.0 nmol/l per year, $p = 0.007$), but was successively more prominent in subgroups without current or previous lithium treatment or any mood stabilization treatment (-7.7 nmol/l per year, $p = 0.001$ and -11.4 nmol/l per year, $p = 0.004$, respectively). The general population demonstrated a correlation between age and post-DST cortisol (-1.4 nmol/l per year, $p = 0.038$) that however was non-significant when adjusted for relevant confounders. Basal morning cortisol correlated to age with similar magnitude as the correlations between post-DST cortisol and age. However, these correlations were not always significant, possibly due to fewer patients in these analyses.

These results corroborated our hypothesis as well as results from studies on other stress-related disorders[74, 136, 137]. Some support is also found in depression where an association between HPA-axis hypoactivity and depression to a large degree has been reported in study samples with older participants and among patients with long-lasting or chronic depressive symptoms[138-142]. An inverse correlation between number of previous episodes and urinary cortisol levels has also been reported[140]. Further support for the results in Paper II are found in a study of the general population where long-term accumulation of temporary employment was associated with suppressed cortisol secretion during most parts of the day[202]. In contrast, the normal aging process has in previous studies been associated with either no change or a slight increase in HPA-axis activity[100, 203-205]. Our control sample also exhibited non-significant changes in cortisol measures with age in adjusted analyzes, thus supporting these latter studies.

Age was used as a proxy for lifelong cumulative stress load in Paper III. Age is a crude indicator of lifetime stress, and its main drawback is that it involves all aspects of aging. However, as previously mentioned, normal aging has not yet been associated with decreased HPA-axis activity. Bipolar patients suffer from affective symptoms up to 50% of the time, on average; thus, each year, a bipolar patient will live six months with affective symptoms. With an age at onset of around 20 years, bipolar patients in their sixties could have been exposed to 20 years with syndromal and subsyndromal affective symptoms. If affective symptoms are considered stressors, it may be said that older patients have experienced more stress than younger patients, on a group level.

One argument for using age as a proxy for cumulative stress is that it is difficult to find other measures that can retrospectively evaluate the stress accumulated over a lifetime. A drawback of possible alternatives such as number of major episodes, number of hospitalizations, suicide attempts, etc. is that only a few events of large impact are known. These alternatives probably do not capture the daily wear and tear of low grade subsyndromal and syndromal affective symptoms. The most tempting alternative was to use disease duration (defined as the time from first affective episode until day of examination) instead of age as a general proxy for stress exposure; this was done in Paper II. However, the use of disease duration as proxy for accumulated stress load also has its drawbacks. For example, childhood stress in the form of negative life events are overrepresented in bipolar disorder compared to the general population[206-208], and childhood stress has been suggested to be of importance for later HPA-axis dysregulations[209, 210]. Furthermore, the onset of the affective disorder is often preceded by prodromal symptoms and age at onset of the disorder can therefore later in life be hazardous to define.

4.5.2 Lithium prophylaxis, age, and hypocortisolism in bipolar disorder (Paper III)

Paper III hypothesized that mood stabilizers in bipolar disorders could protect the HPA-axis from the development of hypocortisolism. The abovementioned findings of Paper III in which the correlation between age and post-DST cortisol was most pronounced among patients without current or previous mood stabilizers (lithium, antiepileptic, second generation antipsychotics) gave some support to this hypothesis.

A protective effect of lithium was further supported by the lack of significant correlation between age and post DST cortisol among patients with a higher proportion of life time with lithium prophylaxis (-0.5 nmol/l per year, $p = 0.735$). In contrast, a significant negative correlation was observed for patients with a lower proportion of life time with lithium prophylaxis (-7.0 nmol/l per year, $p < 0.001$). At older ages (>47 years median age in the bipolar sample), hypocortisolism was identified in a significantly greater proportion of patients with a history of insufficient lithium prophylaxis, than in patients with a greater proportion of lithium prophylaxis

and controls (50% vs. 19%, p = 0.003 and 50% vs. 24%, p = 0.005, respectively). Similar results were produced by analyses evaluating the effect of any mood stabilizer i.e. not only lithium prophylaxis. It should be noted that 88% of lifetime days with mood stabilizer treatment consisted of lithium treatment. Therefore only the long-term effect of lithium will be discussed in this section.

