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Timo Liukkonen

LOW-GRADE INFLAMMATION IN DEPRESSION, ANXIETY AND SLEEP DISTURBANCES

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INSTITUTE OF CLINICAL MEDICINE, DEPARTMENT OF PSYCHIATRY;
EAST-SAVO HOSPITAL DISTRICT;
OULU UNIVERSITY HOSPITAL, UNIT OF GENERAL PRACTICE;
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Professor Markku Timonen
Professor Pirkko Räsänen

Reviewed by
Professor Jyrki Korkeila
Docent Olli Kampman

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University of Oulu, Faculty of Medicine, Institute of Health Sciences, General Practice, Institute of Clinical Medicine, Department of Psychiatry, P.O. Box 5000, FI-90014 University of Oulu, Finland; East-Savo Hospital District, P.O. Box 111, FIN-57101 Savonlinna, Finland; Oulu University Hospital, Unit of General Practice, P.O. Box 5000, FIN-90014 Oulu, Finland; Oulu Health Centre, P.O. Box 27, FI-90015 Oulu, Finland

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Abstract

Depression, anxiety and sleep disorders have been reported to be associated with low level of inflammation, *i.e*., low-grade inflammation, but mainly in males. The evidence has mainly been based on laboratory or clinical studies with small sample sizes or epidemiological studies with elderly subpopulations. In this study the association of low-grade inflammation with depression, anxiety, and sleep disturbances was investigated using the Northern Finland 1966 Birth Cohort (NFBC 1966). In women, the effect of hormonal factors, menopause and the use of oral contraceptives/hormone replacement therapy on the association between low-grade inflammation and depression was also studied by using the Pieksämäki Study data.

In 31-year follow-up of NFBC 1966 (N=6007), the depressive and anxiety symptoms were assessed by Hopkins Symptom Checklist-25 (HSCL-25) and sleep disorders by 15-D questionnaires, while the marker of low-grade inflammation, plasma concentration of high sensitivity C-reactive protein (hs-CRP), was measured. In the Pieksämäki study a representative sample of inhabitants in the town of Pieksämäki were invited to clinical examination. Depressive symptoms were obtained by Beck's Depression Inventory-21, and hs-CRP was measured (512 women).

The results of this study revealed that at epidemiological level, elevated hs CRP levels of ≥1.0 mg/L increased the probability of current depressive symptoms of single depressive episode in the two highest subgroups (*i.e*., HSCL-25 mean scores ≥1.75 and ≥2.01) 1.4- and 1.7- fold in males, respectively. In addition, anxiety symptoms (HSCL-25 anxiety scale mean score ≥1.75) increased independently the probability of elevated hs-CRP levels (>3.0 mg/L) in males over 2-fold. Risk ratio of 1.3 was found for males with moderate to severe sleep disturbances and elevated hs-CRP levels (≥1.0 mg/L). Regarding females, a positive correlation between elevated hs-CRP levels and depressive symptoms was found only among peri- and postmenopausal women not using exogenous hormones.

The results suggest that low-grade inflammation is associated not only with depression but also with anxiety and sleep disturbances in young adult men. In women, hormonal factors may have an effect on the association between low-grade inflammation and depression. Further investigations are called for to confirm these findings and furthermore, to determine the possible role of low-grade inflammation in the pathophysiology of these disorders.

Keywords: anxiety disorders, C-reactive protein, cohort studies, depressive disorder, hormone replacement therapy, low-grade inflammation, oral contraceptives, sleep disorders

Liukkonen, Timo, Matala-asteinen tulehdustila depressiossa, ahdistuneisuudessa ja unihäiriöissä.

Oulun yliopisto, Lääketieteellinen tiedekunta, Terveystieteiden laitos, Yleislääketiede, Kliinisen lääketieteen laitos, Psykiatria, PL 5000, 90014 Oulun yliopisto; Itä-Savon sairaanhoitopiiri, PL 111, 57101 Savonlinna; Oulun yliopistollinen sairaala, Yleislääketieteen yksikkö, PL 5000, 90014 Oulu; Oulun terveyskeskus, PL 27, 90015 Oulu

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Tiivistelmä

Depressio, ahdistuneisuushäiriöt ja unihäiriöt on yhdistetty elimistön matala-asteiseen tulehdustilaan, joskin pääasiallisesti vain miehillä. Tulosten yleistettävyyttä ovat rajoittaneet tutkimusten pienet otoskoot tai painottuminen iäkkäisiin väestöaineistoihin. Tässä tutkimuksessa selvitettiin matala-asteisen tulehduksen yhteyttä depressioon, ahdistuneisuuteen ja unihäiriöihin Pohjois-Suomen syntymäkohortti 1966 -aineistossa. Lisäksi Pieksämäki-tutkimuksen aineistossa selvitettiin naisilla menopaussin ja ehkäisyvalmisteiden/vaihdevuosihormonikorvaushoidon vaikutusta depression ja matala-asteisen tulehduksen väliseen yhteyteen. Pohjois-Suomen syntymäkohortti 1966 -tutkimuksen 31-vuotisseurannassa kartoitettiin 6007 henkilöltä masennus- ja ahdistuneisuusoireita Hopkins Symptom Checklist-25 -arviointiasteikolla (HSCL-25) ja unihäiriöitä 15-D-kyselyllä. Lisäksi mitattiin matala-asteisen tulehduksen mittarina käytetyn herkän C-reaktiivisen proteiinin (CRP) pitoisuus. Pieksämäki-tutkimuksessa edustava otos Pieksämäen asukkaista kutsuttiin kliiniseen tutkimukseen ja depressiivisiä oireita kartoitettiin Beckin 21-osioisella arviointiasteikolla ja mitattiin herkkä CRP (512 naista).

