Depression, adverse childhood events (ACE) and sleep disturbances link to metabolic and immune alterations. Immunomodulatory transmitters regulate the immune system and contribute to functions such as fibrinolysis and formability of the nervous system. Our results illustrate that levels of IL-5, PAI-1 and adiponectin are altered in individuals with persistent symptoms of depression, and the levels may vary depending on the presence of ACEs and sleep disturbances. This thesis aims to clarify the pathophysiology of depression, which may encourage more personalized treatments.
IMMUNOMODULATORY CHANGES IN DEPRESSION, ADVERSE CHILDHOOD EXPERIENCES AND SLEEP DISTURBANCES
IMMUNOMODULATORY CHANGES IN DEPRESSION, ADVERSE CHILDHOOD EXPERIENCES AND SLEEP DISTURBANCES

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**ABSTRACT**

Major depressive disorder (MDD) is one of the leading causes of disability. MDD increases the risk of developing metabolic disturbances, whereas an onset of somatic illness increases the likelihood of developing depression. Although the underlying mechanisms are poorly understood, adverse childhood events (ACE) associate with both MDD and metabolic disturbances. ACEs and MDD are also linked to sleep disturbances, which may independently expose individuals to psychiatric and somatic morbidity.

The physiological disturbances related to depression, ACEs and sleep might partly be orchestrated by a disturbed immune system. Cytokines, such as interleukins (IL) and adipokines, are potent innate regulators of the immune system. They modulate inflammatory responses, fibrinolytic processes, central nervous system (CNS) plasticity, and even the responses of the hypothalamus-pituitary-adrenal (HPA) axis. MDD, ACEs and sleep disturbances are all characterized by a dysregulated HPA axis, which is a central mediator of stress reactions. This study aimed to clarify other immunomodulatory mechanisms in these associated adverse conditions.

This study formed part of a population-based, seven-year follow-up cohort (n = 3004) and its clinically evaluated subsample (n = 333) (Kuopio Depression Study, KUDEP). Data were acquired from questions concerning aetiological factors, such as ACEs, and thorough psychometric evaluations and venous blood samplings. The results illustrated that alterations in serum cytokines and related fibrinolytic factors characterize MDD and mentally symptomatic populations reporting ACEs and sleep disturbances. Serum levels of IL-5 were elevated in individuals with MDD, while lowered levels of adiponectin characterized individuals with a history of childhood maltreatment. Elevated levels of plasminogen activator inhibitor 1 (PAI-1) linked with a history of alcohol abuse in the childhood home, whereas lowered PAI-1 levels associated with an increased prevalence of sleep disturbances. The investigated biomarkers could contribute to individual well-being through several mechanisms, such as the regulation of blood–brain barrier permeability, sleep–wake cycles and the systemic anti-inflammatory buffering capacity.

National Library of Medical Classification: WA 325, WL 108, WM 167, WM 171.5, WM 274, QW 920  
Medical Subject Headings: Adiponectin; Alcoholism; Biomarkers; Comorbidity; Cytokines; Depression; Immune System; Interleukin-5; Plasminogen Activator Inhibitor 1; Sleep.


Luokitus: WA 325, WL 108, WM 167, WM 171,5, WM 274, QW 920
Yleinen Suomalainen asiakasastu: adiponektiini; alkoholiangemat; immuunijärjestelmä; interleukiinit; kaltoinkohtelu; masennus; mielenterveys; unihäiriöt.
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This thesis had been originally imbued by the work of the researchers in the psychiatric departments of the University and University Hospital of Kuopio (KUH). Furthermore, inspiring researchers across the globe – including the work of several research groups in Finland – have further convinced me that studying the mind is indeed filled with novelty.

Throughout the nearly five years of work leading to this thesis, several individuals have laudably catalysed my process of scientific learning and supported my simultaneous graduation to a Licentiate of Medicine. None of it would have been possible without the commendable work of the primary supervisor, Docent Soili Lehto, and the secondary supervisor Professor Heimo Viinamäki – who each in their personal way have put tremendous effort and positive energy into this thesis. In addition, the publications of the thesis have been blessed with the support and valuable insights given by the coauthors (Ilkka Harvima, Karl-Heinz Herzig, Jukka Hintikka, Kiri Honkalampi, Heli Koivumaa-Honkanen, Leo Niskanen, Heli Ruotsalainen, Sanna Sinikallio, Tommi Tolmunen, Kaisla Uimonen, Anne Huotari, Minna Valkonen-Korhonen), allowing the maintenance of successful publishing and science. In addition to the co-authors, I thank Sara Remes, Kaisa Haatainen and Tarja Saharinen for their contributions on KUDEP and this thesis. For astonishingly great language reviews I want to thank Roy Siddall. The integrity of the work would not have been possible without the knowledge of the respected reviewers, Professor Timo Partonen and Professor Jussi Jokinen. The persistent work would not have been possible without the help of several close friends, family and relatives – who I am sincerely indebted to. Hobbies, such as movie making, beach volley, weightlifting and traveling, have given me the critical energy for achieving this milestone in my scientific career. Most of all, I am grateful to my beloved partner, Outi Nieminen, for her sound support throughout the work. Outi was predisposed to observing countless of hours of reading, writing, rewriting and creative pain, but endured them laudably. Thanks to all the contributors, I feel inspired to continue my research endeavours in the future.

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List of original publications

This dissertation is based on the following original publications:


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### Abbreviations

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<th>Description</th>
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<tbody>
<tr>
<td>15D</td>
<td>15-dimensional questionnaire</td>
</tr>
<tr>
<td>ACE</td>
<td>adverse childhood events</td>
</tr>
<tr>
<td>ACTH</td>
<td>adrenocorticotropic hormone</td>
</tr>
<tr>
<td>AVP</td>
<td>arginine-vasopressin</td>
</tr>
<tr>
<td>BBB</td>
<td>blood–brain barrier</td>
</tr>
<tr>
<td>BD</td>
<td>bipolar disorder</td>
</tr>
<tr>
<td>BDI</td>
<td>Beck Depression Inventory</td>
</tr>
<tr>
<td>BDNF</td>
<td>brain-derived neurotrophic factor</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CHD</td>
<td>coronary heart disease</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CRH</td>
<td>corticotropin-releasing hormone</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CSF2RB</td>
<td>colony stimulating factor 2 receptor b</td>
</tr>
<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
</tr>
<tr>
<td>DHEA</td>
<td>dehydroepiandrosterone</td>
</tr>
<tr>
<td>DSM</td>
<td>Diagnostic and Statistical Manual of Mental Disorders</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>ERK</td>
<td>extracellular signal-regulated kinases</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
</tr>
<tr>
<td>GR</td>
<td>glucocorticoid receptor</td>
</tr>
<tr>
<td>HAM-D</td>
<td>Hamilton Depression Rating Scale</td>
</tr>
<tr>
<td>HDL</td>
<td>high-density lipoprotein cholesterol</td>
</tr>
<tr>
<td>HPA</td>
<td>hypothalamus-pituitary-adrenal cortex</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>high-sensitivity C-reactive protein</td>
</tr>
<tr>
<td>ICD</td>
<td>International Classification of Diseases</td>
</tr>
<tr>
<td>IFN</td>
<td>interferon</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>JAK</td>
<td>Janus kinase</td>
</tr>
<tr>
<td>KUDEP</td>
<td>Kuopio Depression Study</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>LS</td>
<td>Life Satisfaction scale</td>
</tr>
<tr>
<td>MADRS</td>
<td>Montgomery–Åsberg Depression Rating Scale</td>
</tr>
<tr>
<td>MDD</td>
<td>major depressive disorder</td>
</tr>
<tr>
<td>MetS</td>
<td>metabolic syndrome</td>
</tr>
<tr>
<td>MR</td>
<td>mineralocorticoid receptor</td>
</tr>
<tr>
<td>NCEP ATP III</td>
<td>National Cholesterol Education Program Adult Treatment Panel III</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartic acid</td>
</tr>
<tr>
<td>NSAID</td>
<td>non-steroidal anti-inflammatory drug</td>
</tr>
<tr>
<td>OR</td>
<td>odds ratio</td>
</tr>
<tr>
<td>OSA</td>
<td>obstructive sleep apnoea</td>
</tr>
<tr>
<td>PAI-1</td>
<td>plasminogen activator inhibitor-1</td>
</tr>
<tr>
<td>REM</td>
<td>rapid eye movement</td>
</tr>
<tr>
<td>SCID</td>
<td>Structured Clinical Interview for DSM-IV</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SERPINE</td>
<td>serine protease inhibitors</td>
</tr>
<tr>
<td>SNRI</td>
<td>serotonin-noradrenaline reuptake inhibitors</td>
</tr>
<tr>
<td>SOCS</td>
<td>suppressors of cytokine signalling</td>
</tr>
<tr>
<td>SSRI</td>
<td>selective serotonin reuptake inhibitor</td>
</tr>
<tr>
<td>STAT</td>
<td>signal transducers and activators of transcription</td>
</tr>
<tr>
<td>TAS</td>
<td>Toronto Alexithymia Scale</td>
</tr>
<tr>
<td>TG</td>
<td>triglyceride</td>
</tr>
<tr>
<td>Th-cell</td>
<td>T-helper cell</td>
</tr>
<tr>
<td>TNF</td>
<td>tumour necrosis factor</td>
</tr>
<tr>
<td>t-PA</td>
<td>tissue-type plasminogen activator</td>
</tr>
<tr>
<td>u-PA</td>
<td>urokinase-type plasminogen activator</td>
</tr>
<tr>
<td>vmPFC</td>
<td>ventromedial prefrontal cortex</td>
</tr>
<tr>
<td>WBC</td>
<td>white blood cells</td>
</tr>
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</table>
1 Introduction

In 2004, major depressive disorder (MDD) was predicted to become the world’s most common cause of disability by the year 2030 (WHO 2004). In 2013, the Global Burden of Disease study ranked MDD as the second leading cause of disability, and if all mood disorders were accounted for, the first rank had already been reached in 2010 (Ferrari et al. 2013, Global Burden of Disease Study 2013 collaborators 2015). MDD has also been predicted to be one of the most significant causes of death globally. Indeed, mental illnesses account for 90% of all suicides, with over a half of these due to mood disorders (Arsenault-Lapierre et al. 2004, OECD 2013). In Finland, suicides account for a mortality rate of 0.016%, that is, a total of 870 individuals with mood disorders are expected to die from suicide each year (OECD 2013). However, the harm induced by depression is not limited to suicides. In addition to attenuating cognitive function, MDD contributes to a myriad of somatic diseases, such as cardiovascular diseases (CVD). CVDs alone cause more than 38 000 deaths each year, from which 11 000 individuals are under 64 years of age (Finnish Statistical Database).

The disability caused by MDD is characterized by typical symptoms such as a lack of motivation, dependency, fatigue and social isolation. Only during the past decades have clinicians begun to take an interest in the question of “why” MDD patients are also characterized by a myriad of metabolic risks. The risk is so pronounced that a recent expert panel of the American Heart Association (Goldstein et al. 2015) included MDD as a tier-II risk factor for CVD, based on its pathophysiological evidence indicative of accelerated atherosclerosis.

Individuals with CVD are characterized by low-grade inflammation, such as elevations of C-reactive protein (CRP) by 0.7–1.0 units (mg/l) and IL-6 by 0.4–1.0 units (ng/l) on average (Yudkin et al. 2004). Similar low-grade inflammation characterizes MDD. The low-grade inflammation also associates with several other factors, such as smoking, sleep disturbances and a sedentary lifestyle. However, such factors have not fully accounted for the relationship between MDD and CVDs. Thus, MDD has been suggested to contribute to CVDs through independent pathways (Mosovich et al. 2008, Leonard and Maes 2012, Black et al. 2015).

Preventive actions against both CVDs and depression focus on determinants of health, such as obesity, alcohol consumption and smoking. According to a report by the
Organisation for Economic Co-operation and Development (OECD 2013), 40% of Finns on average have reported repeated drunkenness in their lifetime beginning as early as at the age of 15 years. Although adult smoking in Finland had decreased by 24% between the years 2001 and 2011, the consumption of alcohol had increased by 3%. In the United Kingdom, almost a third of children live with at least one binge-drinking parent (Manning et al. 2009). Although some studies have suggested that parental alcohol abuse may not endanger children, over two-thirds of cohort studies illustrate adverse outcomes (Rossow et al. 2015).

The biology underlying the adverse effects of alcohol abuse in the childhood home are unknown, although some clues have been presented. First of all, gestating mothers expose their foetuses to adverse outcomes via ethanol and its metabolites, thus promoting, for example, foetal alcohol syndrome (Drew et al. 2014). Secondly, an extensive Scandinavian study on adopted children revealed that the tendency for alcohol abuse carries a significant genetic predisposition (Kendler et al. 2015), and regardless of the genome, adoptive parents can also transmit their alcohol drinking tendencies (Mares et al. 2013). Nevertheless, serious alcohol abuse is often the mere tip of the iceberg, tied with severe primary mental issues and deficient socioeconomic traits (Rossow et al. 2015), which increase the risk of a child experiencing adverse childhood events (ACE) such as neglect (Dunn et al. 2002). Consequently, or not, individuals experiencing ACEs may indulge in metabolic risk behaviours such as the alcohol consumption (Gilbert et al. 2009).

Facing one form of ACE increases the likelihood of experiencing other forms. A review by Gilbert et al. (2009) suggested that annually as many as 16% of children in high-income countries are exposed to physical abuse, and up to 15% of children face neglect. However, estimating the prevalence of ACEs is difficult, as differences between agency reports and self-reports are substantial. The prevalence of neglect, in particular, is considered underestimated (Gilbert et al. 2009). Nevertheless, prospective studies have associated ACEs with adverse psychological conditions such as behavioural problems, affective disorders and criminality (Afifi et al. 2013). Most types of ACEs increase the risk of violence in terms of being both the victim and a perpetrator in intimate relationships (McMahon et al. 2015). The individuals reporting ACEs have an increased prevalence of suicide attempts and depression (Hoertel et al. 2015), and also a poorer outcome in the treatment of depression (Nanni et al. 2012). Increasing evidence illustrates that ACEs also predict adverse altered immune responses and metabolic outcomes. Some studies have suggested that the adverse outcomes could be
independent of depression, and could thus cumulate the likelihood of metabolic disorders (Rohde et al. 2008, Danese et al. 2009).

In addition to the aetiological factors of depression, such as ACEs, several physiological processes can have potent systemic effects. Indeed, disturbance of sleep is one of the symptoms of MDD, and ACEs associate with up to a doubled risk of sleep disturbances (Chapman et al. 2011). Retrospective studies have illustrated that sleep disturbances are common in adults with a history of ACEs. Furthermore, prospective studies have suggested the ACEs expose individuals to poorer sleep quality and increased self-reported symptoms of insomnia (Kajeepeta et al. 2015). Sleep disturbances also have several ways to crosstalk with MDD, such as similar immune responses and sleep–wake cycle behaviours (Irwin and Miller, 2007, Motivala et al. 2011).

ACEs can induce sleep disturbances and depression, which may become chronic and catalyse metabolic complications. Nevertheless, some researchers, such as Almeida et al. (2013), have concluded that the presence of CVD does not significantly influence the course of depression, thus doubting the need for concepts such as “vascular depression”. However, psychoneuroimmunological perspectives support the positive effects of treatment for depression on somatic diseases, and vice versa. As reviewed in this dissertation, a cascade of similar immunological factors link ACEs, sleep disturbances and MDD. Understanding the immunological interplay between these conditions is a prerequisite for developing optimal pathophysiological models. Thus, based on the pathophysiological mechanisms and references reviewed within this study, I suggest that more personalized approaches to treating depression are required.