Although our results are based on cross-sectional data, they indicate that prophylactic lithium treatment over several years and decades may protect the HPA-axis from an age-related decrease in HPA-axis activity. Earlier reports on the relationship between HPA-axis activity and lithium medication evaluated short-term (up to one year) effects of lithium medication. Results from these studies are divergent. One study in a mixed sample of affective patients that measured cortisol levels after one year of lithium treatment showed a decrease in cortisol levels and concluded that the finding was associated with decreased depressive symptoms[211]. In contrast, lithium augmentation in depression patients has been associated with increased HPA-axis activity[212, 213], and in one of these studies the increased activity was associated with treatment response[213]. In light of these studies, it should be noted that none of the patients in Paper III initiated lithium medication close to the start of the study. In addition, the correlations between age and post-DST cortisol were adjusted for current lithium use. Thus a direct short-term effect of lithium on HPA-axis activity is not likely to have confounded our results.

A long-term protective effect of lithium against stress in bipolar disorder was indirectly supported by the finding that in bipolar patient samples where most patients did not receive lithium medication patients exhibited shorter mean telomere length and higher percentage of short telomeres than controls[197, 198]. On the other hand, lithium-treated bipolar patients possessed longer leukocyte telomere lengths than controls[196]. Paper I also showed that short telomere length is associated with hypocortisolism. Altogether, these results agree with our finding that lithium treatment of bipolar disorder may protect patients from developing hypocortisolism. There are, however, several other possibilities. For example, lithium may exert a direct protective effect on the HPA-axis that is not mediated by stress reduction. Another prospect is that patients on long-term lithium prophylaxis are likely to be lithium responders, a previously-suggested distinct subgroup of bipolar patients[214].

There are also studies that did not support our findings of an association between lower HPA-axis activity and older age or longer disease duration in bipolar disorder. However, none of these studies addressed long term effects of mood stabilizing prophylaxis. Manenschijn et al. did not find any association between hair cortisol levels and age or disease duration, although they reported higher hair cortisol levels among late onset (> 30 years) bipolar disorder compared to both early onset (< 30 years) bipolar patients and controls. That study consisted of 100 bipolar patients between 20-82 years of age, 68 were on lithium treatment and there was no difference in hair cortisol levels between patients with or without current lithium treatment[205]. Fries et al. reported a positive correlation between numbers of past affective episodes and post-DST saliva cortisol concentrations among 24 euthymic

bipolar patients[215]. They did not report on an association between post-DST cortisol and age or disease duration. Patients in that study had a similar mean age (47 vs. 48 years) but a somewhat shorter disease duration compared to patients in our study (21 vs.25 years)[215]. In a study on 36 remitted patients (mean age 46 years) from a lithium clinic Havermans et al. found that patients with more previous episodes had higher mean salivary cortiol levels over the day but a flatter diurnal cortisol slope and an impaired cortisol response to negative events compared to patients with fewer episodes[216].

Contrary to our findings in bipolar patients, there was no association between hypocortisolism and age or disease duration in the recurrent depression sample in Paper I. This contradiction may reflect a distinction between recurrent depression and bipolar disorder. However, Paper I did not specifically aim to addresses this question, and thus did not, for example, evaluate possible effects of lifetime antidepressant medication (37% of the patients were on lithium treatment and 89% were on antidepressants).

In summary, our results indicate that the natural course of bipolar disorder involves decreased HPA-axis activity that eventually develops into relative hypocortisolism at older ages. Prophylactic lithium treatment seems to prevent this development. It is argued that this development is due to the increased cumulative stress load of persistent affective symptoms. As a first step in supporting or rejecting the described results, future cross-sectional studies investigating the relationship between old age and low HPA-axis activity in light of mood-stabilizing treatments would be valuable. Although longitudinal studies are, methodologically, the best way to evaluate the proposed associations between age, HPA-axis activity and a protective lithium effect, long study periods is a practical obstacle.