Nuorilla aikuisilla miehillä, joiden herkkä CRP oli kohonnut (≥1.0 mg/l), todettiin 1.7-kertainen masennusoireiden riski, kun katkaisupisteenä käytettiin HSCL-25-kyselyn masennuskeskiarvopistettä ≥2.01. Ahdistuneisuusoireet (HSCL-25-kyselyn ahdistuneisuuskeskiarvopisteet ≥1.75) lisäsivät kohonneen herkän CRP:n riskiä (>3.0 mg/l) yli kaksinkertaiseksi miehillä. Keskivaikeasta tai vaikeasta unihäiriöstä kärsivillä todettiin 1.3-kertainen kohonneen herkän CRP:n (≥1.0 mg/l) riski. Naisilla positiivinen yhteys masennuksen ja kohonneen herkän CRP:n välillä todettiin vain peri- ja postmenopausaalisilla naisilla, jotka eivät käyttäneet hormonikorvaushoitoa tai suun kautta otettavia ehkäisyvalmisteita.

Tutkimustulokset viittaavat matala-asteisen tulehduksen liittyvän depressioon, ahdistukseen ja unihäiriöön nuorilla aikuisilla miehillä. Naisilla hormonaaliset seikat mahdollisesti vaikuttavat depression ja matala-asteisen tulehduksen väliseen yhteyteen. Tulevaisuuden tutkimushaasteena on selvittää matala-asteisen inflammaation mahdollinen merkitys depression, ahdistuneisuuden ja unihäiriöiden patofysiologiassa.

Asiasanat: ahdistus, c-reaktiivinen proteiini, depressio, hormonaalinen ehkäisy, hormonikorvaushoito, kohorttitutkimus, matala-asteinentulehdustila, unihäiriö

To Kaija, Akseli and Santeri

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Savonlinna, October 2011

Abbreviations

BDI-21	Beck's Depression Inventory-21
BMI	Body Mass Index
CRH	Corticotropin-releasing hormone
CRP	C-reactive protein
DM	Diabetes mellitus
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
GAD	Generalized anxiety disorder
GR	Glucocorticoid receptor
HRT	Hormone replacement therapy
ICD-10	International Classification of Diseases, Tenth edition
IL-6	Interleukin-6
hs-CRP	High sensitivity C-reactive protein
HPA axis	Hypothalamic-Pituitary-Adrenal axis
HSCL-25	Hopkins Symptom Checklist-25
MR	Mineralocorticoid receptor
NFBC 1966	Northern Finland 1966 Birth Cohort
OCD	Obsessive-compulsive disorder
PTSD	Post-traumatic stress disorder
TNF-α	Tumor necrosis factor alpha

List of original publications

This thesis is based on the following original publications, which are referred to in the text by the Roman numerals I–IV.

I Liukkonen T, Silvennoinen-Kassinen S, Jokelainen J, Räsänen, P, Leinonen M, Meyer-Rochow VB & Timonen M (2006) The association Between C-Reactive Protein Levels and Depression: Results from the Northern Finland 1966 Birth Cohort Study. Biol Psychiatry 60: 825–830.

II Liukkonen T, Räsänen, P, Ruokonen A, Laitinen J, Jokelainen J, Leinonen M, Meyer-Rochow VB & Timonen M (2007) C-Reactive protein Levels and Sleep disturbances: Observations Based on The Northern Finland 1966 Birth Cohort Study. Psychosom Med 69: 756–761.

III Liukkonen T, Vanhala M, Jokelainen J, Keinänen-Kiukaanniemi S, Koponen H & Timonen M (2010) Effect of Menopause and use of contraceptives/ hormone therapy on association of C-reactive protein and depression: A population-based study. Journal of Psychosomatic Research 68(6): 573–579.

IV Liukkonen T, Räsänen, P, Jokelainen J, Leinonen M, Järvelin M-J, Meyer-Rochow VB & Timonen M (2011) The Association between anxiety and C-reactive protein (CRP) levels: Results from the Northern Finland 1966 Birth Cohort Study. European Psychiatry 26(6): 363–369.

Contents

1 Introduction

Psychiatric disorders, especially depression, but also anxiety and sleep disorders, have become an important public health concern. A study by the World Health Organization ranked depression as the fourth global burden disease and the largest non-fatal burden of disease (Ustun *et al.* 2004). In Finland, according to the Health 2000 Study, 5% of adults had suffered a major depression episode during the preceding 12 months. Anxiety and sleep disturbances are often co-occurring with depression. On the other hand, depression, anxiety and sleep disturbances associate with an increased risk of developing cardiovascular disease and type 2 diabetes (Alvarez & Ayas 2004, Huffman 2010), and chronic low-grade inflammation has also been found to associate with these somatic diseases (Åkerstedt & Nilsson 2003, Pearson *et al.* 2003, Huffman 2010).

A growing body of evidence is suggesting unipolar depression to be associated with chronic low-grade inflammation (Penninx *et al.* 2004) and possibly low-grade inflammation to be a link between depression and the above-mentioned increased risk of somatic diseases (Danner *et al.* 2004, Ford & Erlinger 2004). Evidence has come mainly from studies concerning clinical samples or elderly people (Kop et al., Penninx *et al.* 2004). In epidemiological datasets with elderly participants the association between depression and the marker of low-grade inflammation, C-reactive protein (Ford & Erlinger 2004), has been found in men and women (Panagiotakos *et al.* 2004), but regarding the younger population only in men (Ford & Erlinger 2004, Danner *et al.* 2004). A different hormonal environment between young adult men and women is expected to affect these findings (Ford & Erlinger 2004). Regarding the association between sleep disturbances and anxiety with low-grade inflammation, the evidence is based on laboratory findings or clinical studies, while epidemiological studies are rare (Pitsavos *et al.* 2006). Population-based studies are therefore called for on this issue to confirm the association of chronic low-grade inflammation with depression, anxiety, and sleep disturbances.

This thesis is based on findings in two different study databases. The first one is the psychiatric part of the Northern Finland Birth Cohort 1966, known as the Northern Finland Health and Wellbeing Study. This database gives an opportunity to study the association between anxiety/ depression (measured by Hopkins Symptom Checklist-25) and chronic low-grade inflammation (measured by high sensitivity C-reactive protein). The presence of the health-related quality of life instrument, 15D (Sintonen 2001), also allowed focusing on sleep disturbances

and high sensitivity C-reactive protein (hs CRP) in order to study the association between them.