I believe one of the most configurable connections between MDD and somatic comorbidity is the immunomodulatory cascade, including adverse changes in cytokines, lymphocyte subsets and cascades modulating stress responses and sympathetic arousal. This thesis study focused on selected parts of these key components, and sought to illustrate new immunomodulatory mechanisms associated with MDD, ACEs and sleep disturbances.
2 Review of the literature

2.1 IMMUNOMODULATION AND STRESS IN A NUTSHELL

White blood cells (WBC) are the cells responsible for systemic immune responses. They can be divided into monocytes, eosinophils, basophils, neutrophils and lymphocytes. Lymphocytes are most prevalent in lymphatic tissue and have a characteristic histology of a large nucleus; they can be categorized into natural killer (NK) cells and T and B lymphocytes. B cells are mainly responsible for humoral responses (i.e. antibody-mediated immunity), whereas T cells are involved in cell-mediated immunity. Both B cells and subtypes of T cells are meaningful for adaptive immunity. The adaptive immunity identifies invading pathogens, such as bacteria, and attempts to prevent them in the future, normally through a process of antigen presentation, elimination and memorization. The process involves a number of subsequent processes, such as the regulation of inflammatory markers (Chaplin 2010).

T cells develop from hematopoietic cells originating from bone marrow, but their major source of maturation is the thymus. T cells can be subtyped according to their surface glycoproteins, such as CD4* (T-helper, Th) and CD8* cells (cytotoxic T cells, Tc). The first subtype of Th cells (Th1) contributes to cell-mediated immunity orchestrated by the Tc cells, whereas the second subtype (Th2) participates in humoral immunity via B-cell actions. Th1 cells are characterized by the production of cytokines such as interleukin (IL)-2 and interferon (IFN)-γ, and Th2 cells by IL-4, IL-5 and IL-13, respectively (Parkin and Cohen 2001). The cells can be further categorized into several subtypes, such as Th17, regulatory T cells (Treg), and γδ T cells, which all have characteristic roles in the immune system (Awasthi et al. 2008, Chaplin 2010, Koch and Radtke 2011). The inflammatory markers such as the cytokines are presented in detail on page 19.

2.1.1 Hypothalamic–pituitary–adrenal axis

The hypothalamic pituitary adrenal (HPA) axis is an important regulator of stress reactions. The axis can be modulated by internal factors, such as immune cells, and external
stimuli, such as psychological stress. The HPA axis controls the immune system, the autonomic nervous system, and even reproduction (Whirledge and Cidlowski 2013, Li et al. 2014). The mechanisms involving the HPA axis are vital for understanding the plethora of immunomodulatory alterations associated with depression and ACEs.

The hypothalamus secretes neurotransmitters such as corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP). These hormones, among other tasks, promote adrenocorticotropic hormone (ACTH) secretion from the anterior pituitary gland. ACTH contributes to the regulation of the adrenal cortex, which is an endocrine organ located superiorly to the kidney. The adrenal cortex has three main layers: the zona glomerulosa, zona fasciculata and zona reticularis. The cortices regulate several physiological processes. For example, the zona glomerulosa controls blood pressure through mineralocorticoids such as aldosterone, and the zona reticularis regulates hormones associated with sexual development, such as testosterone and dehydroepiandrosterone (DHEA). The middle layer,
i.e. the zona fasciculata, is responsible for the secretion of glucocorticoids. The most abundant of the endogenous glucocorticoids is cortisol. As illustrated in Figure 1 on page 5, cortisol is primarily responsible for an inhibitory feedback action on both the hypothalamus and the anterior pituitary gland. Thus, an increase in cortisol levels leads to decreased expression of CRH and ACTH. The loop between the hypothalamic, pituitary and adrenal cortex hormones is defined as the axis.

Glucocorticoids have a significant impact on both peripheral tissues and the central nervous system (CNS). During transportation across the body fluids, endogenous glucocorticoids are bound to corticosteroid-binding globulin and albumin. The glucocorticoids attach to two main types of receptors: glucocorticoid (GR) and mineralocorticoid (MR) (Reul and de Kloet 1985). Both receptor types have high affinity for glucocorticoids and they bind to similar sequences on nuclear DNA. As reviewed by Hayashi et al. (2004), glucocorticoids activate gene expression through transcription factors such as the Signal Transducers and Activators of Transcription (STAT) family of proteins, nuclear factor-kappaB and activator protein-1. However, glucocorticoids can also induce persistent epigenetic changes, such as histone acetylation of DNA and chromatin reformation. Through these mechanisms, glucocorticoids regulate a number of immunomodulatory peptides, such

![Figure 2](image)

*Figure 2. Simplified process of T-cell differentiation and primarily secreted immunomarkers. The white boxes illustrate markers investigated in the publications of this study.*

IFN-γ = interferon-γ, IL = interleukin, Tc = cytotoxic T cell, Th = T-helper, Treg = regulatory T cell.
as chemokines, IL-5 and -6, tumour necrosis factor (TNF) α and adhesion molecules, and related signalling molecules such as secretory leukocyte inhibitory protein, IL-1 receptor type 2, IL-1 receptor antagonist, and β2-adrenoceptor.

In order to get to the CNS, the transporting glucocorticoids have to pass the blood–brain barrier (BBB), which by default allows lipid soluble steroids such as cortisol to enter (Karssen et al. 2001). On the other hand, the transportation of synthetic glucocorticoids, such as dexamethasone, is controlled by BBB gatekeepers such as multidrug resistance P-glycoproteins (Meijer et al. 1998). However, before international recommendations restricting the use of synthetic glucocorticoids during pregnancy, repeated doses were often used, which was associated with negative neurological outcomes in children (Matthews et al. 2002). Similar adverse outcomes have been illustrated in relation to an excess of endogenic glucocorticoids in animals (Holmes et al. 2005, Dubovsky et al. 2012). The negative outcomes may result from various sources, including the excitation of neurotransmitters such as N-methyl-D-aspartic acid (NMDA) and γ-aminobutyric acid (GABA) (Pattison et al. 2009). Furthermore, the excess glucocorticoids and excitatory activation can lead to gene-mediated alterations in GRs and MRs. The following reactions, such as calcium-influx, can alter depolarization trends in neurons. In adults, the acute phase responses could protect neurons and may sometimes serve a favourable behavioural purpose. The glucocorticoids appear to guide stressed individuals towards “selfish” nucleus caudate-based stimulus-response learning that leads to immediate self-comfort, instead of hippocampus-related spatial learning, which may guide allocentric actions such as sympathy (Joëls 2011). Nevertheless, the effects of glucocorticoids on the CNS could be modulated by interrelated steroids, such as dehydroepiandrosterone (DHEA), which moderate the effects of glucocorticoids throughout the body (Pattison et al. 2009).

**Glucocorticoids and the CNS**

As described by Herbert et al. (2006), glucocorticoids have typical patterns in their daily secretion (i.e. diurnal secretion), being typically highest in the morning and declining throughout the day. In addition, the NK and T cells of the blood have characteristic diurnal patterns (Trifonova et al. 2013) that follow the levels of hormones such as cortisol (Bollinger et al. 2010). The axis innervates to anatomical areas associated with diurnal regulation, such as the suprachiasmatic nucleus (SCN) located in the hypothalamus. For example, the levels
of cortisol can vary up to eight-fold during the day. However, the activity of the HPA axis is also affected by factors such as sympathetic signalling. Chung et al. (2011) reviewed the daily patterns related to the HPA axis and its sympathetic innervation, suggesting that even extracortical organs such as the adrenal cortices could contribute to the regulation.

In addition to the SCN, the hippocampus has attracted particular interest due to its central role in memory and behaviour. Joëls (2011) noted that acute exposure to glucocorticoids can lead to the release of glutamate-containing vesicles in hippocampal areas such as cornus ammonis 1 and 3. Glutamate is an abundant neurotransmitter and a precursor for GABA, but it also serves a metabolic role in processes such as glucose regulation. Neuroanatomical areas such as the basolateral amygdala and dentate gurys are enhanced by glucocorticoids in a noradrenergic-dependant manner, which may have a beneficial contribution to coping mechanisms after an acute sympathetic crisis, such as the “flight or fight” responses (Joëls 2011, Suri and Vaidya 2013).

Indeed, normal stress-responsive regulation of glucocorticoids is vital for the protection of neurons (Lupien et al. 2005, Wingenfeld and Wolf 2015), whereas dysregulation of glucocorticoids due to stress may have a number of adverse effects. Even mild chronic stress reduces cell survival in central areas such as the hippocampus (Lee et al. 2006). The exact mechanisms for the reduced cell survival are still unclear, although both decreased neurogenesis (Leuner and Gould 2010) and increased cellular apoptosis (i.e. regulated cell death) appear to contribute. The chronic excess of glucocorticoids also increases glutamatergic transmission in the hippocampus and could impair long-term potentiation (LTP) (i.e. the strengthening of nervous synapses), which is considered a prerequisite for learning (Joëls 2011). A significant molecule contributing to these neuronal changes is brain-derived neurotrophic factor (BDNF). BDNF can be moderated by glucocorticoids through multiple signalling cascades, such as the extracellular signal-regulated kinase (ERK) pathways (Suri and Vaidya 2013). Animals with chronically elevated glucocorticoids display reductions in hippocampal synaptic plasticity, which is seemingly attributable to a decreased expression of BDNF (Wosiski-Kuhn et al. 2014). Increasing evidence suggest that levels of neurotrophic factors, such as the BDNF, have a role in the degeneration of vital CNS structures such as the hippocampus (Duman and Monteggiq 2006). Lowered serum BDNF and other neurotrophic factors, such as vascular endothelial growth factors (VEGF), have characterized depressed individuals, and may be involved in the therapeutic effects of
antidepressants. The VEGF associates with neuroprotective trends, as it influences synaptic plasticity, the moderation of stress responses, and neurogenesis of vital areas such as the hippocampus (Clark and Halaris 2013). A degenerated hippocampus and atrophy of the medial prefrontal cortex (mPFC) is commonly associated with the progression of MDD (Mervaala et al. 2000, Treadway et al. 2015).

The cascade of events leading from stress responses to macroscopic outcomes such as atrophy is complex and in many ways unknown. One of the likely mediators of the cascade is, however, inflammation.

2.1.2 Cytokines

Physiology of cytokines

As reviewed by Dinarello (2007), cytokines derive from primitive signalling cascades and are thus prevalent in most living organisms. Unlike hormones, cytokines can be secreted from most cells. They are highly potent, soluble factors with diverse functions in both local and systemic cell–cell interactions, but also in autologic signalling. The majority of cytokines exhibit pleiotropism (i.e. they have diverse biological roles). For humans, they are lymphocyte growth factors, transmitters of anti- or proinflammatory responses, and even regulators of immune reactions in response to antigens. Cytokines participate in positive immunological responses such as vaccines, but can also drive negative effects, for example, in autoimmune diseases.

The cytokine superfamilies

Interferons (IFN) were the first set of cytokines to be discovered. In the 1960s, the first reports associated them - namely the IFN type 1 (IFN1) - with viral actions (Isaacs and Lindenmann 1957). IFN-γ was later observed to originate from lymphocytes, and was thus named lymphokine. Soon, new cytokines were discovered, each with characteristic traits. The second group, named chemokines, has similar roles in the migration of cells in response to chemical stimuli. Interleukins were discovered shortly after, being the first superfamily associated with leukocytes. Finally, tumour necrosis factors (TNF) formed an individual superfamily.
Cytokine regulation

Several factors influence the levels of circulating cytokines and cells expressing them. Cytokine expression may fluctuate during the course of a disease, and can be unexpectedly moderated by concurrent life-style factors and comorbidities. The potentially biasing factors for soluble measurements of inflammatory markers include, but are not limited to, the following: age (Bruunsgard et al. 2001), obesity (Gustafson 2010), exercise (Rosenblat et al. 2014), sleep duration (Meier-Ewert et al. 2004, Vgontzas et al. 2004), alcohol abuse (González-Quintela et al. 1999), smoking (Tsunoda et al. 2003), time of day (Altara et al. 2015, Izawa et al. 2013), CVDs (Han et al. 2007, Gustafson 2010), metabolic syndrome (Yudkin et al. 2004), autoimmune diseases such as Graves disease (Zhu et al. 2010), asthma and allergies (Katon et al. 2010, KleinJan et al. 1999), affective disorders such as MDD (Lehto et al. 2010, Young et al. 2014), post-traumatic stress disorder (Gill et al. 2008, Pace and Heim 2011), other psychiatric diseases (Kalia and Costa E Silva 2015) and antidepressant use (Sutcigil et al. 2007).

The available information on confounding factors far exceeds human indexing capabilities. However, advances in bioinformatics have yielded great opportunities, as future applications may automatically index studies and compile the results for further analysis. A few of such applications have been collected and managed by the National Center of Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/).

Cellular signalling of cytokines

A plethora of functions has been associated with the cytokines. For example, IL type 1 (IL-1) has been observed to control body temperature, modulate the synthesis of liver proteins and alter T-cell responses to antigens and mitogens (Dinarello et al. 2007), whereas TNF type α (TNF-α) can both support cell survival and cause cell death, depending on which receptor it attaches to (Brenner et al. 2015).

The cytokines act within blood and tissues, and in order to make changes at the cellular level, they have to use intracellular signalling pathways. Comprehending the signalling between the cell surface and the nucleus can be overwhelming. However, a few cytokine-associated major pathways are worth mentioning. The same physiological pathways contribute to autoregulation and are considered as central pathways, for example, for the development of “immortal”, cancerous cell lines.
When a protein, such as a cytokine, attaches to a cell surface receptor, a cascade of intracellular proteins starts interacting. The interactions within the cytoplasm usually involve core of the cell, i.e. the nucleus. When the interactions reach the nucleus, either through surface or intranuclear receptors, they start contributing to the production of ribonucleic acid (RNA) sequences. The produced RNA sequences are transported across the cell and participate in tasks such as intracellular regulation and the manufacture of new proteins. The signalling can also induce epigenetic changes, such as changes to the packaging of deoxyribonucleic acids (DNA), which may alter the production of the RNA sequences.

The intracellular signalling by cytokines has been laudably reviewed by Yoshimura (2009). Proinflammatory cytokines, such as IFN-α and -γ, activate cell-surface proteins, such as Janus kinase (JAK) proteins. JAKs activate cytoplasmic signalling such as STAT pathways, although the STATs are also expressed in the cell nucleus. In addition to the STATs, some cytokines, such as IL-5, may involve pathways such as the Ras-extracellular signal-regulated kinase (ERK) pathway. The Ras-proteins are guanosine triphosphate (GTP) hydrolyzing enzymes contributing to cellular signalling in practically all cells. The outcome of activation of the pathways is dependant on factors such as the cytokine and duration of the stimulus. For example, prolonged activation of STAT3 by IL-10 is associated with anti-inflammatory outcomes, whereas attachment of IL-6 to the STAT3 receptors can lead to transient proinflammatory arousal (Yoshimura et al. 2009, Suzuki et al. 2015). Another set of signalling markers, Spread and Sprouty, are intranuclear proteins especially contributing to cytokine-dependant growth factor signalling. For example, Spread1 participates in haematopoiesis and can suppress IL-5-dependant cell proliferation by inhibiting the proteins of the ERK pathway. On the other hand, cytoplasmic suppressors of cytokine signalling (SOCS) and cytokine-inducible SH2-containing (CHI) proteins have been associated with cytokine autoregulation, i.e. autologic signalling. Indeed, proteins such as SOCS1 can inhibit JAK-STAT pathways. However, SOCS1 is also essential for Th-cell differentiation and many other immunological processes. SOCS3 seems to promote Th2 development and is suggested to contribute, for example, to allergic reactions. The nuclear toll-like receptors (TLR) are also considered central moderators of cytokine production, meanwhile being potent inducers of SOCS (Yoshimura et al. 2009).