4.6 Hypocortisolism, obesity, and metabolic syndrome (Paper IV)

Excess cortisol secretion over time is believed to promote central obesity and metabolic disturbances[73]. This is most evident in Cushing's syndrome, which is associated with a very high incidence of obesity and metabolic syndrome[57, 58, 97]. Studies of the association between obesity and HPA-axis activity in the general population have, however, been highly heterogeneous. Obesity has been associated with both high and low HPA-axis activity, and recent reviews conclude that no general conclusion can be drawn[104-107]. In effort to explain the lack of consensus, it has been proposed that background factors such as chronic stress may influence the relationship between obesity and HPA-axis activity[104-106]. Hypocortisolism is believed to develop after long-term chronic stress, and Paper IV hypothesized that hypocortisolism is associated with obesity, dyslipidemia, and metabolic syndrome.

For both the general population and the affective sample (recurrent depression and bipolar disorders), hypocortisolism was associated with a worse metabolic profile compared to eucortisolism and hypercortisolism (together, non-hypocortisolism)

based on anthropometric measures, dyslipidemia, and metabolic syndrome (Figure 4). However, there were generally no significant associations between hypocortisolism and neither hypertension nor high blood sugar. The metabolic differences between hypocortisolism and non-hypocortisolism were in general more pronounced in the affective sample than in the control sample. From this perspective, however, it should be noted that affective patients with hypocortisolism exhibited lower mean post-DST cortisol levels than control subjects with hypocortisolism. Non-hypocortisolism controls (reference group) exhibited the least deranged metabolic conditions. Control subjects with hypocortisolism and non-hypocortisolism patients showed similar proportions of metabolic disorders, whereas patients with hypocortisolism exhibited the worst metabolic profiles (Paper IV, Table IV). There were considerable differences in metabolic disturbances between patients with hypocortisolism and the reference group: obesity (OR = 5.1); overweight (OR = 4.2); large waist (OR = 5.3); large waist-hip ratio, (WHR; OR = 5.8); high triglycerides (OR = 3.9); high cholesterol (OR = 2.2); high LDL (OR = 4.7); low HDL (OR = 3.6); high LDL/HDL ratio (OR = 2.9); high TC/HDL ratio (OR = 3.7); and metabolic syndrome (OR = 3.9).

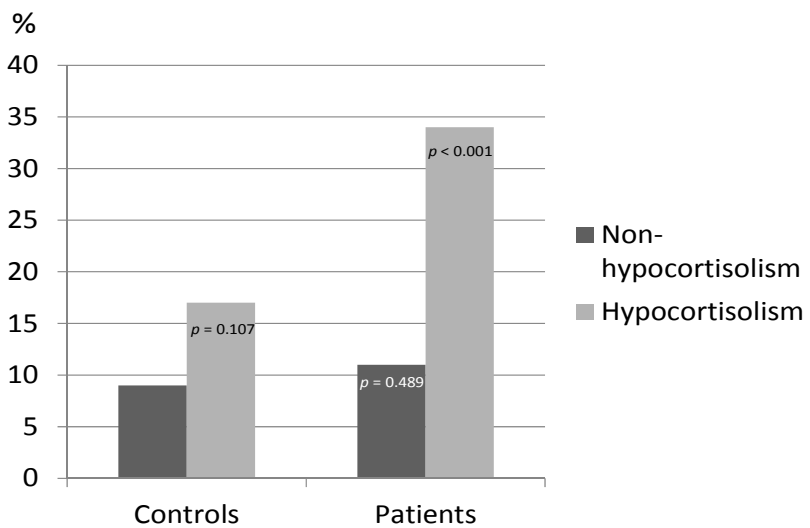

Figure 4. Percentage of obesity among patients and controls subdivided into hypocortisolism and non-hypocortisolism (eucortisolism + hypercortisolism). Analyzes compare the reference group of non-hypocortisolism controls with the three other groups consisting of hypocortisolism controls, non-hypocortisolism patients and hypocortisolism patients.