The Pieksämäki study database collected in 1997–98 in Pieksämäki (Finland) offered a possibility to compare the association between low-grade inflammation and depression among pre-, peri- and postmenopausal women. The entire age classes of those born in 1942, 1947, 1952, 1957 and 1962, and living in Pieksämäki, were invited (N = 1,294); out of 730 women 512 (70.1%) participated in this cross-sectional study. Depressive symptoms were assessed by Beck's Depression Inventory-21 (Beck *et al.* 1961) and high sensitivity C-reactive protein was used as an indicator of low-grade inflammation.

2 Review of the literature

2.1 The immune system and inflammation

Immunity is divided into two systems: innate and acquired immune system. Inflammation is a consequence of the activation of innate immunity. The innate immune system is a non-specific primary defense mechanism against environmental threats, such as microbial infection and physical or chemical injury. (Ruotsalainen 2009, Eklund 2009) Unlike acquired immune system, the innate immune system does not exhibit a memory response, and reacts similarly to a variety of organisms and threats (Ruotsalainen 2009). The term innate immunity is sometimes used to include physical, chemical, and microbiological barriers, but more commonly it encompasses the elements of immune systems such as neutrophils, monocytes, macrophages, complement, cytokines, and acute phase proteins, which provide immediate host defense (Parkin & Cohen 2001).

The classical signs of inflammation – redness, swelling, heat and pain – are seen locally, whereas a systemic reaction is known as an acute-phase response, which is characterized by changes in the blood concentrations of acute phase reactants. The blood concentrations of some of these proteins rise, such as C-reactive protein (CRP), fibrinogen, and serum amyloid A, while concentrations of some, for example albumin and transferrin decrease. Acute phase proteins are mainly synthesized in the liver and their production is stimulated by cytokines of the innate immune response. (Eklund 2009, Ruotsalainen 2009)

Besides the classical clinical signs of inflammation, the grade of inflammation is usually defined by inflammatory markers in the blood, of which one of the most commonly used is CRP. In acute-phase reaction the blood concentration of CRP can rise up to 1000-fold, and it is therefore widely used as part of the diagnostic workup to monitor disease status and treatment results. (Ford *et al.* 2003, Eklund 2009) CRP is considered to reflect clinically significant inflammatory state when the concentration in blood elevates over 10mg/L. CRP is mainly produced in the liver and mainly regulated by cytokines, especially interleukin-6 (IL-6), but also by IL-1β and IL-17 (Eklund 2009).

2.1.1 Low-grade inflammation

In recent years, researchers have paid attention to the possible significance of sub-clinical elevation of inflammatory markers, which have been found to associate with many common disorders. Minor elevation of inflammatory markers above baseline in the blood is defined as low-grade inflammation. Consecutively, a mild inflammation is therefore present in the body. For practical reasons, the inflammation is defined as low-grade when CRP concentration is < 10 mg/L. (Eklund 2009)

In population-based studies, serum CRP concentrations are broadly distributed among apparently healthy people and associated with a variety of demographic, metabolic, and socioeconomic factors. CRP concentrations have been found to increase slightly with age (Eklund 2009, Ford *et al.* 2003), with females having higher concentrations compared to males (Eklund 2009, Ford *et al.* 2003). In females, hormonal factors have been found to have an effect on CRP levels. CRP levels have been found to be higher in females using oral contraceptives or hormone replacement therapy (Ford *et al.* 2003), or depending on the phase of the menstrual cycle, CRP level being highest at midcycle (Jilma *et al.* 1997).

CRP concentrations have been found to associate positively with heavy alcohol intake, smoking, and body mass index (BMI) (Eklund 2009). On the other hand, the level of CRP has been found to associate inversely with socioeconomic position (Nazmi & Victora 2007), Mediterranean-style diet (high in oleic acid or monosaturated fatty acid content, fiber, and antioxidants) (Esposito *et al.* 2006) and with birth weight (Tzoulaki *et al.* 2008). CRP levels have also been found to vary between racial/ethic groups, being lowest among Caucasians and blacks, whereas Hispanic and South Asian populations have the highest levels (Nazmi & Victora 2007). In addition, elevated concentrations of C-reactive protein in adulthood have been found to associate with childhood adverse experiences (Danese *et al.* 2009).

Chronic systemic low-grade inflammation has been proposed to associate with various common somatic diseases, such as metabolic syndrome (Sutherland *et al.* 2004), diabetes mellitus type 2 (DM2) (Pradhan *et al.* 2001), atherosclerosis (Ross 1999), and some types of cancer (Heikkilä *et al.* 2009). As a marker of low-grade inflammation, CRP has been found to be a valid risk assessment tool for cardiovascular disease (CVD) events by the American Hearth Association (AHA).

C-reactive protein levels between 1 and 3 mg/L have been suggested to represent an average risk, while levels over 3mg/L represent high risk for CVD events. (Pearson *et al.* 2003)

2.2 Depression

2.2.1 Definition of depression

Depression can be regarded as an illness, but also as a feeling or a certain state of mood. Feeling of depression is a normal emotional reaction to crisis in life, for example to loss. Depressed mood, one of the typical symptoms of depressive illness (*i.e.* depressive disorder) is characterized by more of a stable and continuous experience of low mood. (Isometsä 1999) Depression as a disorder is usually diagnosed according to criteria in either of the two main diagnostic systems: DSM-IV (the fourth edition of Diagnostic and Statistical Manual of Mental Disorders) (American Psychiatric Association 1994) or ICD-10 (International Classification of Disorders) (World Health Organization 1992). In Finland, the latter is in clinical use, and diagnosing depression is based on typical (depressed mood, loss of interest and enjoyment, increased fatigability) and other common symptoms (reduced concentration and attention, reduced self-esteem and self-confidence, ideas of guilt and unworthiness, bleak and pessimistic views of future, ideas or acts of self-harm or suicide, disturbed sleep, diminished appetite). (World Health Organization 1992) For clinical diagnosis, the symptoms are expected to be present daily, covering the greater portion of the day and lasting a minimum of two weeks. Depression can also be classified by severity as mild, moderate or severe depression and as psychotic depression in case when either delusions or hallucinations are present. Depression is also classified by the duration of depressive symptoms (*e.g.* chronic or recurrent), but also by the quality of symptoms. The clinically relevant subgroups of depression are psychotic, melancholic, atypical, postpartum depression, chronic depression and seasonal affected disorder as listed by Isometsä (1999).