Ras-ERK is a neural plasticity-related pathway interacting with several other intracellular signallers, such as JAK2 and STAT5. Ras-ERK controls neural stem cell functions and
maintains specific brain structures (Phoenix et al. 2010). Hyperactivity of the Ras-ERK pathways causes deficits in synaptic plasticity and hippocampus-related learning in mice (Denayer et al. 2008). The JAK-STAT pathways, on the other hand, play important roles in cell proliferation and survival in both the CNS and the periphery through specific and diverse effects on cell responses to hormones, growth factors and cytokines (Heim et al. 1996, Zambrano et al. 2010). The activation of JAK-STAT can lead to symptoms of depression through glucocorticoid signalling (Hu et al. 2009). Inflammatory markers such as IFN-α and -γ activate the JAK family kinases and phosphorylate STATs (Darnell et al. 1994), which may consequently induce depressive symptoms (Felger et al. 2007).

The aim of this section was to illustrate the potentially diverse physiological effects of cytokines at both extra- and intracellular levels. Although the focus of this dissertation study was on illustrating stress- and depression-related immunomodulation as a systemic process, the exact mechanisms involving intracellular signalling are beyond the scope of this literature review.

Interleukins 5 and 13

As reviewed by Wynn (2015), IL-5 and -13 are expressed by Th2 cells, eosinophils, innate lymphoid cells, basophils and mast cells. IL-5 appears to be especially linked with eosinophilic activities, that is, the main focal point for allergic and parasitic reactions. Substantial evidence indicates that overproduction of Th2 cytokines such as IL-5 contributes to the harmful pathogenesis of asthma and severe allergies. Meanwhile, some Th2 cytokines may have beneficial roles in autoimmune diseases such as Crohn’s disease and multiple sclerosis (Tomasiak-Lozowska et al. 2010, Zhu et al. 2010, Chung et al. 2015). The various cell lines involved may explain the discrepancies in the pathogenetic roles of cytokines.

Adipocyte-derived cytokines

Adipocytes are cells of fat tissue. Only during the recent decades have we realized how significant an endocrinological role they play. Indeed, leukocytes are not the sole expressors of cytokines. Adipokines are a subtype of cytokines primarily associated with adipocytes (Lau et al. 2005). Leptin, adiponectin and resistin are the most studied of the adipokines, although current terminology mostly refers to them as adipose-derived hormones.
Adiponectin and resistin

Adiponectin has several oligomeric forms, with a high molecular state suggested to present its primary active form. It has anti-inflammatory traits through the inhibition of macrophages (Tilg et al. 2006), TNF-α and several other pro-inflammatory cytokines (Gustafson 2010). Adiponectin also attenuates sympathetic nervous system activity (Tanida et al. 2007), and may in turn be attenuated by sympathomimetic agonists such as catecholamines and dopamine (Fasshauer et al. 2001). The serum levels of adiponectin correlate negatively with adiposity; that is, the more obese a person is, the less adiponectin circulates in the blood. Indeed, adiponectin is predominantly secreted by adipose cells and seems to serve an antiatherogenic and insulin-sensitizing role, thus contributing to the pathogenesis of diabetes and CVDs (Han et al. 2007). Lowered levels of adiponectin characterize metabolic cluster conditions such as obesity, type 2 diabetes and atherosclerosis, whereas individuals with higher levels have a diminished likelihood for naïve coronary events (Han et al. 2007). However, a recent meta-analysis suggested an opposite association in the case of occurred cardiac events, as elevated adiponectin related to increased post-ischemic mortality (Wu et al. 2015). A review by von Frankenberg et al. (2014) and Han et al. (2007) suggested that adiponectin levels can be elevated through supplementation with n-3 polyunsaturated fatty acids, physical activity and weight loss, although verification through randomized controlled trials is lacking.

In contrast, resistin is an adipose-derived hormone that is positively associated with metabolic disorders (Steppan et al. 2001). As reviewed by Badoer et al. (2015), resistin is often related to adiposity and adipocytes, although in humans it appears to be mainly produced by macrophages. Indeed, macrophages can infiltrate adipocytes, and may thus be overexpressed within the tissues of an obese person. The circulating levels of resistin correlate positively with adiposity, and as such, conversely with adiponectin. Receptors through which resistin exerts its effects are relatively unknown. Nevertheless, its expression induces sympathetic nervous system activation.

A third extensively studied adipokine is leptin. The physiology of leptin has been reviewed, for example, by Münzberg and Morrison (2015), suggesting it has similar characteristics to resistin, including a stimulatory role in sympathetic tone. However, leptin was beyond the scope of this study, and is thus not further introduced.
Cytokines and neuroanatomy

Both overexpression and an absence of cytokines modulate CNS functions such as synaptic plasticity and neurogenesis. The modulation involves activity within dopamine-sensitive areas such as the basal ganglia and frontal cortex (Nadjar et al. 2005, Capuron et al. 2005 and 2007, Nelson et al. 2002). For example, basal ganglia are associated with functions such as procedural learning, emotions, cognition and voluntary movement, whereas the frontal cortex contributes to traits such as motivation, expression of emotions, attention, planning and long-term memory. A review by Kemptom et al. (2011) suggests that MDD is characterized by distinct neuroanatomical changes in both the above-mentioned areas, in addition to other landmarks. Such neuroanatomical variations may distinguish MDD from other psychiatric diseases such as bipolar disorder (BD).

Cytokines and pathology

Although the associations between biomarkers and psychopathology are often small, they may be clinically relevant, as even slight alterations in the levels of cytokines can modulate CNS functions (Pollmächer et al. 2002). The roles of cytokines vary between diseases and cytokine types. Some cytokine abnormalities, such as elevated IL-5, associate with many diseases, but could do so because they may provide protection to the CNS (Hendrix et al. 2007). Such accumulation of therapeutic signalling markers is referred to as protective autoimmunity.

Cytokines are immune system signalling molecules that affect the synthesis, release, and cell reuptake of monoamines (McAfoose et al. 2009). However, only a proportion of cytokines are secreted within the CNS, while the rest are transported across the BBB from the peripheral circulation (Banks et al. 2005, Raison et al. 2006). As reviewed in a previous section starting from page 4, the HPA axis is involved with cytokines. The connection between activation of the HPA axis and inflammatory arousal is observable during “sickness behaviour”, i.e. when an inflammatory disease, such as a fever and flu, induces depression-like symptoms, such as fatigue and lowered mood. Some have suggested that the elevated circulatory cortisol levels in MDD patients could cause Th2 cytokine dysregulation (Pavón et al. 2006). However, the association between the HPA axis and inflammatory markers is probably bidirectional (Turnbull and River 1999).
2.1.3 Prothrombosis and fibrinolysis

Factors such as obesity and chronic inflammation provoke a systemic imbalance in terms of thrombosis, most often causing a prothrombotic condition (Samad and Ruf et al. 2013). Prothrombotic conditions associated with mood disorders especially include vascular hypercoagulability and atherothrombosis (von Känel et al. 2001).

During a typical prothrombotic condition, such as when a wound occurs, fibrinogen is polymerized by the protease thrombin, thus forming an elastic protein called fibrin. The fibrin connects with platelets to form a haemostatic clot. The clot is an important physiological process in preventing excessive bleeding, but may also pathologically contribute to, for example, atherothrombosis. However, the fibrinolytic system attempts to dissolve any unnecessary fibrin clots. If fibrinolysis fails and the clot is not dissolved or the area otherwise revascularized, the tissue suffers ischemia, which leads to necrosis of the targeted tissue.

Plasminogen activator inhibitor type 1 (PAI-1)

Plasminogen activator inhibitor type 1 (PAI-1) is a central component in the process of fibrinolysis. When a need to start dissolving occurs, an enzyme called plasmin starts to be converted from its precursor, plasminogen. The conversion is orchestrated by two main enzymes: tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA). PAI-1 inhibits both t-PA and u-PA.

PAI-1 is an unstable molecule and is expressed in many tissues, including adipocytes (Van De Craen et al. 2012). Approximately 80% of PAI-1 is in its active form (Dellas et al. 2005). The majority of it is stored in platelets, but about one-fourth lingers in plasma (Van De Craen et al. 2012). It belongs to a superfamily of serine protease inhibitors (SERPINE). The superfamily is divided into non-inhibitory and inhibitory serpins, with PAI-1 belonging to the latter (Van De Craen et al. 2012).

Regulation of PAI-1

PAI-1 is a stress-induced acute phase reactant (Tsai 2006). It has characteristic diurnal secretion (Irokawa et al. 1998), the expression being regulated by a family of CLOCK and CRY genes (Ohkura et al. 2006, Oishi et al. 2009). The HPA axis is also regulated by the temporally oriented CLOCK genes (Herbert et al. 2006). The levels of PAI-1 are modulated
by factors such as inflammatory cytokines, growth factors, sympathetic nerves and adiposity (Cesari et al. 2010, Jiang et al. 2011, Schaefer et al. 2006).

The potentially biasing factors for soluble measurements of PAI-1 may include, but are not limited to, the following: age (Yamamoto et al. 2005), gender (Krishnamurti et al. 1988), smoking (Ozaki et al. 2010), obesity (Allman-Farinelli 2011), serum insulin levels (Agren et al. 2008), metabolic syndrome (Coffey et al. 2011), cardiovascular diseases (Meier-Ewert et al. 2004), thrombotic diseases (Yamamoto et al. 1998), use of statins (Kruithof 2008), asthma (Cho et al. 2011), sleep disordered breathing (Rångemark et al. 1995, Matthews et al. 2010, Toraldo et al. 2012), acute stress (von Känel et al. 2002, Tsai 2006), depression (Lahlou-Laforet et al. 2006, Huotari et al. 2010, Baune et al. 2012), and antipsychotic medication, although the effect is possibly mediated by the body mass index (BMI) (Carrizo et al. 2008).

**Neuroprotection and PAI-1**

Both sleep disturbances and chronic stress can cause a hypercoagulable state, a condition related to elevated levels of serum plasminogen activator inhibitor 1 (PAI-1) (Rångemark et al. 1995, von Känel et al. 2001 and 2007). However, in addition to balancing out blood coagulation, the fibrinolytic system involves additional mechanisms related to the CNS.

As reviewed before, PAI-1 cleaves plasminogen into plasmin. In addition to dissolving fibrin clots, plasmin can contribute to the cleaving of proBDNF into BDNF (Figure 3, p. 17). BDNF stimulates mitogen-activated protein kinase (MAPK)/ERK signalling, which has been associated with neuronal survival, neuronal morphogenesis and neuronal plasticity (Numakawa et al. 2010). Such associations have led to investigations into whether elevated levels of PAI-1 could be a desirable condition.

Soeda et al. (2008) suggested that the neuroprotective effects of PAI-1 could also be exerted through direct stimulation of the MAPK/ERK pathways. The potential mechanisms include inhibition of neuronal death through the modulation of NMDA-related calcium influx (Soeda et al. 2008), a process critical for synaptic plasticity (Docagne et al. 2002).
Psychopathology and PAI-1

Dysregulation of the HPA axis, such as in sleep disturbances and chronic stress, is associated with a hypercoagulable state. The hypercoagulation is a condition related to elevated levels of serum plasminogen activator inhibitor 1 (PAI-1) (Rångemark et al. 1995, von Känel et al. 2001 and 2007). Increased expression of PAI-1 has been associated with pathological conditions such as regular smoking (Ozaki et al. 2010), elevated BMI (Allman-Farinelli 2011), metabolic syndrome (MetS) (Coffey et al. 2011), CVD (Meier-Ewert et al. 2004), several thrombotic conditions (Yamamoto et al. 1998), sleep-disordered breathing (Rångemark et al. 1995, Matthews et al. 2010, Toraldo et al. 2012) and asthma (Cho et al. 2011). Elevated PAI-1 also characterizes remitted depression in elderly populations, as well as mood-disordered individuals with comorbid metabolic diseases (Lahlou-Laforet et al. 2006, Baune et al. 2012). A study on a small sample of premenopausal women illustrated similar results (Eskandari et al. 2005), suggesting that PAI-1 may contribute to the pathophysiology of depression.

2.1.4 Role of the blood–brain barrier

The BBB has an important regulatory role in the exchange of biomarkers between the CNS and peripheral blood. However, the clinical relevance of the regulation is often undervalued. A study on orthopaedic surgeries reported that most cytokines are post-operatively activated both in cerebrospinal fluids (CSF) and in blood. The post-operative cytokine-effects appear unrelated and even more pronounced in the CSF than in the serum (Bromander et al. 2012).
On the other hand, some infections, such as rabies virus (lyssavirus), are notorious for causing severe effects on an individual’s behaviour. During a rabies infection, the tight junction proteins of the BBB are downregulated in a cytokine-dependant manner, hypothetically to improve the transportation of immune system effectors (Chai et al. 2014). Indeed, many of the cytokines, such as TNF-α, can have potent effects on the CNS, one being the moderation of BBB permeability (Rochfort and Cummins 2015). Such observations highlight the role of cytokines as central factors in BBB regulation and illustrate the potency for peripheral stress to induce inflammatory marker activation within the CNS.

*In vitro* models have illustrated that PAI-1 also tightens endothelial tight junctions of the blood–brain barrier (Dohgu et al. 2011). Thus, PAI-1 may moderate the transportation of biomarkers across the BBB. These observations may relate to the plamin-associated cleavage of proBDNF to BDNF, which may explain the therapeutic effects of statins on conditions such as MDD (Tsai et al. 2007).

As reviewed in this section, immunity may play a major role in several psychopathological conditions. The scope of changes related to depression reaches from macroscopic events, such as the atrophy of neuroanatomical areas, to gene-level interactions, such as epigenetic changes.

### 2.2 Major depressive disorder

#### 2.2.1 Diagnosis of major depression

The diagnosis of depression is based on a relatively numerous selection of coexisting symptoms. There are two main guidelines for diagnosis: the Diagnostic and Statistical Manual of Mental Disorders (DSM; American Psychiatric Association) and the guidelines of the Classification of Mental and Behavioural Disorders (ICD; World Health Organization). The presented studies used the criteria of DSM version IV (DSM-IV). DSM-V, released in 2013, is the most current version of the guidelines, including only minor changes to the diagnosis of MDD.

The criteria used for a major depressive episode according to DSM-IV (American Psychiatric Association 1994):

- Depressed mood and/or loss of interest or pleasure in life activities for at least 2 weeks;
- At least five of the following symptoms that cause clinically significant impairment in everyday life:
a) Depressed mood most of the day, either subjective report (e.g., feels sad or empty) or objective (e.g., appears tearful);

b) Markedly diminished interest or pleasure in most activities, either subjective or objective;

c) Significant weight loss without dieting (i.e. >5% of body weight in a month), or change in appetite;

d) Insomnia or hypersomnia;

e) Psychomotor agitation or retardation, which is observable by others;

f) Fatigue or loss of energy;

g) Feelings of worthlessness or excessive or inappropriate guilt;

h) Diminished ability to think, concentrate or make decisions, either subjective or objective;

i) Recurrent thoughts of death or suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide.

In addition:

- The symptoms do not meet the criteria for a mixed episode;

- The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning;

- The symptoms are not due to the direct physiological effects of substance abuse or a somatic medical condition;

- The symptoms are not better accounted for by bereavement.

The additional criteria for major depressive disorder (MDD):

- The major depressive episode is not better accounted for by schizoaffective disorder and is not concurrent with schizophrenia, schizophreniform disorder, delusional disorder, or psychotic disorder not otherwise specified;

- There has never been a manic or a hypomanic episode (i.e. unipolar).

The additional subcriteria for recurrent MDD:

- Two or more major depressive episodes.

2.2.2 Epidemiology of depression

The MDD is one of the world’s most common cause of disability and death (WHO 2004, Ferrari et al. 2013, Global Burden of Disease Study 2013 collaborators 2015). Retrospective studies suggest that 17% of the population experience an MDD episode during their lifetime.
(Kessler et al. 2005), women almost twice as often as men (Alonso 2007). However, the retrospective observations are susceptible to recall bias, i.e. people “forget” having an episode. Prospective studies controlling for such bias suggest that the lifetime prevalence could be over double the figures reported in retrospective studies (Moffitt et al. 2010). In 2011, the 12-month prevalence of MDD in the Finnish general population aged over 30 was up to 7.4% (Markkula et al. 2015).