Although previous studies have produced inconsistent results[104-107], hypocortisolism was strongly associated with metabolic disturbances in Paper IV. The causality of the association between hypocortisolism and metabolic disturbances observed cannot be determined from the study because of its cross-sectional design. However, we suggest that our findings are best understood if hypocortisolism is considered an indicator of

increased cumulative stress and cortisol exposure. Through Papers I-III, we reinforced the evidence for hypocortisolism as a marker of long-term chronic stress. Several methodological aspects of study IV also made it suitable for evaluating chronic stress as an important factor of obesity and metabolic changes. First, patients with either recurrent depression or bipolar disorders are exposed to a high cumulative stress load. Second, the very-low-dose weight-adjusted DST is believed to be a sensitive measure of stress-induced hypocortisolism. Third, the high median age (53 years) and long median disease duration (27 years) rendered a patient sample in which a large number of the patients could have developed both hypocortisolism and metabolic disturbances.

In opposition to the proposed hypothesis stands the observation that hypercortisolism is often associated with melancholic depression that, in turn, often is associated with decreased appetite and weight loss[59, 62]. It is possible that subsyndromal depressive symptoms and subtle cortisol excess over time contribute to the individual's metabolic condition, whereas shorter periods of melancholic depression may have the opposite effect. However, existing properties of the individual's current state of hypocortisolism could also cause or further exacerbate the metabolic changes. As aforementioned, hypocortisolism has been associated with atypical depression which often involves symptoms of increased appetite and weight gain[59, 62]. Furthermore, and as also aforementioned, there is evidence for hypocortisolism as a condition consisting of insufficient immune modulation and increased inflammation. Inflammatory interleukins may produce metabolic effects, and this has spurred the idea that prolonged inflammation can contribute to metabolic syndrome[183, 217, 218]. Later, we discuss the notion that hypocortisolism could also involve local or global glucocorticoid hypersensitivity, resulting in an increased net glucocorticoid effect despite low concentrations in circulation. Moreover, there are the possibilities of an inverse relationship in which obesity causes cortisol changes, or a bidirectional relationship as well as a co-association without causal links between stress, cortisol, and obesity.

One aspect of the conflicting results from previous research on the cortisol-obesity relationship is that HPA-axis activity can be influenced by both current and preceding stress episodes. Yet if stress causes obesity, the process likely occurs over a prolonged period, so that cumulative stress and cortisol exposure are important and a "snapshot" of the current stress level is insignificant. Stress exposure and transient cortisol increase on the day or week of examination could confound analyses of the HPA-axis-obesity relationship. Hypocortisolism may be a better representative of the history of cumulative stress and cortisol excess than HPA-axis hyperactivity in stress-related disorders. Blood pressure and blood glucose can also be influenced by prior stress exposure but are probably more susceptible to the control of current hormonal activity than obesity. The fight-or-flight response associated with increased stress responses, including cortisol increase, is normally aimed at an acute increase in both blood pressure and blood glucose. This may explain why we did not observe an

association between hypocortisolism and blood glucose or hypertension in our study samples.

In summary, hypocortisolism was associated with anthropometric measures, dyslipidemia, and metabolic syndrome in the affective disorder sample. A similar, but less pronounced, pattern was also seen in the general population. The previous literature is highly heterogeneous and more studies, preferably with longitudinal designs, of study samples exposed to high stress are needed to further elucidate the complex associations between stress, cortisol, obesity, and metabolic syndrome

4.7 General aspects (Papers I-IV)

4.7.1 Is hypocortisolism a state of cortisol deficiency or cortisol hypersensitivity?

In Papers I and IV, hypocortisolism was associated with the classic features of both excess cortisol exposure, such as obesity and metabolic syndrome, and cortisol deficiency, such as increased inflammation. Hypocortisolism is most commonly viewed as a state of cortisol deficiency[136, 137]. However, one of the core characteristics of hypocortisolism is an increased sensitivity to negative feedback at the pituitary gland. This indicates increased glucocorticoid sensitivity, at least locally. One possibility that should be considered is that a presumably stress-induced state of hypocortisolism, defined as low concentrations of post-DST cortisol, is instead a state of glucocorticoid hypersensitivity that is either local to some tissues or present throughout the whole body. Low basal cortisol levels could then be a consequence of adaption to this sensitivity, such that the body might be exposed to normal or surplus cortisol signaling, despite the low circulating cortisol levels. The studies in this thesis do not address this complexity, but it will be of paramount importance for future research aimed at treating depression by restoring HPA-axis balance.