2.2.2 Prevalence of depression

Depression is a disorder of great public health concern worldwide: depression was calculated to be the fourth leading cause of disease burden globally in the

Global Burden of Disease 2000 study by WHO. It causes the largest amount of non-fatal burden, which accounts for nearly 12% of all total years lived with disability (Ustün *et al.* 2004). In the cross-sectional WHO World Health Survey (WHS), which was conducted in all regions (60 countries, n = 245404) of the world, consisting of adult participants over 18 years of age, the 1-year prevalence of ICD-10 depressive episode was 3.2% (95% CI 3.0–3.5) (Moussavi *et al.* 2007). In patients with several different medical illnesses, the prevalence of depression was higher than in general population (Moussavi *et al.* 2007), being 5–10% among patients in primary care (Katon & Schulberg 1992).

In Finland, the Health 2000 Study (a nationally representative sample of 6,005 persons aged 30 years or over) reported that 5% of participants had suffered a major depressive episode during the previous 12 months (Pirkola *et al.* 2005). Generally, depression is more common in women. In the Health 2000 Study the prevalence was 6.3% in women and 3.4% in men (Pirkola *et al.* 2005). The FINHCS'96 (Finnish Health Care Survey, including 5,993 Finns aged 15 to 75 years) study found age-adjusted prevalences for females and males of 10.9% (95% CI 9.7–12.0) and 7.2% (95% CI 6.2–8.2), respectively, for 1-year major depressive episode (Lindeman *et al.* 2000).

2.2.3 Etiology of Depression

Monoamine hypothesis

The monoamine hypothesis was developed during the 1960s to explain the pathophysiology of depression based on both pharmacological and monoamine-depletion studies. According to this hypothesis, there is a dysfunction in the monoamine systems, namely noradrenergic, serotonergic, and dopaminergic systems of the brain in individuals with depression. Although monoamine systems are known to be involved in the pathophysiology of depression, the dysfunction of single neurotransmission systems comprises a small part of the complex neuromodulatory mechanisms behind depression. (Thase 2009, Belmaker & Agam 2008)

Stress and HPA axis

In many cases a stressful life event (SLE), such as loss or humiliation, is known to precede the onset of depression (Kendler *et al.* 2003). Although the correlation between a stressful life event and depression has been shown to be causal (Kendler *et al.* 2006), the stressful life event is not always followed by depression. Vulnerability to SLE, *i.e.*, life event-associated stress is supposed to vary between individuals. Furthermore, the personal characteristics of individuals, *i.e.*, cognitive style, have been proposed to be associated with a certain type of symptom pattern after SLE. On the other hand, there are findings supporting the symptom pattern to be related to the type of SLE. (Keller *et al.* 2007)

Normally, in case of acute stress, the body forms a stress response to maintain homeostasis, which involves two neuroendocrine systems: the hypothalamic-pituitary-adrenal (HPA) axis and the sympatomedullary axis. As a stress response, the hypothalamus releases corticotropin-releasing hormone (CRH), which induces adrenocorticotropin (ACTH) release from the pituitary, which subsequently causes corticosteroid (mainly cortisol in human) release from the adrenal cortex. Cortisol normally exerts a negative feedback effect to shut down the stress response after the threat has passed, acting upon pituitary and hypothalamus levels. As a major stress hormone cortisol acts on many organs and brain areas through two types of receptors: mineralocorticoid (MR) and glucocorticoid receptors (GR) (Bao *et al.* 2008, Pariante & Lightman 2008).

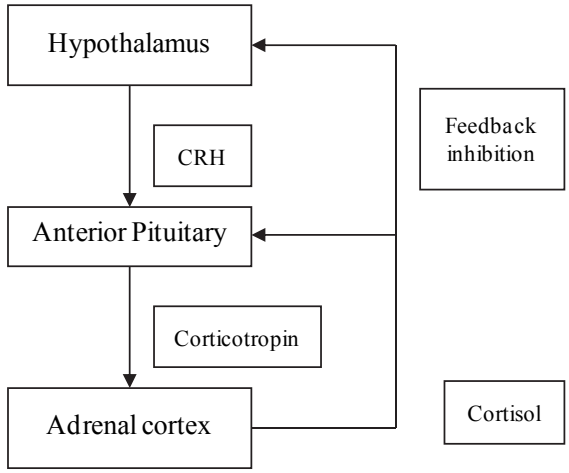

Fig. 1. The hypothalamic-pituitary-adrenal axis (adapted from Belmaker & Adam 2008).

Hyperactivity of the HPA axis is one of the most consistent biological findings in major depression (Pariante & Lightman 2008). One part of this mechanism is supposed to be impaired glucocorticoid-mediated feedback inhibition, *i.e.*, GR resistance (Bao *et al.* 2008).

Heritability

Depression is known to aggregate in families. Although environmental factors are found to be important in the etiology of depression, the familiarity seems mostly to result from genetic influence, according to adoption and twin studies. (Sullivan *et al.* 2000) Depression is a genetically heterogeneous disorder, and so far no single gene variant has been able to explain the development of depression. Gene-environment interaction has been proposed to be of importance in the development of depression, *i.e.*, possible genetic vulnerability might be expressed only if an individual is exposed to SLE. The serotonin transporter gene, 5-HTTLPR (serotonin-transporter-linked promoter region) short allele, together with stressful life events, has been proposed to elevate the risk of becoming depressed (Uher 2008), although these findings have been questioned later (Risch *et al.* 2009). The genes coding GR and CRF are also supposed to modulate the development of depression (Uher 2008, El Hage *et al.* 2009).