Up to 90% of all the suicides are accounted for by mental illnesses (Arsenault-Lapierre et al. 2004, OECD 2013). Most often, the individuals at risk of suicide are comorbid with medical conditions such as mood, personality or anxiety disorders or alcohol dependence (Rojat et al. 2014, Wang et al. 2015). Socioeconomic factors such as unemployment and financial difficulties increase the risk of both depression and suicide (Viinamäki et al. 1995, Hintikka et al. 2000). Indeed, MDD associates with an increased prevalence of suicide; however, MDD also associates with an increased likelihood of several somatic conditions such as CVD, diabetes, cancer, asthma and allergic conditions (Zorrilla et al. 2001, Timonen et al. 2003 Pace et al. 2007). The current economic burden of MDD and its somatic and psychiatric comorbidities in the United States has been estimated at $210.5 billion (Greenberg et al. 2015, Gustavsson et al. 2011), and the respective value for Europe is €91.9 billion (Olesen et al. 2012). However, the overall increase in morbidity caused by the somatic associations is difficult to predict.

2.2.3 Pathogenesis of depression

During the 1980s, scientists had already suggested a key role for the HPA axis in the pathophysiology of depression (Carrol and Curtis 1976). A dysregulated HPA axis has explained alterations linked to depression, such as the increased levels of endogenic glucocorticoids and sympathetic arousal (Kronfol and House 1985). The dysregulation was also investigated in relation to immune parameters; for example, Charles et al. (1989) observed that resistance of lymphocytes to exogenous glucocorticoids (i.e. glucocorticoid-insensitivity) predicts the risk of an MDD relapse. Similar glucocorticoid insensitivity was illustrated in individuals with hypercortisolemia (e.g. Cushings disease), but also in healthy individuals undergoing experimental stress (Sauer et al. 1995).

At the turn of the 1980s, a number of scientists observed an increased prevalence of somatic complications in psychiatric patients and started to investigate their immune parameters (Dvorakova et al. 1980, Kronfol and House 1985, Maes et al. 1989). Whether MDD
is related to consistent immune-cell alterations remained inconclusive until early meta-analytic reviews. For example, Herbert and Cohen (1993) concluded that clinical depression associated with lowered NK cell activity, altered white blood cell counts and reduced proliferative responses of lymphocytes. Indeed, according to the monocyte-T-lymphocyte hypothesis of major depression, the pathophysiology of MDD precedes immune system activation (Maes et al. 1995). The theory still appears - in principle - correct, although the causalities remain unclear.

Meta-analyses conducted in the 2010s have verified that MDD has several distinct biological features, including increased levels of lymphocyte subsets, such as natural killer cells (NK), and hypersensitivity of neurotransmitters, such as catecholamines. Features such as these contribute to the altered immune responses and neuronal communication within the brain tissue (Zorrilla et al. 2001, Irwin and Miller 2007). Individuals with MDD have also illustrated an imbalance between Th1 and Th2 cells (Sredni-Kenigsbuch 2002) and biomarkers related them (Maes et al. 1994, Myint et al. 2005, Huang and Lee 2007). Ever since the discovery of the immunomodulatory potencies of cytokines, they have been sought to explain both symptoms and somatic risks related to depression (Felger et al. 2013). Nevertheless, as reviewed by Zunszain et al. (2011), evidence continues to indicate that MDD is also characterized by states of both hypersecretion and hyperreactivity of circulating cortisol, elevated cerebrospinal CRH, and activation of the pituitary and adrenal glands. The reduced effectiveness of glucocorticoids in causing normal physiological effects (i.e. glucocorticoid insensitivity) appears to characterize chronic depression (Holsboer et al. 2000, Parker et al. 2003a). Stressful states illustrating excess glucocorticoids have also associated with altered gene expression and epigenetic modifications, such as deacetylation of histones (Hayashi et al. 2004) and DNA methylation (Babenko et al. 2015).

It is fair to note that conflicting results have frequently been reported from studies investigating immune markers and depression. Some of the irregularity is probably explained by differences in the study settings and populations, namely the phase of the illness (Eyre et al. 2014), and the severity of the symptoms and symptom clusters (Maes et al. 2012). Most of all, a small sample sizes often leave the results inconclusive, and as the same theme is investigated from various points of view, the metabolic associations are often omitted (Kunugi et al. 2015). Indeed, MDD associates with an increased likelihood of several metabolic disturbances such as CVDs and diabetes, and somatic diseases such as cancer,
asthma and allergic conditions (Timonen et al. 2003). Current evidence suggests that depression, psychological stress and at least the metabolic cluster diseases are all characterized by an altered inflammatory state (Wolkowitz et al. 2001, Dowlati et al. 2010).

2.2.4 Inflammatory markers related to depression

C-reactive protein

Perhaps one of the most common markers for measuring inflammation is C-reactive protein (CRP). It is clinically used as an unspecific marker for inflammation. High-sensitive CRP (hsCRP) allows the quantification of over 100-times lower concentrations of CRP than classical methods. A slight above-average elevation of CRP is commonly referred to as low-grade inflammation.

Reactive signal markers, such as the acute phase proinflammatory cytokine IL-6, activate CRP production in the liver. However, low-grade inflammation, as indicated by elevated hsCRP, is considered more of a chronic condition. hsCRP has gained rapid interest, as it has a robust association with several important public health diseases, such as CVDs (VanGilder et al. 2014, Di Napoli et al. 2005) and MDD (Liukkonen et al. 2006, Valkanova et al. 2013, Young et al. 2014). Consistent evidence suggests that elevated hsCRP is a feasible marker for assessing the CVD risk during both secondary and tertiary prevention (VaGilder et al. 2014, Di Napoli et al. 2005). Elevated CRP may also characterize non-dementic cognitive decline (Yang et al. 2015), as well as conditions such as sleep disturbances (Liukkonen et al. 2007), but also psychiatric diseases such as schizophrenia (Miller et al. 2014). The state of low-grade inflammation may also be clinically relevant in depressed patients, as it appears to predict treatment outcomes. Elevated baseline levels of CRP predict poor treatment responses in MDD, whereas some other markers of inflammation, such as IL-6, decrease during treatment. Some of the alterations may be attributed to the independent effects of antidepressants (Strawbridge et al. 2015).

We do not know whether decreasing the low-grade inflammation of MDD patients attenuates the somatic risks, such as unfavourable CVD outcomes. Indeed, individuals with MDD represent an extremely heterogeneous group with a vast range of health issues. Thus, whether or not elevated CRP is causal to the symptoms of depression remains debated. A small (n = 20/20) clinical trial by Cubała and Landowski (2014) following strict exclusion criteria investigated this matter on drug-naïve first-episode major depressive patients, and
suggested that early-stage first-episode MDD is not characterized by elevated CRP, although altered HPA axis activity is present. Such observations could suggest that inflammation does not provoke the depressive symptoms per se, but could rather alter during the progression of the disease because of symptoms and accumulating health adversities. These “after-effects” and their biological manifestation are probably augmented by altered neuroimmunomodulatory responses, which may have been dysregulated by previous experiences such as ACEs, states of low-grade inflammation and previous depressive episodes.

Cytokines and depression

IFN-γ is one of the most studied cytokines in MDD (Kim et al. 2007). It is often elevated in psychiatric disorders, especially in the case of suicide, although the evidence lacks consistency (Ganança et al. 2015). In addition, elevated levels of TNF-α have fairly consistently characterized MDD patients (Young et al. 2014) and like CRP, consistently elevated TNF-α may predict a poor treatment outcome for depression (Strawbridge et al. 2015). TNF-α, but also other cytokines such as IL-1, may serve a neuroplasticity-modulating role in MDD (Khairova et al. 2009). In some cases, anti-TNF-α treatment has proven to be beneficial in alleviating the depressive symptoms (Rosenblat et al. 2014). Large clinical studies have also associated MDD with elevated levels of IL-4 and IL-13 (Pavón et al. 2006, Hernández et al. 2008).

Relatively little is known about the role of the Th2 cytokines IL-5 and IL-13 in MDD. Studies have failed to illustrate differences in IL-5 in MDD patients (Simon et al. 2008). However, individuals with, for example, asthma suffer from an increased risk and severity of depression, whereas MDD patients with comorbid asthma suffer from greater impairments in asthma symptoms (Katon 2010). Although very little data have covered the potential mechanisms connecting asthma and MDD, Th2 cytokines appear to play a role in the pathophysiology of both of these diseases (Pavón et al. 2006, Levine and Wenzel 2010, Shelton et al. 2011, Bhakta et al. 2011).

Altered levels of adipocyte-derived cytokines, namely elevated levels of adiponectin and lowered resistin, characterize individuals with major depressive disorder and adverse psychological symptoms (Leo et al. 2006). However, meta-analyses have illustrated non-consistency in the results, and bias has been attributed to population characteristics and the
applied laboratory methods (Carvalho et al. 2014). For example, both depression and childhood adverse events associate with obesity (Rohde et al. 2008), which associates with increased numbers of adipocytes and low-grade inflammation.

## 2.3 Adverse Childhood Events

### 2.3.1 Definition of adverse childhood events

Adverse childhood events (ACEs) are sets of traumatic events that are expected to result in negative outcomes for the children experiencing them. Martins et al. (2011) described four main types of ACEs (*Figure 4*, p. 24):

- **Physical abuse** includes actions violating the physical boundaries of a child. The actions inflict pain or harm, either directly or through instruments or toxins.

- **Sexual abuse** involves a child encountering sexual activity beyond her or his full consent or appropriate stage of development. Adults or children alike may inflict the abuse. Boundaries between abuse and other forms of sexual contacts are culture specific.

- **Emotional abuse** (i.e. psychological abuse) involves offensive or otherwise damaging behaviour from a caretaker to a child. The damaging behaviours are non-physical, such as threatening, frightening, restriction, rejection, hostile behaviour and ridiculing.

- **Neglect** is a pattern of insufficient support from a caretaker, such as disregard of the child’s emotions, physical health and nutrition or general safety.

*Figure 4. The four main types of adverse childhood events (ACEs).*

Other types of maltreatment suggested to lead to adverse development include events such as maternal or paternal deprivation, death of a caretaker, and a hostile living
environment (Martins et al. 2011). In the literature, several structured, validated questionnaires have been used to assess the history of ACEs in adult populations, such as the childhood trauma questionnaire (Bernstein et al. 1994), Adverse Childhood Experiences Study questionnaire (Felitti et al. 1998) and Stressful Life Events Screening Questionnaire (Goodman et al. 1998).

2.3.2 Alcohol abuse in the childhood home

In addition to the types of maltreatment listed above, adults who report experiencing alcohol abuse in their childhood homes have an elevated risk of psychological disorders in adulthood. They are at increased risk of disorders persisting throughout late adolescence and during young adulthood (Brook et al. 2010), such as sleep disturbances (Koskenvuo et al. 2010), and psychiatric diseases such as somatoform dissociation (Maaranen et al. 2004) and MDD (Hill et al. 2008). Individuals living with alcohol-abusing caretakers are more likely to experience other adverse events in early life, such as emotional abuse, which may contribute to the increased likelihood of later psychological symptomology (Anda et al. 2002) and suicide (Dube et al. 2001).

2.3.3 Early life stress and morbidity

Over 60% of individuals experiencing mood or anxiety disorders report events of early life stress (Martins et al. 2011). Furthermore, adults with a history of ACEs share pronounced vulnerability to rapidly increasing public health concerns such as obesity and CVD (Rohde et al. 2008, Danese et al. 2009, Gilbert et al. 2009). Such associations are also observable in Finnish populations, at least in individuals with a disadvantaged residential location, such as accommodation distant from city centres (Halonen et al. 2015).

Some hypotheses have suggested that the long-term adverse effects of ACEs are mediated by an increased prevalence of interrelated factors, such as living in a disadvantaged environment or having an unhealthy or dangerous lifestyle. Indeed, it is likely that individuals with ACEs are susceptible to persistent stress and health adversities. However, the risk, for example, of CVDs appears independent from known metabolic risk factors, such as obesity (Danese et al. 2009), suggesting an alternative pathophysiological mechanism for the somatic comorbidity.
2.3.4 Biology of adverse childhood events

The ACEs can have permanent effects on an individual’s neurobiology (McEwen et al. 2007). Physical violence and sexual abuse are both characterized by severe violation of an individual’s physical boundaries and may elicit similar neurobiological responses (Weber et al. 2004), but the non-physical forms of maltreatment also have severe consequences. Significant biological differences exist between individuals who have experienced ACEs and those who have not, or more precisely, those who do not report experiencing such. It is relevant to distinguish between early life (normally <7 years of age) and later childhood adverse events, as, for example, the morphological changes in the CNS differ (Baker et al. 2013). As reviewed by De Bellis and Zisk (2014), ACEs have been associated with alterations in many biological processes. The processes involved may vary depending on the time and duration of the ACEs. Dysregulation of pathways such as serotonin, oxytocin and locus coeruleus-noradrenergic systems, but also epigenetics, are involved in the pathogenetic models. However, perhaps the most studied entity related to ACEs is the limbic HPA axis.

The CNS is first vulnerable during the foetal period, as maternal stress during pregnancy puts the offspring at risk of both metabolic and neurocognitive disorders (Talge et al. 2007, Kinsella and Monk 2009). Persistent vulnerability can also occur during adolescence, due to ACEs. Some have suggested that the HPA axis is “reprogrammed” in the presence of stress during critical ages (de Kloet et al. 2005). The reprogramming may manifest as pathological emotional stability and, for instance, Axis II personality disorders (PD). PDs such as borderline personality often follow severe childhood adverse experiences (Afifi et al. 2011), co-occur with depression and anxiety, and associate with their poor long-term treatment outcomes (Riihimäki et al. 2014).

In individuals reporting ACEs they are often accompanied by psychiatric symptoms and vulnerability to later stress (Penza et al. 2003). Not all individuals experiencing ACEs develop psychiatric symptoms, but depression, anxiety and substance abuse in individuals with ACEs associate with a greater severity of the symptoms, as well as morbidity (Teicher and Samson 2013). The vulnerability may involve mediators of stress responses and subsequent persistent sensitization of the CNS (Heim et al. 2001). For over a decade, the pathogenetic mechanisms of ACEs have been associated with altered functioning of the HPA axis and the autonomic nervous system (Heim et al. 2001). Indeed, the responses of both the HPA axis and the sympathetic nervous system, such as heart rate variability, seem blunted in
individuals reporting ACEs (Voellmin et al. 2015). Acute stress can increase HPA activity, whereas chronic stress can lead to reciprocal outcomes, such as decreased stable state CRH and glucocorticoids (Pinnock et al. 2001). ACEs also associate with an imbalance between the glucocorticoid receptor subtypes (de Kloet 2013).

The neurons of individuals experiencing ACEs may suffer from excess pruning, which is the selective removal of neuronal connections (McLaughlin et al. 2014). Animal studies suggest that ACEs could impair neurogenesis and neuroplasticity in the CNS, and decrease neuronal activity in vital areas for cognition, such as the hippocampus (Kim et al. 2006). Although the development of the subcortical nervous system is not entirely similar in rats and humans, for most species the hypothalamus and the pituitary gland seem to be important pathophysiological areas for ACEs (Kalmakis et al. 2015). In addition, the amygdala may be of importance, as it contributes to how one experiences sensory stimuli. Early life stress could alter gene-expression patterns in the amygdala, and possibly induce anxiety-related disorders later in life (Sarro et al. 2014). Indeed, some psychiatric patients, such as post-traumatic stress disorder patients with a history of ACEs, have a smaller right-side amygdala (Veer et al. 2015). The timing of the stress is important, as early life stress may induce the most detrimental alterations (Koppensteiner et al. 2014). In addition, areas participating in the regulation of the amygdala, such as the ventromedial prefrontal cortex (vmPFC), appear to inactivate and wither under constant stress. The vmPFC contributes to decision making and emotional responses, as reviewed by McLaughlin et al. (2014). Nevertheless, in addition to such CNS changes, ACEs are linked to broader systemic imbalance.