4.7.2 Other steroids and hormone systems

The studies in this thesis have focused on HPA-axis activity and cortisol levels in recurrent affective disorders. However, it is important to note that CRH, ACTH, and cortisol interact with other hormonal systems, several of which may also be associated with depression.

The adrenal cortex produces three main types of hormones: mineralocorticoids (aldosterone, deoxycorticosterone), glucocorticoids (cortisol, corticosterone), and sex steroids (the androgen precursors dehydroepiandrosterone (DHEA) and androstenedione)[73]. Aldosterone is controlled by angiotensin II and potassium, as well as ACTH, though to a lesser degree[73]. Corticotrophin stimulates the secretion of DHEA and androstenenedione, which have similar diurnal rhythms to cortisol[73]. Cortisol also activates the renin-angiotensin system that regulates mineralocorticoid release[73]. Circulating cortisol is bound to CBG, and estrogens increase CBG

concentrations[73]. Furthermore, there is close interaction between the HPA-axis and the sympathomedullary system. Catecholamines from the sympathomedullary system stimulate HPA-axis activity and CRH, which stimulates ACTH release in the HPA-axis, also stimulates the release of norepinephrine and epinephrine in the sympathomedullary system[62]. CRH and cortisol downregulate the thyroid, gonadal, and growth hormone axes[62, 72]. On the other hand hyperthyroidism results in increased cortisol metabolism and clearance[62].

Dysregulation of several of the aforementioned hormone systems has been associated with depression: Aldosterone[219], DHEA[220], DHEAS[220], allopregnanolone[220], estrogen[221] and the thyroid axes[49-51]. Overall, there are complex bidirectional interactions between the HPA-axis and several other hormone systems. Both hypocortisolism and hypercortisolism are likely associated with disturbances in some of these systems. These disturbances could be part of the pathways through which cortisol dysregulations influence mood, activity, metabolism, and aging. Although this is particularly interesting, these questions go beyond the scope of this thesis.

4.8 Clinical implications (Paper I-IV)

The results of Papers I-IV do not impose any immediate changes to clinical praxis. However, they do support the importance of current efforts at early identification and mood-stabilizing (lithium) treatment of bipolar disorder. In patients already exhibiting hypocortisolism, these study results are still far too limited to contribute to clinical praxis. Yet the results may be a good starting point for future efforts to improve quality of life and decrease depressive symptoms and somatic comorbidity, as well as normalize life expectancy in these disorders.

4.8.1 Can the identification of hypocortisolism help to improve treatment of depression?

If the association between hypocortisolism and depression in bipolar disorder is replicated and found to be causal, there are several opportunities for which the identification of hypocortisolism could help improve treatment. First of all, several existing antidepressant medications affect HPA-axis activity[222, 223]. It would be interesting if one's current state of HPA-axis activity could be used as a guide for determining the best antidepressants for treatment; this should be investigated in future studies. Second, based on a very small pilot study cortisone substitution could be an alternative treatment for depression with HPA-axis hypoactivity[143]. Third, studies that generally did not address HPA-axis activity still identified some effect of glucocorticoids as well as glucocorticoid antagonist treatments[169-173]. It would have been interesting to evaluate treatment efficiency within these studies from a homeostasis perspective where both hypocortisolism and hypercortisolism are acknowledged. Finally, if we could identify potential downstream molecular pathways of hypocortisolism that are associated with depression, we could develop new, more specific, treatment strategies

4.8.2 Hypocortisolism, cardiovascular disease, and longevity in affective disorders

The results in Papers I and IV connect hypocortisolism to short telomeres, high hs-CRP, and somatic comorbidities such as obesity, dyslipidemia, and metabolic syndrome. These factors have all been identified as risk factors for CVD and early death. Few studies have evaluated hypocortisolism relative to these endpoints. However, studies of the general population found that subjects with low HPA-axis activity had increased risk of death after a myocardial infarction[224, 225]. If hypocortisolism is associated with CVD and early death it could, in the future, contribute to risk assessment and guidance for prophylactic CVD treatment.