Immune functions

Immune functions are proposed to be involved in the pathophysiology of depression. Depressed patients are found to have elevated levels of proinflammatory cytokines, such as interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) in the blood (Miller *et al.* 2009a, Dowlati *et al.* 2010). Cytokines are supposed to have an impact on the monoamine system, HPA axis, and also on GR functioning, which are all found to be dysfunctional in depression. Besides somatic infectious disease, psychosocial stress can also activate inflammatory response. (Miller *et al.* 2009b) This mechanism involves both the sympathetic nervous system and the HPA axis. A major stressful life event in childhood, *e.g.* childhood maltreatment, has been proposed to have a major effect on inflammatory functions, showing elevated levels of inflammatory markers among adults with a history of childhood maltreatment (Danese *et al.* 2007).

Gender differences

The prevalence of depression differs greatly between genders during the reproductive years of women. The difference between genders starts at puberty, ending at menopause in women. Higher incidence of depression is especially found postpartum and during the transition to menopause. In addition, during the premenstrum, women are known to suffer from symptoms overlapping (premenstrual syndrome) with depression, including lowering of mood. (Young & Korszun 2010, Bao *et al.* 2008, Cohen *et al.* 2006, Freeman *et al.* 2006) The gonadal hormones in women, especially estrogen, are thought to have an effect on the pathophysiology of depression, since all those above-mentioned time periods of women's lifespan are characterized by lowering of estrogen levels.

Imaging studies

Some structural changes as well as functional changes have been found in MRI and PET imaging studies in patients with depression. For example, increased amygdala reactivity to negative stimuli has been demonstrated (Savitz & Drevets 2009). Hippocampal volume reduction is also rather a common finding among depressive patients. However, these findings are non-specific to depression and also found in several other psychiatric disorders. MRI scans show an increased number of deep white matter hyperintensities. These findings have led to a hypothesis of vascular depression, a condition associated with multiple cortical infarcts of ischemic origin. (Savitz & Drevets 2009)

2.3 Anxiety

2.3.1 Definition of anxiety

Anxiety and fear are two of the core negative emotions in humans. As an experience they are much alike, although they differ as to context. Fear is more a reaction to presentation of overtly dangerous stimuli, whereas anxiety is more of a reaction to signals predicting, but not immediately presenting danger. Broadly defined, anxiety can be understood as a fear-like emotion without real existing danger. (Pine 2009)

Anxiety is normally experienced in everyday life. Clinically significant anxiety is justified by distress and functional impairment caused by it, which

forms a basis of diagnosis of different anxiety disorders. In addition to the emotion of anxiety, symptoms of autonomic nervous system are usually present in different anxiety disorders, and separate disorders differ by their clinical appearance, but also by the context where or when the symptoms appear. Anxiety disorders in the ICD-10 classification (World Health Organization 1992) are categorized under the title "Neurotic, stress-related and somatoform disorders" (Table 1). In general, the diagnoses in the DSM-IV are the same, except the hierarchy in panic disorder and agoraphobia. In the ICD-10 the phobic disorder is primary, but in the DSM-IV the panic disorder is primary and subcategorized by whether agoraphobia exists or not. (Isometsä 1999). In clinical practice, the five most debilitating disorders are panic disorder (PD), obsessive compulsive-disorder (OCD), social anxiety disorder (SAD), posttraumatic stress disorder (PTSD) and generalized anxiety disorder (GAD). (Garner *et al.* 2009)

Table 1. The ICD-10 Classification of mental and behavioral disorders, neurotic, stress-related and somatoform disorders (main classification shown).

F40	Phobic anxiety disorders (incl. agoraphobia and social phobia)
F41	Other anxiety disorders (incl. panic disorder and generalized anxiety disorder)
F42	Obsessive-compulsive disorder
F43	Reaction to severe stress and adjustment disorders (incl. post-traumatic stress disorder)
F44	Dissociative disorders
F45	Somatoform disorders
F48	Other neurotic disorders

2.3.2 Prevalence of anxiety

Anxiety disorders are a group of the most prevalent mental disorders. The World Health Organization (WHO) World Mental Health (WMH) Initiative Survey conducted in 14 countries worldwide found anxiety disorders to be the most prevalent mental disorders in all but one country during the previous 12 months (prevalence ranging from 2.4% to 18.2%, IQR (interquartile range) 5.8–8.8%) (Demyttenaere *et al.* 2004). In Finland, the Mini Finland Health Survey reported a prevalence of 6.2% for anxiety or phobic neurosis during the previous 12 months among young adults, being 7.5% among women and 4.6% among men (Lehtinen *et al.* 1990). The Health 2000 Study reported that 4.8% of women and 3.7% of men had suffered from anxiety during the previous 12 months (Pirkola *et al.* 2005).

Single anxiety disorders are commonly co-occuring with other anxiety disorders. In the World Health Organization (WHO) World Mental Health (WMH) Initiative Survey, significant comorbidity was found between panic disorder and agoraphobia, social phobia and agoraphobia, and also between anxiety and depression (Kessler *et al.* 2011).

2.3.3 Etiology of anxiety

Etiology of feeling of anxiety and different anxiety disorders includes various complex mechanisms that have been found on many different levels: psychological, structural, and functional. One uniform model may not be able to explain the etiology of different anxiety disorders. However, there are some common findings reflecting the pathophysiology of anxiety disorders.

Stressful life events, temperament and environmental factors

Stressful life events are found to precede anxiety disorders. The threat events were found to precede anxiety disorders, while loss or humiliation was found to precede depression, although loss was also found to precede GAD. (Beesdo *et al.* 2009) Temperament and parenting style have also been shown to associate with anxiety disorders. Furthermore, behavioral inhibition, shown as tendency to display fear and to withdraw from unfamiliar situations, is also shown to be a risk factor for development of anxiety disorders, especially social phobia (Beesdo *et al.* 2009). Parental overprotection has been revealed to increase the risk of anxiety disorders, but interestingly, not of pure depression, which is associated with rejection by a parent (Garner *et al.* 2009). Finally, childhood adversities are associated with a wide range of psychiatric disorders in adulthood, including anxiety disorders (Beesdo *et al.* 2009).