Persistent evidence suggests that ACEs associate with increased levels of circulating inflammatory markers, such as proinflammatory cytokines and CRP, but also with activation of the fibrinolytic system, such as elevation in the levels of fibrinogen (Coelho et al. 2014). A prospective longitudinal cohort study by Danese et al. (2008) illustrated that the history of ACEs is characterized by persistent proinflammatory state. The presence of depressive symptoms augmented the association between inflammation and ACEs; nevertheless, they suggested that inflammatory changes in their sample were mainly attributable to the childhood experiences. However, such assumptions may be too linear, as will be discussed in the following sections.
Inflammation and ACEs

ACEs have been associated with elevated levels of high-sensitivity C-reactive protein (hs-CRP) (Danese et al. 2007, 2008 and 2009). A recent preliminary study suggested that during stressful situations, individuals with a history of childhood maltreatment display greater IL-6 concentrations than those not reporting an adverse background (Carpenter et al. 2010). Elevated IL-6 and CRP during childhood may predict later depression and suicide in a dose-dependant manner (Khandaker et al. 2014). A review by Coelho et al. (2014) illustrated an association between ACEs, elevated CRP and proinflammatory cytokines; however, they concluded the evidence regarding anti-inflammatory cytokines to be insufficient.

Adiponectin and ACEs

Activation of the sympathetic nervous system by synthetic agonists has been reported to suppress adiponectin gene expression (Fasshauer et al. 2001), and pronounced sympathetic activity has been associated with lower levels of adiponectin, at least in diabetic men (Takahashi et al. 2007). On the other hand, ACEs could lead to persistent hyperactivity of the sympathetic nervous system (Charmandari et al. 2003). Such a relationship between sympathetic tone and adiponectin could be bidirectional, since in an animal model, adiponectin administration attenuated sympathetic nervous system activity (Tanida et al. 2007). The mechanism by which adiponectin and the sympathetic network interwined might be direct (Fasshauer et al. 2001, Tanida et al. 2007), but could also be linked to pronounced secretion of pro-inflammatory cytokines such as TNF-α and IL-6 (Liu and Liu 2009).

2.4Sleep disturbances

Sleep disturbances can be evaluated with objective variables, such as the polysomnographic duration of sleep. However, evaluation can also be subjective, such as self-reported daytime symptoms. As I only investigated self-reports in this study, here I mostly discuss subjective evaluation.

2.4.1Definition of sleep disturbances

Sleep can be disturbed due to several reasons. The vast diagnostic pool of sleep disorders includes hypersonnias, parasomnias, circadian rhythm sleep disorders, narcolepsy and cataplexy, sleep-related movement disorders and sleep–wake disorders such as nightmare disorder. One of the most common conditions concerning sleep is insomnia, which can be
defined as follows: persistent difficulty initiating sleep, difficulty in maintaining sleep, waking up too early, or sleep that is chronically non-restorative or poor in quality. The abovementioned disturbances should co-occur with daytime impairment related to adverse sleep, such as tiredness. Depending on the guidelines, the disturbances may or may not co-occur with a psychiatric disease, or other contributing factors such as certain medicines or somatic diseases (DSM; American Psychiatric Association, ICD; World Health Organization). Nevertheless, insomnia is most often a comorbid condition, and several diseases may cause the disorder, such as gastroesophageal reflux disease or chronic pain (Katz et al. 1998, Roth 2007, Van Cauter et al. 2008). As many as 40% of individuals reporting insomnia have a psychiatric illness, the most common being depression and anxiety disorders (Roth 2007). On the other hand, hypersomnia, i.e. that is prolonged sleep, occurs in up to 76% of adults with MDD (Kaplan and Harvey 2009). Despite their common nature, insomnia and concurrent depression present a multidimensional challenge for healthcare.

Due to the ambiguity of the word “insomnia”, I discuss the issue by using the term “sleep disturbances”, which is more descriptive for the subjective experiences of impaired sleep.

2.4.2 Epidemiology and aetiology of sleep disturbances

Sleep disturbances are common, as symptomatic individuals may comprise up to 30% of adults across populations (Roth 2007). Sleep disturbances are associated with various adversities, such as a lowered quality of life (Zammit et al. 1999, Roth 2007), psychiatric morbidity and suicide (Ford and Kamerow 1989, McCall et al. 2010). Poor sleep also predisposes to significant somatic risks (Kupperman et al. 1995) such as developing and dying from CVDs (Sofi et al. 2014). In the elderly, the disturbances precede cognitive impairment and dementia (Yaffe et al. 2014).

Most individuals with depression report sleep disturbances in both active and residual symptom phases. On the other hand, the prevalence of sleep disturbances increases the likelihood of both primary depression and its relapse (Baglioni et al. 2011, Harvey 2011, Perlis et al. 2006). Indeed, the link between sleep disturbances and mood is probably bidirectional. However, associations between depression and some forms of sleep disorders, such as obstructive sleep apnoea (OSA), are not as well established (Gupta and Simpson 2015).
2.4.3 Pathophysiology of sleep disturbances

Self-reported symptoms of sleep disturbance seem to predict mortality more strongly than the actual measured sleep time (Dew et al. 2003, Kaplan and Harvey 2009). Studies in high school students, the elderly and military veterans have suggested that elevated self-reported sleep disturbance may indeed reflect overall distress rather than the functional sleep time (Alapin et al. 2000, Dew et al. 2003, Waldron-Perrine et al. 2012). Interestingly, several inflammatory markers have characteristic differences between subjective sleep disturbances and actually altered sleep duration (Okun et al. 2009, Patel et al. 2009, Dowd et al. 2011).

Physiological alterations of sleep are complex, as both autonomic and endocrine dysfunctions are present in a psychosocial context. The disturbance of sleep has several potential ways to crosstalk with MDD, such as similar inflammatory marker expression and rapid eye movement (REM) sleep behaviours (Irwin and Miller, 2007, Motivala et al. 2011). The mechanisms linking sleep disturbances and adverse somatic outcomes are also multifactorial. For example, an increased appetite may contribute to the CVD risks associated with sleep loss. Indeed, sleep can contribute to the regulation of the appetite through signalling from cells such as adipocytes. “Hungry” cells moderate signalling markers, leading to an increase in the gastrointestinal hormone ghrelin and a decrease in leptin. Ghrelin and leptin are peptides from the orexin family and contribute to the feeling of hunger and glucose metabolism, but also to wakefulness. Thus, sleep disruption can contribute to craving for food, obesity, sedentary lifestyles, and weakened glucose tolerance (i.e. an increased risk of diabetes), as reviewed by Van Cauter et al. (2008). However, the gastrointestinal hormones are by far not the only biomarkers involved.

Cytokines and sleep disturbances

Proinflammatory markers such as hsCRP and IL-6 correlate positively with the extent of sleep deprivation (Meier-Ewert et al. 2004, Vgontzas et al. 2004). Elevated cytokine levels such as IL-6 have been positively associated with alterations in polysomnographic rapid eye movement (REM) registrations (Mills et al. 2007), which may represent a disturbance in sleep dynamics. The altered levels of potent immune markers are often regarded as pathological phenomena, although they may simultaneously serve a fundamental physiological role in minimizing damage caused by surrounding physiological processes. One such interesting
physiological process is the fibrinolytic process, as many of its mediators are responsive to cytokines.

**PAI-1 and sleep disturbances**

Breathing disordered sleep disturbances associate with elevated levels of PAI-1 (Rängemark et al. 1995, von Känel et al. 2007), although sleep deprivation per se seems to block the recovery of circulating PAI-1; thus, dampened levels of PAI-1 could be expected (Irokawa et al. 1998). Nevertheless, due to the fluctuating diurnal levels of PAI-1, its potential role in circadian mechanisms has been investigated.

Dysregulation of circadian mechanisms involves a hypofibrinolytic risk, especially in individuals with metabolomic disturbances (Oishi 2009). The circadian CLOCK and CRY genes can upregulate proinflammatory cytokines, such as IL-6, but also PAI-1 (Ohkura et al. 2006, Narasimamurthy et al. 2012). The circulating levels of PAI-1 could regulate sleep patterns; however, studies have reported contradictory conclusions. Sakakibara et al. (2011) illustrated that peritoneal administration of PAI-1 had only a slight effect on the awake time of rats, suggesting that PAI-1 is a marker rather than a mediator of sleep disturbances. Mou et al. (2009), on the other hand, suggested that PAI-1 could contribute to circadian pacemaker regulation. Their in vitro study concerning rats illustrated that PAI-1 may inhibit glutamate-induced phase shifts in the suprachiasmatic nucleus, which is one of the most central neuroanatomical parts for the circadian cycle. They also observed that administering tPA counteracted the inhibition, suggesting that sleep modulation is related to the products of the fibrinolytic cycle, such as plasmin and BNDF.

An elevated PAI-1 level and consecutive phase-shift inhibition may lead to shortened REM latency, a phenomenon associated with depression-related sleep disruption decades ago (Coble et al. 1976, Pillai et al. 2011, Palagini et al. 2013). Elevated PAI-1 may also contribute to poor perceived sleep as a result of sympathetic activation and disturbed suprachiasmatic circadian regulation, and consecutively altered REM and non-REM cycles. Previous studies in both healthy (Mills et al. 2007) and depressed populations (Motivala 2011) have concluded on similar associations between other inflammatory markers and the sleep patterns.

Studies on humans suggest that cytokines and stress may sensitize the nervous system and provoke a nocturnal shift towards an increased sympathetic tone (Bonnet and Arand
The sympathetic tone associates with increased levels of catecholamines (Gunnar and Quevedo 2007, Wong et al. 2012) such as noradrenaline, which attach to adrenoreceptors. Although the majority of PAI-1 is stored in platelets, PAI-1 may also be released from catecholamine storage vesicles, at least in rat chromaffin cells (Jiang et al. 2011). Cells, including adipocytes, may overexpress catecholamine vehicles upon stressful stimuli (Kvetnansky et al. 2012). Animal studies suggest that the upregulation of PAI-1 occurs through the expression of a stress-related gene, SERPINE1 (Tsai 2006). Nevertheless, the opposite effects could result from chronic stress, as stimulation of the β1 adrenoreceptor-mediated reduction of prostacyclins and downregulation of β2 adrenergic receptor function may both result in lowered levels of PAI-1 (von Känel et al. 2001). Indeed, Irwin et al. (2003) illustrated that only non-depressive individuals with insomnia express increased levels of catecholamines when compared to depressed individuals or healthy controls.

2.5 IMMUNOMODULATORY APPLICATIONS FOR DEPRESSION

An accumulation of T cells, especially Th2 cells, may play a protective role for the CNS. Compounds favouring Th2-cell expression, such as aluminium hydroxide, glatiramer acetate, Freund’s solvents and statins, seem to induce a favourable increase in axon regeneration and neuronal survival, at least in animal models (Hendrix et al. 2007). Epidemiological studies on humans support these observations, as statins have associated with a reduction in the risk of depression (Redlich et al. 2014), while on the contrary, nonpersistent use of statins increased the likelihood of cognitive disorders (Lilly et al. 2014). Furthermore, in a small (n = 68) Iranian randomized double-blind placebo controlled trial, a group of MDD patients benefited from lovastatin-augmented treatment with the selective serotonin reuptake inhibitor (SSRI) fluoxetine (Ghanizadeh et al. 2013). However, such benefits from statins may not be generalizable (Glaus et al. 2015) and may only involve subtypes of MDD patients.

Anti-inflammatory and anti-cytokine agents have been routinely used to treat diseases in immunology and oncology, i.e. diseases often characterized by excess proinflammatory cytokines. Rosenblat et al. (2014) reviewed the effectiveness of several anti-inflammatory therapeutics as adjuvant treatments in MDD. For example, anti-TNF-α agents have been helpful in treating depression in certain populations, such as individuals with psoriasis. Although the effect may be emphasized in individuals with atypical features of depression, compounds such as non-steroidal anti-inflammatory drugs (NSAID) (Rosenblat et al. 2014)
and curcumin taken in doses of 500 mg (88% curcuminoids) twice daily have been suggested to have antidepressive efficacy through a decreased inflammatory load and normalization of HPA axis function (Lopresti et al. 2014 and 2015). Some preliminary evidence suggests that the use of tetracycline antibiotics, such as doxycycline, may alleviate the symptoms of depression, but mainly due to the anti-inflammatory, anti-oxidant and anti-glutamatergic effects of the antibiotics (Rosenblat et al. 2014). Directly, glucocorticoid-related treatments, such as antiglucocorticoids, have been considered for treating depression (Murphy 1997), but their efficacy remains debated (Gallagher et al. 2008, Ferrier et al. 2015). In addition, some glucocorticoid-related hormonal modulators, such as DHEA, may alleviate the symptoms of some depressive patients (Schmidt et al. 2005, Peixoto et al. 2014). However, double-blinded clinical trials with appropriate sample sizes are still lacking for most anti-inflammation-related antidepressant treatments. Furthermore, several trials have observed them to only be effective in some subtypes of depression. Thus, more specific sample selection is needed in order to conclude on their clinical usefulness. Nevertheless, medical substances such as NSAIDs and glucocorticoids can result in severe adverse events, such as peptic ulcer, and should only be used after careful consideration by a doctor familiar with the medications.

Depression not only increases the risk of cardiovascular disease and stroke, but has consistent adverse effects on outcomes after a stroke (Kutlubaev et al. 2011). Whether or not antidepressants relieve CVD morbidity has been debated (Spindelegger et al. 2014, Monte et al. 2009). However, impressive retrospective studies suggest that at least the SSRIs help diminish the risk of cardiovascular complications (Acharya et al. 2013, Rahman et al. 2013, Cooper et al. 2014), and similar conclusions may be expected from serotonin-noradrenaline reuptake inhibitors (SNRI) (Xue et al. 2012). A prospective follow-up study, although post hoc, suggested that aggressive treatment of depression before the onset of CVD halved the risk of severe outcomes (Stewart et al. 2014). Some of the benefits may be attributable to the effects of SSRIs on blood coagulation and fibrinolytic cascades (Geiser et al. 2011). SSRIs appear to be fairly safe medications for supporting CVDs (Ramasubbu 2004), although factors such as multipharmacology may increase the risk of complications such as cerebrovascular haemorrhage (Shin et al. 2015). A recent review by Teply et al. (2015) suggested SSRIs and SNRIs as primary medications for depressive patients with comorbid ischemic cardiac disease. They attributed the choice mainly to its general safety and antiplatelet activities. However, in addition to the effects of SSRIs on blood coagulation and
fibrinolytic cascades (Geiser et al. 2011), they may also exert benefits by harmonizing inflammatory markers and central neuroendocrine systems (Leonard 2014).

Nevertheless, almost certain therapeutic and cytokine-alleviating effects are expected from healthy life styles. Benefits from physical exercise, a healthy diet including omega-3 polyunsaturated fatty acids, and avoidance of toxic compounds, such as smoking and alcohol, are supported by epidemiological, pathophysiological and trial-based evidence (Rosenblat et al. 2014).
3 Aims of the studies

Publication I
To investigate the levels of the Th2 cytokines IL-5 and IL-13 in MDD and healthy controls. We also investigated IFN-γ due to its comparative role to the Th2 cytokines as a Th1 cytokine.

Publication II
To investigate the levels of the adiponectin and the resistin in mentally symptomatic adults reporting and not reporting a history of childhood maltreatment.

Publication III
To investigate the levels of PAI-1 in mentally symptomatic adults reporting and not reporting a history of alcohol abuse in the childhood home.