4.8.3 Hypercortisolism in recurrent affective disorders

This thesis has mainly focused on the role of hypocortisolism in affective disorders, but the results might also contribute to an understanding of HPA-axis hyperactivity in these disorders. Despite substantial evidence for its existence, the association between HPA-axis hyperactivity and depression has not led to any well-established present-day clinical uses for prognosis or treatment. One challenge involved in translating these findings about HPA-axis hyperactivity into clinical applications could be that HPA-axis hyperactivity, to some extent, may be compared to samples with an unknown mixture of patients with either eucortisolism or hypocortisolism. For example, studies evaluating the potential antidepressant effects of glucocorticoid antagonists could have benefitted from separate analyses of results obtained from patients with HPA-axis hypoactivity and hyperactivity. We, therefore, suggest that by studying both hypocortisolism and hypercortisolism within the same patient sample it might help produce clearer, more specific results. This, in turn, could help us better understand and treat both hypocortisolism and hypercortisolism.

4.8.4 Hypocortisolism and stages of bipolar disorder

In recent years, there has been increased interest in a staging process for bipolar disorders[226-228]. At an early stage, patients often exhibit good treatment responses and a high degree of inter-episodic recovery with little or no impairment of global functioning. In later stages, bipolar disorder is associated with a worse treatment response, high degrees of residual and chronic affective symptoms, reduced global functioning, and increased somatic comorbidity[226-228]. Furthermore, it has been indicated that long treatment delays, from age at onset of the first episode until start of medication, is associated with a worse long-term prognosis[229]. Another proposal is that lithium prophylaxis may be able to halt the progression of the disorder[230]. In Papers II and IV, hypocortisolism was associated with depression, low quality of life, low global functioning, obesity, dyslipidemia, and metabolic syndrome. In Paper III, hypocortisolism was most common among older bipolar patients that, throughout life, had received little or no lithium medication. Altogether, it seems like a plausible suggestion that the development of hypocortisolism is associated with the

progression of bipolar disorder from an early to a late stage. In other words, hypocortisolism could be one of the biological underpinnings of late stage bipolar disorder, thus patients would have acquired a second disorder, namely a stress-induced relative hypocortisolism, in addition to their bipolar disorder. The proposal of an association between HPA-axis hyperactivity and late stage bipolar disorder contradicts the above hypothesis[215]. However, this proposal is, as earlier discussed, based on a much smaller patient sample (n = 24) than that of Paper III.

Despite the gaps in knowledge, we propose the following speculative and simplified schematic model of HPA-axis activity relative to age and the stages of bipolar disorder: At an early stage, cortisol levels will alternate between normal and high based on the current stress level. In sensitive individuals, high cortisol levels will be accompanied by depressive symptoms and low quality of life, whereas normal levels will be associated with clinical remission. With repeated episodes, the HPA-axis will eventually become exhausted (or become hyper-sensitized), and may develop a reduced ability to mount adequate stress responses. Hypocortisolism, once developed, is proposed to be a relatively chronic state associated with low quality of life, depression, inflammation, and metabolic disorders (Figure 5).