Neurocognitive model

Development of anxiety and anxiety disorders involves numerous neurobiological mechanisms. Several structures, such as the amygdala, prefrontal cortex (PFC), hippocampus, and HPA axis are possibly the most important structures concerning fear-formation, forming a response to stress, and development of anxiety. (Berretta 2005, Garner *et al.* 2009, Drevets *et al.* 2009) The amygdala has been demonstrated to be involved in learning to fear a previous stimulus, whether it is

neutral or harmless, and later the amygdala responds (automatically) to learned stimuli which predicted danger earlier (Shin & Liberzon 2010). PFC has been found to have an important modulating role together with the hippocampus by setting a stimulus in the context when learning whether a stimulus is neutral or harmless, and later PFC has a role in controlling and down-regulating the amygdala (Fig 2.) (Berretta 2005, Cisler & Coster 2010, Groeneweg *et al.* 2011). The neurocognitive model of anxiety proposes a dysfunction in amygdala-PFC circuitry, which causes biased emotional processing, *e.g.* selective attention to threat, interpretation of ambiguous emotional stimuli, and acquisition and extinction of conditioned fear (Garner *et al.* 2009). Clinically anxious individuals demonstrate a readiness to selectively attend to threat cues and to interpret emotionally ambiguous stimuli in a negative manner (Garner *et al.* 2009).

The amygdala itself consists of a functionally integrated complex of cellular groups that play a crucial role in coordinating and modulating autonomic, hormonal, neurochemical and motor aspects of responses to stressful events. The amygdala receives visceral, nociceptive, and humoral information from a broad range of inputs and provides substantial inputs to the hypothalamus, which mediates sympathetic and HPA axis activation. (Berretta 2005)

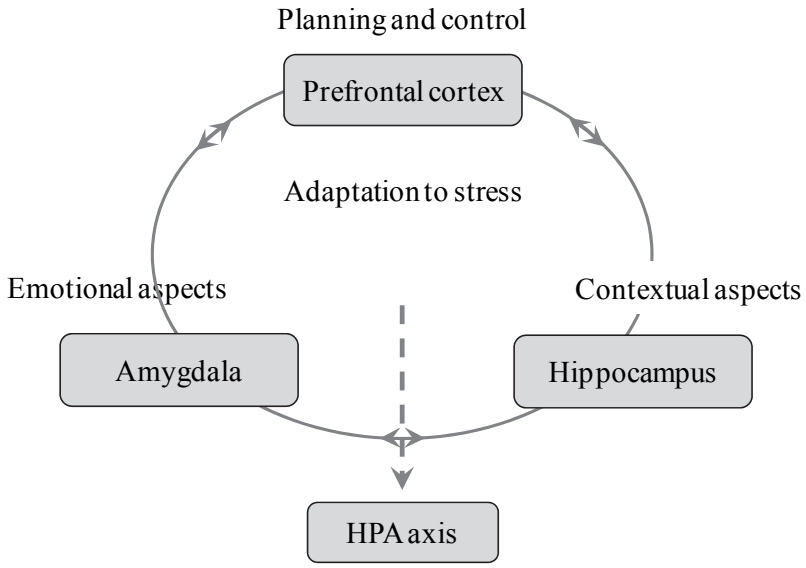

Fig. 2. Limbic system in fear-formation and in forming a response to stress (adapted from Groeneweg *et al.* 2011).

Neuroendocrine systems

Dysfunction of numerous neuroendocrine systems may also play a significant role in the etiology of different anxiety disorders. In general, neurochemicals released during stress or prolonged anxiety can result in chronic alteration in the neuroendocrine systems. In many anxiety disorders, early life stressors or trauma may result in a predisposition to development of anxiety disorder later in life, possibly due to the altered neuroendocrine systems or direct neurotoxic effect. The neuroendocrine systems found to have an impact on anxiety disorders are the noradrenergic, dopaminergic, serotonergic, benzodiazepine and glutamate system and the HPA axis. A number of neurochemicals have also been found to play a role in anxiety disorders. These include neurosteroids, arginine vasopressin, neuropeptide Y, cholecystokinin, and opioid peptides. (Garakani *et al.* 2009)

HPA axis and immune functions

Dysfunction of the HPA axis has been proposed to play a role in the pathophysiology of anxiety disorders (Greaves-Lord *et al.* 2009). Individuals who are vulnerable to anxiety symptoms are supposed to have a lower threshold for HPA axis activity following stimuli and are therefore more easily prone to stress reactions and anxious and withdrawing behavior when trying to avoid stressful situations. HPA axis activation is also known to cause activation of the immune system, which is found to be associated with anxiety (Pitsavos *et al.* 2006). Since anxiety is also found to be associated with infectious and autoimmune diseases, it has been hypothesized that immune functions are involved in the pathophysiology of anxiety (Garcia-Bueno *et al.* 2008).

2.4 Association of chronic low-grade inflammation with depression and anxiety

2.4.1 Chronic low-grade inflammation and depression

Depression has been found to associate with somatic diseases, especially cardiac heart disease (CHD). Rather solid scientific evidence also shows an association between CHD and low-grade inflammation. Furthermore, elevated CRP, as a marker of low-grade inflammation, is a good predictor for CHD events. (Pearson *et al.* 2003) According to these findings, the association between depression and

low-grade inflammation has been proposed earlier. In addition, a possibility of low-grade inflammation being an underlying link between CHD and depression has been suggested.

The association between depression and low-grade inflammation has been studied in several clinical samples. Among population-based studies, the evidence came first from studies with mainly elderly participants. Among these studies Kop *et al.* (2002) compared mean CRP level among 4268 participants over 65 years of age (mean 72.4 ± 5.5 years) between depressive and non-depressive subpopulations and found depressive participants to have higher mean CRP levels (3.51 ± 0.21 vs. 3.31 ± 0.10; p = 0.0008), but after adjusting for cardiovascular risk factors, the result attenuated to non-significant (p = 0.056). In addition, Tiemeier *et al.* (2003) demonstrated results suggesting a positive association between CRP and depressive disorder, but the association disappeared after adjustment for confounding factors. Penninx *et al.* (2003) showed an elevated risk for depression (OR 1.48, 95% CI 1.04–2.04, p = 0.03) among those with elevated CRP (≥ 3.17mg/L). Ladwig *et al.* (2003) revealed a corresponding association only in the subgroup of obese men 45–74 years of age.