Publication IV
To investigate the levels of PAI-1 in depressed individuals with less than mild and more than moderate sleep disturbances.
4 Methods

4.1 Kuopio Depression Study (KUDEP)

The Kuopio Depression (KUDEP) is a four-phase general population study involving adults aged 25–64 years in the province of North Savo in Eastern Finland.

4.2 Study subjects

In 1998, a random sample of 3004 participants from the National Population Register was invited to join the study. The participants were followed up in 1999 and 2001, and invited to a clinical study arm in 2005 (Figure 5, p. 37).

The baseline sample (1998) included 2050 participants, the first follow-up sample (1999) 1722 participants, and the second follow-up sample (2001) 1593 participants. The second follow-up questionnaire was only sent to those subjects who responded in either of the preceding surveys. Questionnaires were re-sent to the non-respondents one month later after each follow-up. Altogether, 1347 individuals responded all three times.

In 2005, a fourth-phase study sample of 427 participants was invited to take part in a clinical evaluation and laboratory testing, based on the presence (high adverse mental symptoms (HMS) group) or absence (low mental symptoms (LMS) group) of self-reported adverse mental symptoms (Viinamäki et al. 2009, Honkalampi et al. 2010). The criteria for belonging to the HMS group were a Beck Depression Inventory (BDI)-21 score >9; or Toronto Alexithymia Scale 20-item scale (TAS)-20 score >58; or Life Satisfaction scale (LS) score >11 in the three assessments, i.e. 1998, 1999 and 2001. Altogether, 209 participants fulfilled the criteria. The sample for the LMS group was selected based on the same age and gender distribution, but without the affective symptoms. Altogether, 218 participants fulfilled the LMS criteria. The final participation rate was 78%, that is, 333 participants.
4.3 Research ethics and consent

All the participants were given a thorough description of the study, after which they provided written consent together with the questionnaire by mail. The study protocols were in accordance with the Declaration of Helsinki. Approval for the studies was obtained from the Ethics Committee of Kuopio University Hospital. The Ethics Committee reference numbers for the original study and the follow-up are 174/97, 34/2001 and 55/2004. Before investigating any of the medical records, the permission of the hospital medical director was obtained. All the participants also gave permission for collection of their medical record data as part of their original consent to participate in the study.

4.4 Data collection

The participants were asked to provide information at four different time points: at baseline, at the first and second follow-ups, and at the clinical evaluation (Figure 6, p. 4137).
4.4.1 Baseline characteristics

At baseline (1998), participants reported the following: basic background characteristics such as their age, gender and marital status; medical diagnoses such as asthma, coronary heart disease (CHD), angina pectoris and rheumatoid arthritis; life-style factors such as daily smoking and alcohol use; use of medications such as antidepressants, statins or lipid lowering drugs, sleep medication, oral glucosteroids and non-steroidal anti-inflammatory drugs (NSAIDs); and physical capacity, such as the ability to complete a 2-km walk without difficulty.

The 1999 KUDEP study questionnaire used six separate questions for assessing the childhood adverse events within this study. The questions enquired about various aspects regarding the childhood home and ACEs, such as:

- Happiness in the childhood home: “Oliko lapsuuden kotinne onnellinen?” (kyllä / ei)
- The nature of parenting in the childhood home: “Millaista oli saamanne kasvatus?” (hyvin lempeä / lempeä / melko ankara / ankaraa)
- The relationship between the parents of the participants during adolescence and childhood: “Millaiset olivat vanhempien keskinäiset suhteet lapsuus- ja nuoruusvuosinanne?” (hyvät ja sopuointuiset / melko hyvät / melko huonot / huonot ja riitaisat / en tiedä)
- Experiences of physical punishment under the age of 15: “Jos saitte ruumiillista kuritusta alle 15-vuotiaana, kuka sitä antoi?” (isä, ei kukaan muu / äiti, ei kukaan muu / sekä isä että äiti / joku muu / en saanut ruumiillista kuritusta)
- Suffering from family violence during adolescence and childhood: “Oletteko kärsinyt itseenne kohdistuvasta perheväkivallasta lapsuus- ja nuoruusvuosinanne?” (kyllä, vain ruumiillisesta väkivallasta / kyllä, ruumiillisesta väkivallasta, joka oli myös seksuaalista / kyllä, vain seksuaalisesta väkivallasta/hyväksikäytöstä / ei, en ole kärsinyt perheväkivallasta)
- Alcohol abuse in the childhood home: “Käyttikö joku lapsuudenkodissanne alkoholia liikaa tai väärin?” (isä, ei kukaan muu / äiti, ei kukaan muu / sekä isä että äiti / joku muu / en saanut ruumiillista kuritusta)

4.4.2 Baseline and follow-up questionnaires

At baseline and at both follow-ups (1998, 1999, 2001), the participants completed the self-report instruments BDI-21, TAS-20 and LS. The questionnaires have been established for
screening individuals at risk of affective disorders, such as MDD. In our studies, we used the Finnish versions of the following questionnaires. Some characteristics, such as the age and the marital status, were enquired at each time point.

The **21-item Beck Depression Inventory (BDI-21)** (Beck et al. 1961 and 1988) is a well-validated questionnaire for screening depression in general populations (Aalto et al. 2012). The items are rated on a 4-point Likert scale (range 0–63), with a score of 63 indicating the most severe depressive symptoms. A cut-off value of >9 has been used for screening depression, providing good sensitivity (0.93) and reasonable specificity (0.75) for at least mild depression within the preceding few weeks (Aalto et al. 2012).

The **20-item Toronto Alexithymia Scale (TAS-20)** (Bagby et al. 1994a and 1994b) is the most widely used instrument for assessing alexithymia, that is, an inability to identify and describe one’s emotions. Alexithymia associates with both the onset and the severity of depression (Joukamaa et al. 2001, Taylor et al. 2003, Li et al. 2015). The items are rated on a 5-point Likert scale (range 20–100), with a score of 100 indicating the highest degree of alexithymia. A common cut-off score of >60 has been established in community settings for identifying individuals with alexithymic features (Parker et al. 2003b).

The **Life Satisfaction Scale (LS)** is a useful scale for predicting long-term health. The modified version (Koivumaa-Honkanen 1998) includes 4 items (range 4–20) with a maximum score of 20 indicating high dissatisfaction with life. High scores associate with conditions such as poor health and behaviour, living alone and depressive symptoms (Koivumaa-Honkanen et al. 2000). A cut-off value of >11 has been used for identifying individuals with significant dissatisfaction, whereas accumulating high scores during a follow-up could be significant indicator of poor mental health and MDD (Rissanen et al. 2011).

### 4.4.3 Clinical evaluation

In the fourth phase of the study (2005), psychiatric interviews were conducted for all participants by two trained, experienced research nurses using the Structured Clinical Interview (SCID-I and SCID-II) from the 4th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association 1994, First et al. 1997 and 2002). The interviewer was unaware of the results of the self-reported questionnaires. The participants also filled out the self-report instruments BDI-21, TAS-20 and LS and were enquired for basic characteristics.
Structured Clinical Interview for DSM-IV-TR disorders (SCID). The SCID has two parts. SCID-I is used for axis I disorders such as depression, whereas SCID-II is used for personality disorders. SCID-I is reliable, regardless of interviewer’s discipline, although training and experience have a strong influence on the credibility of the assessment (Lobbestael et al. 2011). Different versions can be used in clinical and research settings (First et al. 2002). The SCID is well validated (Ramirez Basco et al. 2000, Shear et al. 2000) and often regarded as the gold standard in psychiatric assessment. During the interviews, an attempt was made to rule out organic and physical diseases significantly contributing to the depressive symptoms.

Clinical evaluation of depression

The fourth phase (2015) also included other measures of mental health and well-being. The Hamilton Depression Rating Scale (HAMD) was used for the assessment of depression severity and the atypical features of depression (Williams and Terman 1961), alongside the Montgomery–Åsberg Depression Rating Scale (MADRS) (Montgomery and Asberg 1979). Structured and semi-structured questionnaires were used to estimate the severity of symptoms.

Hamilton Depression Rating Scale (HAMD). A trained healthcare worker can perform an interview with the HAMD. The 21-item questionnaire (HAMD-21) evaluates basic depressive symptoms, and can be supplemented with an 8-item subscale for scoring atypical features of depression (Atypical Depression Supplement). Together, these questionnaires form the HAMD-29. The HAMD-21 includes a more established 17-item version of the HAMD. The atypical depression supplement includes questions concerning weight gain, increased appetite/eating, carbohydrate craving, hypersomnia, leaden paralysis and social withdrawal (Williams and Terman 1961).

Health related quality of life questionnaire - 15D (15D). The 15D measures the general health-related quality of life. Altogether, 15 items are rated on a 5-point Likert scale and weighted based on their relevance to the studied population. The 15D questionnaire is a valid tool for assessing health-related quality-adjusted life years (HRQoLs) (Sintonen 2001). It assesses an individuals’ motility, sight, sense of hearing, breathing, eating, talking, bowel and urinary functions, mental health, inconveniences and symptoms, depression, anxiety, exuberance, sexual life, and the ability to complete ordinary procedures. The questionnaire enquired the severity of subjective sleep disturbances in five categories: 1) none, 2) slight, 3)
moderate, 4) great, or 5) severe problems with sleeping. We used a computed binary variable indicating less than moderate versus moderate or greater sleep disturbances in publication IV of this study.

4.4.4 Registries and medical records

Data concerning the participants' prescription medications were acquired from the nationwide Social Insurance Institute. Medical records were investigated in order to control for possible bias from a diagnosis of obstructive sleep apnoea.

4.5 The subsamples used in this study

The substudies of this study focused on the fourth phase of the KUDEP study. The investigated study population represented individuals with a high level of long-term mental symptomology, recorded at four time points over a follow-up period of seven years. In all study phases, the participants with elevated BDI scores and severe depressive symptoms on clinical evaluation were further directed to mental health care services.

4.6 Laboratory measurements

After the clinical evaluation in 2005, the participants were given a referral to visit the laboratory within the following week.

4.6.1 Venous samples and storage

The sampling and storage procedures followed the standard high quality protocols of the medical laboratory (Islab) of Kuopio University Hospital. The participants came for venous blood sampling at 8 am, after been instructed to fast for the previous 12 hours. The venous
blood samples were stored at −80 °C until analysis. Before routine analyses such as lipids and glucose, the samples were centrifuged for 15 minutes at 3000 rpm. After all the samples had been collected, they were shipped in a container packed in dry ice to the University of Oulu for cytokine analysis.

4.6.2 Metabolic assays

The measurements of serum total cholesterol, high-density lipoprotein cholesterol (HDL), triglyceride (TG) and fasting plasma glucose were carried out according to routine protocols at Islab. Enzymatic methods (Thermo Electron Co., Finland) were used for all measurements (total cholesterol: Konelab CHOLESTEROL, code 981812; HDL-C: Konelab HDL-CHOLESTEROL, code 981655; TG: Konelab TRIGLYCERIDES, code 981301; fasting plasma glucose: Konelab GLUCOSE, code 981304). The total variations in the utilized methods were 1.6%, 3.7%, 4.8% and 3.1%, respectively.

The measurement of serum insulin was carried out with an automated AutoDelfia Insulin Kit (PerkinElmer Finland Oy Turku, Finland). The samples were analysed using a Konelab 60i Clinical Chemistry Analyzer (Thermo Electron Co.). The analysis was based on the time-resolved fluoroimmunoassay (TR-FIA) principle. The within-run variation was 2.4–1.7% and the between-run variation 2.5–3.3%, and the concentration range was 5.6–30.1 mU/l.

Diagnosis of metabolic syndrome (MetS)

MetS was diagnosed according to the modified criteria of the National Cholesterol Education Program (NCEP) (Expert panel on detection, evaluation and treatment of high blood cholesterol in adults 2001, Grundy et al. 2005). The criteria were fulfilled if three or more of the following were true: fasting plasma glucose ≥5.6 mmol/l, serum triglycerides ≥1.7 mmol/l, systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg, and waist girth > 102 cm and serum HDL < 1.0 mmol/l for men and > 88 cm and < 1.3 for women, respectively. The waist circumference was taken at the midpoint between the lowest rib and the iliac crest. Height and weight was measured in light clothing. The body mass index (BMI) was calculated as weight / height² (kg/m²).

4.6.3 Laboratory assays

ELISA is a traditional method for gathering either qualitative or quantitative data on biological analytes, such as a cytokines. Although several advanced techniques have been
developed from ELISA, such as multiplexing, the principles are attributable to a handful of innovative researchers in the late 1970s and early 1980s.

The principle of ELISA is fairly simple. A plate (i.e. the immunosorbent) is housed with an analyte and concentrated with highly specific antibodies. The antibodies form a compound with the analyte, after which an enzyme links to the compound. Finally, the tested substrate is attached, leading the enzyme to react. The presence of the analyte is observable by measuring the expression of the final molecule consisting of the antigen, the antibody, an enzyme and the substrate attached to the enzyme. The final molecule can be washed from unnecessary molecules to allow the emission of light, i.e. electromagnetic radiation. The amount of electromagnetic radiation emitted is extrapolated, usually using spectrophotometry. Modifications of the method involve, for instance, secondary antibodies and even second antigens.

Although ELISA has been a golden standard for cytokine assays for decades, some potential weaknesses have been recognized. The quality of the equipment and the antibodies can vary, and further variance is attributable to human error related to experience and skills (Aziz et al. 1999). Multiplexing assays are automated, advanced assays for measuring multiple biomarkers simultaneously, time and cost effectively, and with less biological material. The multiplexing-methods are regarded as different from ELISA, although their principles are highly similar.

**IL-5, IL-13 and IFN-γ multiplex assays**

IL-5 (pg/mL), IL-13 (pg/mL) and IFN-γ (pg/mL) levels were analysed by multiplexing with Bio-Plex Human Cytokine Panel 1 utilizing a Bio-Plex 200 instrument based on Luminex xMAP technology (Bio-Rad Laboratories Inc., CA, US). Before analyses, the samples were centrifuged for 15 min at 3000 rpm, and diluted 1:2 in a sample matrix. The samples were assayed singly. High-sensitivity range standard settings were utilized. The assay conditions were standardized and pre-optimized to ensure optimal reproducibility of the assays. There were 17 non-detectable samples for IL-5, 30 for IL-13 and 52 for IFN-γ. The results were calculated with BioPlex Manager Software version 4.3 with five-parameter logistic equations (Gottschalk et al. 2005). The values between zero (blank sample) and the lowest standard (IL-5: 0.52 pg/ml; IL-13: 0.71 pg/ml; IFN-γ: 0.30 pg/ml) were extrapolated from the standard curve.
by the software The intra-assay and interassay variation for the kit analyses was 4.6–13.8% and 3.7–17.2%, respectively.

**Adiponectin, resistin and PAI-1 multiplex assays**

Adiponectin, resistin and PAI-1 were analysed with a human serum adipokine (Panel A) LincoPlex kit (Millipore, MA, USA) using a Bio-Plex Suspension Array System (Bio-Rad Laboratories Pty Ltd; Hercules, CA, USA). For the analyses, the samples were diluted in the appropriate sample matrix to 1:400 according to the kit instructions. The assayed kits were from the same lot, which allows better control of inter-assay variability. The beads were incubated overnight with the samples. A minimum of 50 events (beads) were collected for each analyte protein, and the concentrations calculated from the standard curves based on the median fluorescence intensities. BioPlex Manager Software 4.1 was utilized in calculating the concentrations. The intra-assay and interassay variation for the adiponectin and resistin analyses was 1.4–7.9% and 21%, respectively, and for PAI-1 the values were <7.9% and 21%.

All the kit instructions and instrument manuals were followed accordingly.

**4.7 Substudy inclusion and exclusion criteria**

All studies were subjected to exclusion of participants with missing serum sample or background data.