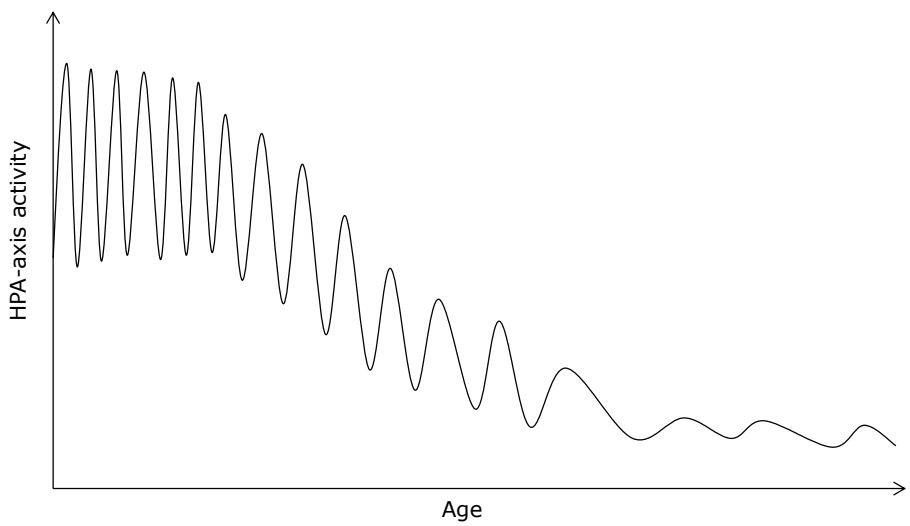

Figure 5. Speculative schematic figure of HPA-axis variation in relation to age in bipolar disorder.

5. CONCLUSIONS AND FUTURE DIRECTIONS

5.1 Conclusions

Relative hypocortisolism was in the recurrent depression sample both more prevalent and more pronounced than in the control sample. In the bipolar sample, relative hypocortisolism had a similar prevalence and magnitude as in the control sample. Relative hypercortisolism was found in similar proportions of recurrent depression patients and bipolar patients compared to the control sample. The magnitude of hypercortisolism was more pronounced in the bipolar sample than in the control sample but did not differ between the recurrent depression sample and the control sample.

Leukocyte telomere length was shorter in the recurrent depression sample than in the control sample. Leukocyte telomere length was also shorter in subjects with hypocortisolism compared to those with non-hypocortisolism (eu- and hypercortisolism) in the recurrent depression sample. In the control sample both HPA-axis hypoactivity and hyperactivity were associated with shorter telomeres compared to the group with normal HPA-axis activity.

Both relative hypocortisolism and hypercortisolism, compared to eucortisolism, were associated with depressive symptoms and lower quality of life in bipolar disorder.

HPA-axis activity was inversely correlated with age in the bipolar sample. There was no correlation between age and HPA-axis activity neither among patients with a high proportion of life time with lithium prophylaxis nor in the control sample. Accordingly, hypocortisolism was most prevalent in older patients with a low life-time proportion of lithium prophylaxis.

Hypocortisolism was compared to non-hypocortisolism (eu- and hypercortisolism) associated with obesity, dyslipidemia, and metabolic syndrome in a combined sample of bipolar disorder and recurrent depression patients. A similar but less pronounced association was also found in the general population sample.

5.2 Future directions

Throughout Papers I-IV, hypocortisolism was associated with: a) core aspects of the disease burden in bipolar disorder (depression, low quality of life, low global functioning, obesity, dyslipidemia, and metabolic syndrome); b) somatic aspects of the disease burden in recurrent depression (obesity, dyslipidemia, metabolic syndrome, and shortened telomeres (early aging)); and c) somatic aspects of disease burden in the general population (increased inflammation, obesity, dyslipidemia, metabolic syndrome, and shortened telomeres (early aging)). Altogether, hypocortisolism emerges as a very unfavorable state of bipolar disorder and, potentially, of recurrent depression and the general population.

First of all, several of these results are novel and must be replicated in other study samples. If these results are replicated, hypocortisolism may emerge as a biomarker of poor psychiatric and somatic health. Moreover, if longitudinal studies provide evidence for any of these associations being causal, then hypocortisolism could be a treatable cause of ill health in patients with affective disorders. Studies investigating the mechanistic underpinnings of the associations described will then come into focus. Elucidating whether hypocortisolism is a state of cortisol deficiency or a paradox state of cortisol hypersensitivity will be of paramount importance for the success of future efforts at restoring the HPA-axis balance

Without a perspective in which both hypocortisolism and hypercortisolism were considered potentially important dysregulations, several of the associations we report would not have been identified. We suggest that future studies evaluating HPA-axis activity relative to mental and physical health should evaluate both HPA-axis hypoactivity and hyperactivity.

Several other psychiatric disorders also involve exposure to chronic stress, and it is tempting to think that both hypercortisolism and hypocortisolism could be present and associated with the clinical expression and progression of disorders such as ADHD, schizophrenia, drug abuse, generalized anxiety disorder (GAD), and personality disorders.

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