In studies consisting mainly of non-elderly adults, positive associations have been shown between depression or depressive symptoms and CRP: Danner *et al.* (2004) found an earlier depressive episode to associate positively with elevated (≥ 2.2 mg/L) CRP (OR 2.77, 95%CI 1.43–5.26) in men but not in women among participants aged 17 to 39 years. Interestingly, this association was stronger when the last depressive episode had been less than 1 month before and weaker if the last episode had taken place 12 months previously (Danner *et al.* 2003). With the same database (NHANES III, Third National Health and Nutrition Examination Survey), Ford and Erlinger (2004) revealed an association between recent (within last year) episode of depression and elevated CRP (OR 3.00, 95% CI 1.39–6.48) in men and also between elevated CRP and recurrent depression episodes (≥ 2 episodes) (OR 3.55, 95% CI 1.55–8.14) in men, but not in women. In the ATTICA study (Health and nutrition Survey in Attica province, Greece), Panagiotakos *et al.* (2004) found a significant positive correlation between depressive symptoms and CRP both in men, and also in women among participants aged 18 to 84 years. Using Cardiovascular Risk in Young Finns Study data (1,201 young adults), Elovainio *et al.* (2006) also revealed an association between depressive symptoms after adjusting for various confounding factors, but after adjustment for obesity and triglyceride levels, the result attenuated to non-

significant. They concluded that an association may therefore be largely attributable to obesity or triglycerides (Elovainio *et al.* 2006).

Regarding gender differences, the association between depression and low-grade inflammation has shown to be more evident among men (Danner *et al.* 2004, Ford & Erlinger 2004). Among young adult women (17–39 years of age), neither Danner *et al.* (2004) nor Ford and Erlinger (2004) found a significant association. On the other hand, in study consisting of 51% of elderly female participants, Penninx *et al.* showed the association under discussion (2003). Also Panagiotakos *et al.* (2006) showed a significant association among men and also among women, although the age range in that study was 18–84 years (men mean 45 ± 13 and women 44 ± 18). Based on these findings many of the authors have concluded that women's different hormonal milieu at premenopause could possibly be the factor explaining the discrepancy in results between men and younger women. (Danner *et al.* 2003, Ford & Erlinger 2004)

2.4.2 Chronic low-grade inflammation and anxiety disorders

Like depression, anxiety has been found to be associated with low-grade inflammation-associated somatic diseases, especially CHD (Huffman *et al.* 2010). Of note is, however, that until now the putative association between low-grade inflammation and anxiety/single anxiety disorders has almost entirely been studied in clinical settings.

Based on the ATTICA study (general population-based health and nutrition survey), Pitsavos *et al.* (2006) found among cardiovascular disease-free people (n = 853) anxiety symptoms to be associated with elevated CRP and interleukine-6 (IL-6) levels in men and women. The association between single anxiety disorders and low-grade inflammation, mainly PTSD, has come under scrutiny in clinical settings. Based on small clinical samples, there is some evidence that systemic low-grade inflammation could be associated with GAD (Bankier *et al.* 2008), panic disorder (Herran *et al.* 2005), anxiety disorder (not otherwise specified) (Bankier *et al.* 2009), and PTSD (Baker *et al.* 2001, Miller *et al.* 2001, Hoge *et al.* 2009, von Känel *et al.* 2010), even though the findings regarding PTSD are to some extent controversial (Maes *et al.* 1999, Söndergaard *et al.* 2004, von Känel *et al.* 2007).

2.5 Sleep disturbances; relation to depression, anxiety and low-grade inflammation

2.5.1 Definition of sleep disturbances

Insomnia is defined by difficulty initiating sleep, maintaining sleep or having nonrestorative sleep for 1 month or longer. The DSM-IV diagnostic criteria of insomnia also include a clinically significant impairment or distress, and insomnia should not be rooted in any other etiological cause, such as substance use (DSM-IV). Sleep disturbance is more of a term defining problems of sleep in general. Insomnia is often divided into three categories of psychophysiological, paradoxal, and idiopatic insomnia. (Pigeon 2010) Paradoxical insomnia, defined by extreme discrepancy between subjective and objective sleep, and idiopathic insomnia, defined by lifelong and unremitting inability to obtain adequate sleep, are found to be rather uncommon. Psychophysiological insomnia is characterized by somatized tension and learned sleep-preventing psychological associations. (Pigeon 2010) The prevalence of insomnia varies according to the criteria applied. Approximately 10% of people suffer from persistent insomnia in industrialized countries, and one third to one fourth suffer from sleep disturbances at some point in their lives (Ohayon & Roth 2002).

2.5.2 Etiology of sleep disturbances

No single etiological model can explain insomnia, although one widely known model suggests that individuals may have predisposing characteristics, such as various forms of hyperarousal, and/or a tendency to worry or ruminate. A precipitating factor, such as stressful life event, may initiate insomnia, and predisposing factors, such as maladaptive behavioral coping, for example daytime napping, may result in continuous arousal and chronic insomnia. Hyperarousal can be understood as elevated basal level of arousal or as a failure to downregulate this level in the evening or at night. In terms of hyperarousal, patients with insomnia are found to have increased activity of the sympathetic nervous system and HPA axis. (Pigeon 2010)

2.5.3 Sleep disturbance; relation to depression and anxiety

Sleep disturbance is one of the common symptoms in depression and forms part of the diagnostic criteria for depression. (DSM-IV, ICD-10). Depressive patients often suffer from difficulty getting to sleep, frequent awakening or nonrestorative sleep (Benca & Peterson 2008). Insomnia is a separate diagnostic entity that often co-occurs with depression. One large epidemiological study (National Institute of Mental Health Epidemiologic Catchment Area, N = 7,954) found a prevalence of 10% for insomnia and 5% for depression, while 23% of insomnia patients had depression, and 42% of depression patients had insomnia (Ford & Kamerow 1989). Insomnia has been studied as a possible risk factor for depression, and according to a rather large body of evidence, both incident and persistent insomnia are found to predict new-onset depression and new episodes of recurrent depression (Pigeon 2010, Johnson *et al.* 2006). Some authors have even suggested possible common neurobiological mechanisms underlying depression and insomnia (Benca & Peterson 2008).