**Publication I:**

*Elevated levels of serum IL-5 are associated with an increased likelihood of major depressive disorder.* We selected the fourth-phase participants with BDI scores indicating depressive symptoms, that is, BDI scores ≥10 consistently in the three study phases (i.e. 1998, 1999 and 2001). Only participants with a SCID-verified primary diagnosis of MDD in 2005 were included. The control group consisted of individuals reporting a non-depressed state (i.e. BDI scores ≤9) at all points of inquiry. The final sample consisted of 58 MDD subjects and 58 controls.

**Publication II:**

*Lowered levels of serum adipokines in adults with a history of childhood maltreatment.* We only included individuals reporting adverse mental symptoms (i.e. the HMS group), since the number reporting childhood maltreatment was too few among the mentally asymptomatic individuals (i.e. LMS group). Due to missing data, the final sample size was 147, of which 72
participants were diagnosed with current MDD. Altogether, 30 reported childhood maltreatment and 117 did not.

**Publication III.**

*Lowered serum PAI-1 levels in adults with history of alcohol abuse in the childhood home.* We only included the HMS group. The final sample for the study was 149 participants, of whom 75 were diagnosed with current MDD. Altogether, 53 reported alcohol abuse in their childhood home and 96 did not.

**Publication IV.**

*Self-reported sleep disturbance is associated with elevated levels of PAI-1 in individuals with a recorded history of depressive symptoms.* We included fourth-phase participants with a history of elevated depressive symptoms (i.e. BDI scores >9) in at least one of the three study phases. Individuals with obstructive sleep apnoea were excluded, as the study focused on non-breathing disordered sleep. The final sample for the study was 127 participants, of whom 70 were diagnosed with MDD. Altogether, 90 participants had slight or no sleep disturbances and 37 had at least moderate sleep disturbances.

### 4.8 Statistical analysis

The data were analysed using SPSS statistical software (SPSS Inc., Chicago, IL).

The outcome variables used in publications II–IV were computed to binary variables as follows: maltreatment in the childhood home (physical violence, sexual abuse or both vs. no), alcohol abuse in the childhood home (father, mother or somebody vs. no) and sleep disturbances (none or slight vs. moderate, great or severe).

Power analyses were not used for determining the sample sizes. Parametric tests were used with normally distributed and non-parametric tests with non-normally distributed variables. Normality was assessed according to Kolmogorov-Smirnov and/or Shapiro-Wilk tests. After considering the sample size and variance, a p-value below 0.05 for the most suitable test was regarded to indicate a non-normal distribution.

**Crude analyses:** Two-tailed p-values below 0.05 were regarded as statistically significant in all analyses. Differences between groups in categorical variables were assessed using the chi-squared test or Fisher’s exact test if an expected frequency was five or less. The parametric Student’s t-test and the non-parametric Mann-Whitney U-test were used for continuous variables. Associations between biomarkers and potential confounding factors were tested
using Pearson’s and Spearman’s correlations for normally and non-normally distributed variables, respectively.

**Multivariate models (i.e. regression analyses):** Differences between the groups in the examined biomarkers were further examined with logistic regression modelling. The fit of the models was evaluated using the Hosmer and Lemeshow test and the predicted values of the models. The models were tested in multiple steps with method: enter. First, an *a priori* defined model included variables either showing differences between the groups or known to affect the levels of the biomarker. Secondly, for more comprehensive evaluation of the effects of potential confounders, we formed models that included the variables in Model 1 together with further adjustments. The cytokines were entered into the models as continuous variables, except for PAI-1, which was divided based on its median into a lower median category (LMC) and higher median category (HMC). Such dichotomization may increase residual confounding (Pizzi et al. 2012). However, in this case, it was considered to increase the interpretability of the analyses. *Post hoc* adjustments for p-values were not performed, as we only compared two groups and estimated that any effects from further adjustments would be minimal.

The non-detectable samples were recorded as the mean value between zero and the lowest detectable value of each cytokine. Although this calculation lacks elegance, it is considered feasible and nearly as robust as other options (Uh et al. 2008). Missing samples were not included in the studies.
5 Results

Detailed results are thoroughly presented in the tables and figures of the original publications.

5.1 Elevated levels of IL-5 are associated with an increased likelihood of major depressive disorder

Elevated serum levels of IL-5 were associated with an increased likelihood of belonging to the group with MDD. No such associations were detectable for IL-13 or IFN-γ.

The participants with MDD (n = 58) had higher scores on all the depression scales (i.e. HAM-D-21, HAM-D-29 and BDI) than the participants without MDD (n = 58); however, they also more often lived alone, smoked and used antidepressant medication. The levels of IL-5, IL-13 and IFN-γ did not differ between the groups in the crude analyses, although the differences in IL-5 hinted at borderline significance (Z = -1.87, p = 0.061). We investigated the borderline significance by constructing multivariate models [Models (M) 1–4].

The a priori logistic regression model (M1) included age, gender, marital status, daily smoking and alcohol use. Supplementary models included the use of antidepressants (M2), use of NSAIDs (M3) and diagnosis of asthma (M4). The models revealed that each 1-unit (pg/ml) increase in the serum IL-5 levels increased the likelihood of belonging to the MDD group by 76–106% (OR 1.76–2.06, 95% CI 1.03–3.64, p = 0.013–0.038). The likelihood was comparable to the reported use of alcohol or the gender. Neither IL-13 (OR 1.06–1.10, 95% CI 0.94–1.24, p = 0.14–0.33) nor IFN-γ (OR 1.01–1.01, 95% CI 0.99–1.05, p = 0.22–0.25) associated with MDD in the regression models.

5.2 Publication II. Lowered levels of adiponectin in adults with a history of childhood maltreatment

A reduction in serum adiponectin levels was associated with a history of childhood maltreatment in individuals with long-term adverse mental symptoms.

The participants who reported experiencing maltreatment as a child (n = 30) had a diagnosis of MDD and were married or living with a partner more often than participants without the history of childhood maltreatment (n = 117), and they had lowered levels of both adiponectin (Z = -2.684, p = 0.007) and resistin (Z = -2.197, p = 0.028). We controlled for potential confounders by utilizing multivariate models (M1–M3).
The *a priori* logistic regression model (M1) included age, gender and BMI. Supplementary models included the marital status (M2) and a diagnosis of MDD (M3). The adiponectin remained significantly lowered in all the models. Each 1-unit (μg/mL) change in adiponectin increased the likelihood of belonging to the group with a history of childhood maltreatment by 165–172% (OR 2.65–2.72, 95% CI 1.26–5.59, p = 0.005–0.01). The serum resistin levels displayed no associations (OR 1.46–1.64, 95% CI 0.84–2.77, p = 0.09–0.183). The results remained essentially unaltered after exclusion of the participants using NSAIDs, oral corticosteroids or antidepressant. The MDD (in M3 adjusted for adiponectin) was strongly associated with belonging to the group with a history of childhood maltreatment (OR 7.89, 95% CI 2.66–23.47, p < 0.001).

### 5.3 Publication III. Lowered levels of PAI-1 in adults with a history of alcohol abuse in the childhood home

A reduction in serum PAI-1 levels was associated with a history of alcohol abuse in the childhood home in participants with long-term adverse mental symptoms.

The participants who reported a history of alcohol abuse in the childhood home (n = 53) had a similar age, gender, lifestyle factors, medication use and cardiovascular conditions as the participants without such a history (n = 96); however, they more often had a diagnosis of MDD ($\chi^2 = 12.48$, p < 0.001) and lowered levels of PAI-1 ($Z = -2.53$, p = 0.011). The PAI-1 correlated significantly with BMI (rho = 0.173, p = 0.035) and had borderline significance in the correlation with serum insulin levels (rho = 0.158, p = 0.054). We controlled for potential confounders by utilizing multivariate models (M1–M3).

The *a priori* model (M1) included age, gender and BMI. Supplementary models replaced the BMI with a diagnosis of MDD (M2), and replaced the BMI with a diagnosis of coronary heart disease, and additionally included the use of cholesterol medication (M3). The lower median category for PAI-1 (i.e. LMC-PAI-1) values ranged from zero to 28.93 ng/mL and the higher median category (i.e. HMC-PAI-1) from 29.69 to 147.35 ng/mL. Belonging to the LMC-PAI-1 increased the likelihood of a history of alcohol abuse in the childhood home by 119% to 143% (OR 2.19–2.43, 95% CI 1.05–4.99, p = 0.016–0.037). MDD (in M2) was strongly associated with belonging to the group with a history of childhood alcohol abuse (OR 3.15, 95% CI 1.49–6.66, p = 0.003).
5.4 Publication IV. Elevated levels of PAI-1 and sleep disturbance in individuals with a recorded history of depressive symptoms

Elevated serum levels of PAI-1 were associated with a high prevalence of sleep disturbances in individuals with a recorded history of depressive symptoms.

The participants with at least moderate sleep disturbance (n = 37) were younger and more often had MDD, reported higher BDI scores, and used more sleep and antidepressant medication than participants with no or mild sleep disturbance (n = 90). There were no differences in the levels of PAI-1 between the participants using antidepressant medication (n = 26) and non-users (n = 101). Neither the BDI nor age correlated significantly with PAI-1, but BMI did (rho = 0.195, p = 0.028). Due to the group differences, we formed multivariate models (M1–M4).

The a priori model (M1) included BMI, BDI, age and gender. Supplementary models included the use of sleep medication (M2), smoking (M3) and the use of statins or lipid-lowering drugs (M4). Although metabolic syndrome also illustrated a borderline level of significance between the groups ($\chi^2 = 3.10$, p = 0.078), it was not included in the models due to a high cross-correlation with both the BMI and the use of statins or lipid-lowering drugs.

The models revealed that each 1-unit ($\mu$g/mL) increase in the serum PAI-1 levels increased the likelihood of belonging to the group with at least moderate sleep disturbances by 23% to 24% (OR 1.23–1.24, 95% CI 1.04–1.48, p = 0.011–0.016).
6 Discussion

6.1 Comparison with the previous literature

6.1.1 Publication I: IL-5, IL-13 and IFN-gamma and persistent symptoms of depression

We demonstrated that adults with persistent symptoms of depression have an increased likelihood of having elevated levels of serum IL-5, but not IL-13 or IFN-γ. Previous data on the connections between IL-5, IL-13 and MDD are scarce.

A study by Simon et al. (2008) revealed no difference in IL-5 levels between an MDD group and controls. Their results, however, were not adjusted for potential confounders such as smoking, which appears to lower the levels of serum IL-5 (Tsunoda et al. 2003). A study by Hallberg et al. (2010) investigated whether exercise-induced cytokine expression would differ between MDD patients and controls, but could not illustrate changes in either IL-5 or IL-13. However, Shelton et al. (2011) examined the expression of genes within the post-mortem frontal cortex tissue of individuals with MDD. Their gene set enrichment analyses suggested that several cytokines other than IL-6 are indeed upregulated, including IL-5 and IL-13. Furthermore, a genetic risk for MDD has been observed in individuals with certain haplotypes of colony stimulating factor 2 receptor b (CSF2RB), which encodes protein beta chains of receptors with a high affinity for IL-5 (Cheng et al. 2011).

In our study, the individuals with MDD had higher overall levels of both IL-13 and IFN-γ, but this difference did not reach statistical significance. A few previous studies have illustrated elevated IL-13 levels and Th1/Th2 ratios (such as IFN-γ/ IL-13) (Hernández et al. 2008) in MDD patients. Some patient population studies have suggested that IFN-γ is downregulated when compared to healthy controls; however, the patient samples have been more severely ill than random general population samples such as ours (Pavón et al. 2006, Kim et al. 2007). The mean HAMD-21 score for the depressed group reported by Hernández et al. (2008) was 20.3, and the HAMD-17 score in the study of Pavón et al. (2006) was 24.2, whereas in our MDD group, the mean HAMD-21 score was 12.0.

A recent study by Shelton et al. (2015) investigated cytokine profiles in an MDD sample treated with L-methylfolate-augmented SSRI antidepressants. Their baseline serum plasma samples illustrated a negative trend for levels of IL-5. However, their MDD group was older and might have had more heterogeneity in the duration of the symptoms than our study,
which focused on persistent symptoms. Although they attempted to disclose biasing factors such as autoimmune diseases, other factors such as smoking were not controlled for. Nevertheless, they did show a significantly lowered adiponectin within the MDD population, regardless of obesity in the individuals.

Elevated IL-5 could be one of the factors underlying depression-related changes in CNS plasticity, and could link to hyperactivity of signalling pathways, such as the RAS-ERKs (Yoshimura et al. 2009) and the JAK-STATs (Heim 1996). The hyperactivation of such pathways could provoke depressive symptoms (Darnell et al. 1994, Felger et al. 2007), possibly through disturbed glucocorticoid signalling (Hu et al. 2009). In addition, dysregulation in these multipotent signalling routes could disturb cell proliferation and apoptosis, synaptic plasticity, and finally, cognitive processes such as learning (Denayer et al. 2008, Zambrano et al. 2010). Nevertheless, further studies are needed to understand the exact biological role of IL-5 in depression.

6.1.2 Publication II: Lowered adiponectin in mentally symptomatic adults reporting ACEs

Altered expression of adipocyte-derived hormones such as adiponectin and resistin have been reported in adults with elevated levels of adverse psychological symptoms such as depression. However, the interplay between somatic conditions, ACEs and adulthood symptoms of depression has remained inconclusive.

Studies have investigated adipokines in the context of depression, but only a few have reported their levels in ACEs. Previous studies have reported a connection between ACEs and adulthood obesity (Dube et al. 2010) and depression (Leo et al. 2006), both of which have been associated with altered serum adiponectin levels (Gustafson 2010). ACEs can also modulate the relationship between depression and inflammatory changes, such as elevated levels of blood CRP (Danese et al. 2008). Although a recent meta-analysis did not manage to illustrate a consistent association between anti-inflammatory cytokines and ACEs, pronounced expression of proinflammatory cytokines may contribute to lowered adiponectin levels (Coelho et al. 2014). Our results illustrated that individuals who experience adverse mental symptoms and report ACEs are characterized by lowered levels of both adiponectin and resistin when compared to mentally symptomatic individuals not reporting ACEs. After adjustments for the potentially biasing factors, only the adiponectin remained significantly lower in the individuals with a history of ACEs. We took into
consideration the lack of differences in age, gender, smoking, BMI, several metabolic and autoimmune diseases, and use of NSAIDs, corticosteroids or antidepressants, and conducted multivariate adjustments for factors such as depression and marital status. We concluded that the ACEs may independently reflect a distinct inflammatory state in mentally symptomatic individuals.

Adiponectin has anti-inflammatory actions at several levels. It inhibits the functions of cytokines such as TNF-\(\alpha\), which is a proinflammatory cytokine. Moreover, adiponectin also inhibits the secretion of several other endothelial pro-inflammatory cytokines (Gustafson 2010). The lowered levels of adiponectin could suggest that individuals with a history of childhood maltreatment may have weaker anti-inflammatory buffer functions compared with persons without such a background. This could increase their vulnerability to depression, as well as to a variety of conditions linked to disturbed inflammatory systems.

Our observations regarding resistin are in line with an inpatient sample studied by Zeugmann et al. (2013), which illustrated non-significant alteration in the levels of resistin. Although they did not assess adiponectin, they observed an association between childhood adverse events and elevated levels of fibrinogen. Indeed, in addition to the inflammatory markers, the fibrinolytic system appears to be dysregulated in childhood adverse events.

6.1.3 Publication III: Lowered PAI-1 in mentally symptomatic adults reporting alcohol abuse in the childhood home

A recent meta-analysis illustrated altered levels of fibrinolytic cascade markers in individuals reporting ACEs (Zeugmann et al. 2013). However, to the best of our knowledge, no previous studies have assessed ACEs related to parental alcohol abuse and the levels of PAI-1. Furthermore, studies comparing these epidemiological factors specifically in mentally symptomatic populations are lacking.

ACEs expose individuals to somatic risks, physiological symptoms such as sleep disturbances, and vulnerability to stress and MDD. The experiences of alcohol abuse in the childhood home has been associated with complications such as suicide and substance abuse in adulthood (Alonzo et al. 2014); however, the reports on their adverse outcomes have been much more inconsistent than those reported after physical, sexual or emotional abuse (Rossow et al. 2015). We observed that individuals reporting persistent mental symptoms and experiences of alcohol abuse in their childhood home had lowered levels of PAI-1 when compared to mentally symptomatic individuals who did not report such abuse. This
association remained significant after adjustment for MDD and other potential confounders, such as BMI.

Our results suggest that a history of alcohol abuse in the childhood home is associated with an increased severity of depression, and independently with lowered levels of PAI-1. Our participants with a history of alcohol abuse in the childhood home displayed median levels of 30.03 ng/mL, while in individuals not reporting them the respective figure was 40.94 ng/mL. The normal range of PAI-1 is suggested to be 5–40 ng/mL (Vaughan 2005). Thus, our results could also indicate that the individuals with a history of alcohol abusive parenting are not characterized by typical alterations associated with depression, that is, elevated levels of PAI-1, which have been observed in remitting or somatically comorbid depression when compared to healthy controls (Baune et al. 2012, Lahlou-Laforet et al. 2006).

The potential benefits of PAI-1 in CNS protection have been illustrated in the review section of this thesis, including its contribution to neuronal death, regulation of the BBB and neuromodulatory factors such as the BDNF (chapter 0, page 15). In addition, as the main inhibitor of PAs responsible for activating the conversion of plasminogen to plasmin, PAI-1 has a central role in the fibrinolytic process. Together, these biological mechanisms highlight the important role of PAI-1 in homeostasis. However, as this was a narrowly defined preliminary study, additional studies are called for in order to clarify the causalities within mentally symptomatic populations.

6.1.4 Publication IV: Elevated PAI-1 and the severity of sleep disturbances in adults with depressive symptoms

In this study, self-reported sleep disturbances were associated with elevation of PAI-1 in individuals with a recorded history of depressive symptoms. Our results illustrated that the elevation of PAI-1 remained significant, even after adjustments for common biasing factors and the severity of depressive symptoms.

In addition to several factors such as age, gender and BMI, remitted or somatically comorbid MDD has been associated with altered levels of PAI-1 (Baune et al. 2012, Lahlou-Laforet et al. 2006). Only a few published studies have investigated subjective sleep disturbances and the levels of PAI-1. For example, Matthews et al. (2010) reported a carefully conducted experiment that suggested no association between self-reported sleep quality or duration and the levels of PAI-1. However, they did observe that the severity of nightly hypoapnoea and, for instance, Chinese ethnicity associated with elevated PAI-1 levels. The
discrepancies between our studies and that of Matthews et al. (2010) can probably be explained by differences in sample selection, such as the presence of depressive symptoms within our groups. Unexpected bias could also result from different levels of somatic comorbidity (see page 15), but also from the study setting, as their study involved an experimental sleep registration.

The elevated PAI-1 in individuals reporting symptoms of depression and at least moderate sleep disturbances may relate to the association between subjective sleep disturbance and sympathetic activation. Indeed, individuals self-reporting sleep disturbances may share a significant stress-related component (Alapin et al. 2000, Dew et al. 2003, Waldron-Perrine et al. 2012). Our results are interesting, as previous studies have suggested that experimentally elevated PAI-1 could induce sleep patterns mimicking depression (Mou et al. 2009). Furthermore, most previous publications concerning the PAI-1 have attributed the elevation to the presence of obstructive sleep disorders (Rångemark et al. 1995, von Känel et al. 2007). However, diagnosed OSA patients were excluded from our sample, suggesting that PAI-1 may also have a more generalized role in sleep pathophysiology.

6.2 **INTERPRETATION OF THE CYTOKINE ANALYSES**

As mentioned in a previous section, studies involving cytokine analyses may yield heterogeneous results. The differences between analyses are highly dependent on the methods used, solvents, and the experience and skills of the staff. The consequent high interassay variations from poor methods could interfere with the ability to detect differences in cytokine levels. The total variance in cytokine levels is also affected by statistical methods such as the imputation of non-detectable samples, that is, replacement of the missing values with a substitute value. Nevertheless, a general guideline indicates that the number of non-detectable samples can be considered small when they comprise less than 30% of the total sample size (Uh et al. 2008).

In addition to the above-mentioned technical considerations, several population-related aspects need to be accounted for when interpreting the results of *in vitro* cytokine studies. For example, cytokine differences are likely to be less pronounced in population-based samples, which often consist of individuals with depression scores lower than those observed in patient samples. Patient samples are, however, often small, which increases the likelihood of type II statistical errors, leading to the lack of detecting an actual difference in the cytokine levels between the study groups. Significant differences may also result from small non-
randomized studies not controlling for biasing factors. The factors often associated with cytokines and related biomarkers are BMI, gender, age, ethnicity, immunomodulatory medication, antidepressants, non-steroidal anti-inflammatory drugs (NSAIDs), alcohol use, smoking, and several somatic diseases such as cardiovascular diseases, autoimmune diseases and allergic diseases (see pages 10 and 15). It is never possible to take into account all the potential biasing factors, and often the nature of a study sample, the budget or the research ethics prevents a randomized controlled trial, which is the gold standard for clinical research. Nevertheless, as opposed to longitudinal studies, cross-sectional investigations can rarely conclude on causalities for the associations observed. However, meta-analyses and systematic reviews are conducted in order to illustrate consistent trends, although they may also have flaws. The systematic approaches may be able to illustrate global trends, but they often conclude on overly heterogeneous concepts, such as MDD. When investigating plurally defined symptom clusters such as MDD, one easily mixes the “apples and oranges”, omitting the fact that the aetiological factors may be countless. Reviews are also subject to publication bias, that is, they do not take into account unpublished studies illustrating, for example, nonsignificant alterations. Furthermore, the exclusion and inclusion criteria of reviews are sometimes unjustified, which has raised criticism. Critical aspects related to meta-analyses have been debated by Hedges et al. (2009), among others.
7 Strengths and limitations

The study population of the KUDEP cohort consisted of individuals of Finnish descent, thus minimizing the confounding effect of ethnicity (Matthews et al. 2010). Although the selection of our original population-based sample was random, the selection of participants for the clinical study arm in 2005 was based on mood-related symptoms in the earlier study phases (1998, 1999 and 2001). The 2005 sample might have favoured enrolment of the individuals at that time with better motivation and functional capacity. However, one of the strengths in this study was the comprehensive evaluation of depressive symptomatology. Nevertheless, recall bias (Moffitt et al. 2010) may have affected our background data collection, such as the questions concerning the ACEs. The follow-up questionnaires, on the other hand, allowed for selecting participants with actual symptoms, minimizing the bias from recalling the symptoms of depression. The main limitation of our study population was thus the small sample sizes, increasing the risk of type II statistical errors, which might have resulted in the lack of detecting actual differences between the groups.

Bipolar mood disorder (BD) is potentially distinct from unipolar depression in terms of treatment responses (Hirschfeld 2014) and biology (Kempton et al. 2011). Many 20th century studies investigating major depressive patients did not differentiate between MDD and BD. Although the phase of depression in BD resembles MDD, at least manic episodes have distinctive pathophysiology (Cuellar et al. 2005). In evaluating and diagnosing MDD, we used the gold standard tools for differential diagnosis, that is, the SCID, as well as self-reported and interviewer-rated depression scales. We minimized the potential bias by excluding individuals with BD.

The laboratory methods may introduce a significant level of heterogeneity to different studies (Aziz et al. 1999). The ELISA technique is the gold standard for peptide immunoassays. The Luminex xMAP multiplexing analyses in our studies were highly correlated with ELISA (de Jager et al. 2003, Elshal and McCoy 2006, Leng et al. 2008). The number of non-detectable samples should comprise less than 30% of the total sample size (Uh et al. 2008). The proportions of non-detectable samples for IL-5 and IL-13 were below the 30% limit, but IFN-γ exceeded this limit. All our imputed values were close to zero, and the effect of the imputation method on the total variance is therefore likely to have been very small. High inter- or intra-assay variations of the laboratory assays could also interfere with
the total variance and the ability to detect differences in cytokine levels. However, our intra-assay variances were 4.6–13.8% and the interassay variances 3.7–21%, that is, moderate.

A large number of conditions affect the serum cytokine and biomarker levels. We did not have data on some somatic conditions related to the secretion of some of the measured cytokines (e.g. allergic rhinitis, atopic dermatitis), which may have affected our findings on, for example, IL-5 and IL-13. Furthermore, our participants were mentally symptomatic and used antidepressant medications such as SSRIs, which may affect the levels of the measured cytokines (Sutcigil et al. 2007) and at least hypothetically the levels of PAI-1 (Hoirisch-Clapauch et al. 2014, Tsai et al. 2008). Although our participants used antidepressants, the number of participants using other medications than antidepressants was low. The study reported in publication I controlled for antidepressant use in logistic models, leaving the significant results unchanged. Thus, the potential bias from medications other than antidepressants was generally low. Despite all the statistical adjustments for known confounders, there remains a potential for residual confounding (Liang et al. 2014). When compared to general populations, well-defined cohorts may be less vulnerable to the effects of residual confounding (Pizzi et al. 2012). Our 2005 subsample was a well-defined cross-sectional insert from a 7-year general population-based cohort. Residual confounding is especially prevalent when dichotomizing occurs (Groenwold et al. 2013), such as in publication III of this thesis, when we divided the values of PAI-1 according to the median. Nevertheless, the interpretability of the multivariate analyses was enhanced due to the dichotomization.

Additional criticism could be directed to the dual hypothesis- and data-driven approach in this study. Indeed, purely hypothesis-driven research makes primary assumptions on the basis of which results are expected to be found. Data-driven studies, on the other hand, investigate results on the basis of which, for example, they contribute to a paradigm. The original principal hypotheses of the KUDEP patient sample in 2005 did not involve detailed hypotheses concerning MDD and IL-5, childhood adversities and PAI-1 or adiponectin, nor did it assume that sleep disturbance involves depression-related changes in the levels of PAI-1. Although some general assumptions had been made concerning affective disorders and altered immunological parameters (i.e. the principal hypotheses), the publications in this study were mainly data-driven (i.e. based on a statistical result). Although fundamental research has praised hypothesis-driven research above all, recent advances in informatics
and the increasing amounts of data available have given a premise for data-driven methods (Kell et al. 2004, Arazi et al. 2013). Data-driven methods in immunology are often used in studies involving high-throughput laboratory analytics. The microplexing used in our studies is not a high throughput method per se, although it can be considered a predecessor for one. Nevertheless, like all research, data-driven methods can be inconclusive, as in the study of van Loo et al. (2012). They attempted to subtype MDD based on symptoms alone, while omitting the current paradigms related to the heterogeneous characteristics of the MDD population. In this thesis, I have focused on integrating our observations with existing paradigms related to MDD, such as the dysregulation of the HPA axis. By doing so, I have aimed to illustrate several plausible mechanisms for our observations related to common biological markers such as PAI-1, adiponectin and IL-5, which could be clinically applicable in the future.

Finally, the observations from publications II, III and IV were restricted to populations with a history of mental symptoms. Due to the cross-sectional study design with clinical samples, we were unable to assess causality or confirm previous suggestions of possible biological routes. Nevertheless, the KUDEP study - despite its weaknesses – has provided feasible data for investigating biological trends in well-characterized study groups. With the supplementary information from the medical records, a satisfactory epidemiological data set could be acquired and the hypotheses tested. However, larger studies with primary endpoints in identifying phenotypes of depression are called for, as well as clinical and pharmacological studies integrating the possible biological mechanisms into clinical practice. Studies on the augmentation of traditional therapies with inflammation- and fibrinolysis-related medications would probably hold much promise; as such, adjuvant treatments could beneficially affect both the depressive symptoms and the somatic risks of a mentally symptomatic individual. However, caution should be exercised in prescribing such treatments before studies have evaluated overall risks and benefits in a randomized and controlled fashion.
8 Conclusions

This study investigated immunomodulatory markers in mentally symptomatic subpopulations. Depressive symptoms, in particular, characterized the subpopulations. The results suggest that individuals reporting epidemiology of ACEs or symptoms of sleep disturbance are biologically distinct from individuals not reporting them. In addition, we observed a new anti-inflammatory trait related to persistent symptoms of major depression. These characterizable immunomodulatory markers probably contribute to the symptoms and somatic comorbidities associated with MDD.

I have reviewed several potential pathophysiological mechanisms related to the altered immunomodulatory markers, that is, IL-5, adiponectin and PAI-1. We observed some of the differences by using inverse logistical regression, which means that we investigated markers on basis of their biological role, even though crude statistical analyses had illustrated non-significant results. Indeed, the somatic morbidity and life-style factors of mentally symptomatic populations can both explain and hide the significantly altered levels of immunomodulatory markers.

The study cannot answer whether the investigated biomarkers may be of benefit in routine clinical practice in this context. However, these fundamental associations highlight a need to consider more personalized therapeutic methods, depending on epidemiological factors such as ACEs and the specific symptoms of depression. However, some conclusive remarks can be made on basis of our observations, when combined with the vast literature available on inflammatory and fibrinolytic alterations related to MDD, ACEs and self-reported sleep disturbance. The remarks are presented in the next - and final - section of this thesis.

8.1 Relevance to future research

Recently, MDD was recognized as an independent risk factor for cardiovascular diseases (Goldstein et al. 2015). However, ACEs and self-reported sleep disturbances could also be considered as contributors to somatic risks, especially in subpopulations such as individuals displaying mental symptoms related to depression.

Studies so far have associated ACEs with altered immunomodulatory markers and increased morbidity. However, the causalities have not been prospectively and interfactorially studied. Epidemiological and pathophysiological observations suggest that ACEs do increase the risk of CVDs (Halonen et al. 2015). Nonetheless, although the study of
Halonen et al. (2015) commendably illustrated that ACEs and concurrent residential disadvantage increase the risk of CVDs, they did not control for the potential effects of affective symptoms such as depression. Our results suggest that the ACEs can independently contribute to adverse immunomodulatory conditions, although the role of some potential ACEs, such as experiences of alcohol abuse in the childhood home, remain inconclusive.

Sleep disturbances are one of the most common conditions related to MDD. PAI-1 could link to intercrossing mechanisms between inflammation, fibrinolysis and central nervous system protection in individuals reporting either ACEs or pronounced self-perceived sleep disturbances. These observations highlight the need for future studies to take into account such subpopulations, in order to identify the principal components underlying these phenomena.

As a conclusion, the results of this study support a nonconventional approach to the investigation of depression, a method referred to as intermediate phenotyping, described by Leuchter et al. (2014) among others. Intermediate phenotyping is an approach aiming to find characterizable MDD subpopulations with, for instance, a likelihood of responding to a certain types of therapeutics. Indeed, some animal studies have already suggested a biological phenotypic role for IL-6 and CRP (Sukoff Rizzo et al. 2012). However, anamnestic factors such as self-reported family relations have also demonstrated potential in distinguishing between the severities of symptoms (Watters et al. 2013). Indeed, in addition to taking into account biological samples, intermediate phenotyping is likely to be most feasible when also accounting for central anamnestic factors such as ACEs and selected symptoms, such as sleep disturbance.
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Depression, adverse childhood events (ACE) and sleep disturbances link to metabolic and immune alterations. Immunomodulatory transmitters regulate the immune system and contribute to functions such as fibrinolysis and formability of the nervous system. Our results illustrate that levels of IL-5, PAI-1 and adiponectin are altered in individuals with persistent symptoms of depression, and the levels may vary depending on the presence of ACEs and sleep disturbances. This thesis aims to clarify the pathophysiology of depression, which may encourage more personalized treatments.