Regarding anxiety disorders, sleep disturbance is one of the symptoms of posttraumatic stress disorder, for example. Insomnia as a separate entity often co-occurs with anxiety disorders. A study with young adult participants having chronic insomnia revealed lifetime prevalences of different anxiety disorders to be 7.8% for GAD, 6.0% for panic disorder, 5.4% for OCD, and 25.2% for phobic disorders. (Breslau *et al.* 1996) Studies concerning the temporal association between anxiety and insomnia suggest anxiety to be prior to insomnia (Johnson *et al.* 2006, Ohayon & Roth 2002).

2.5.4 Sleep disturbance and low-grade inflammation

The sole body of findings from small laboratory studies have shown sleep restriction to be followed by an elevation of proinflammatory cytokines (Shearer et 2001, Vgontsas *et al.* 2004, Irwin *et al.* 2006), and CRP (Meier-Ewert *et al.* 2004) and also an association between impaired night time sleep and increased secretion of proinflammatory cytokines (Vgontsas *et al.* 2003), or both proinflammatory cytokines and CRP among young women (Okun *et al.* 2009) and men (van Leeuwen *et al.* 2009). In addition, one population-based study (n = 188) has revealed an association between sleep disturbance as indicated by latency to sleep, and elevated CRP. (McDade *et al.* 2006) Based mainly on laboratory

studies with small samples, sleep disturbances seem to be associated with low-grade inflammation.

2.6 Summary of the reviewed literature

Depression and anxiety are of great public health concern in Western societies. Rather solid evidence has linked depression, as well as anxiety, with inflammation-associated somatic diseases such as CHD and DM2. Furthermore, there is increasing evidence showing low level of systemic inflammation, *i.e.*, low-grade inflammation, to be associated with depression and also anxiety.
Regarding the etiology of depression and anxiety, there are different findings suggesting complex pathophysiological mechanisms. In the context of psychoneuro-immunology, many findings point to possible dysfunction of the HPA axis in both depression and anxiety. Theoretically, low-grade inflammation could be a reflection of HPA axis dysfunction in these disorders. Therefore, studies concerning possible association of low-grade inflammation with depression and anxiety are called for.

Scientific evidence concerning an association between depression and low-grade inflammation comes mainly from clinical studies or population-based studies mainly consisting of elderly participants. The few studies with non-elderly participants have reported a corresponding association only among young adult men, but not among young adult women, which has been theoretically interpreted to be a consequence of a different hormonal milieu between young adult men and women. Furthermore, whether the association between elevated CRP levels and depression is different among women before and after menopause has not been investigated at epidemiological level in a general population database. According to the earlier literature, it is also not known whether exogenous hormones, namely oral contraceptive use and postmenopausal hormone therapy, have an effect on this association.

Some clinical studies point towards the fact that anxiety could be positively associated with low-grade inflammation. Population-based studies are rare, although they support the results from clinical studies.

Anxiety and depression are commonly co-occuring disorders and both are possibly associated with low-grade inflammation, but according to the current literature it has remained unclear whether co-occuring anxiety and depression are connected with low-grade inflammation.

Sleep disturbances are common symptoms of depression, and *e.g.* insomnia prevalently co-occurs with both anxiety and depression. Laboratory studies with small samples have showed elevation of inflammatory markers following sleep restriction, and small studies based on clinical data suggest that sleep disturbances could be positively associated with low-grade inflammation, but large population based studies are still called for.

3 Aims of the present study

The purpose of this study was to investigate the association of low-grade inflammation with psychiatric disorders, namely depression and anxiety, and sleep disturbances by using population-based databases. In addition, the purpose of this study was to investigate the effect of female hormonal status on the association between low-grade inflammation and depression. The numbers I–IV hereafter refer to the original publications.

The aims of the present study were:

1. To investigate whether the probability for depressive episodes (previous, current single, and recurrent) in general population differs in males and females with different levels of low-grade inflammation. (I)
2. To study whether sleep disturbances in general population associate with low-grade inflammation. (II)
3. To examine whether menopausal status and exogenous hormone use (hormonal contraceptive or menopausal hormone therapy) have an effect on the association between depression and low-grade inflammation in females. (III)
4. To investigate whether anxiety and comorbid state of anxiety and depression in general population are associated with different levels of low-grade inflammation in males and females. (IV)

4 Material and methods

4.1 Materials

This thesis is based on two different databases: the Northern Finland 1966 Birth Cohort and the Pieksämäki study.

4.1.1 The Northern Finland 1966 Birth Cohort

Professor (emerita) Paula Rantakallio started the prospective Northern Finland 1966 Birth Cohort Study (NFBC 1966) with the purpose of studying the risk factors of low birth weight and perinatal deaths (Rantakallio 1969, 1988). The origin of that study was all the mothers with calculated term falling between January 1st and December 31st, 1966 in geographically defined area of the two northernmost provinces of Finland (*i.e.*, Oulu and Lapland). They gave birth to 12,058 live infants; the majority of them were Finns, 60 were Lapps, and 20 were Gypsies (nowadays often referred to as Roma). A variety of biological, socioeconomical and health condition factors, as well as living habits and family characteristics of the cohort members have been collected prospectively from prenatal stages. (Rantakallio 1969, 1988) Until 2010 three main follow-up surveys have been carried out on the NFBC 1966 cohort (Rantakallio 1988, Sorri & Järvelin 1998).

The present study (original publications I, II, IV) belongs to the psychiatric part of the 31-year follow-up survey, known as the Northern Finland Health and Wellbeing Study (Sorri & Järvelin 1998). During 1997–98, in this third follow-up study of NFBC 1966, 11,541 cohort members were sent postal questionnaires, with a response rate of 75.3%. All those living in Northern Finland or in the capital area were invited to a clinical examination (n = 8,463), and 6,007 (70.1%) of this sample attended the study. The data have been regularly linked to national registers such as hospital discharge registers. (Timonen 2003)

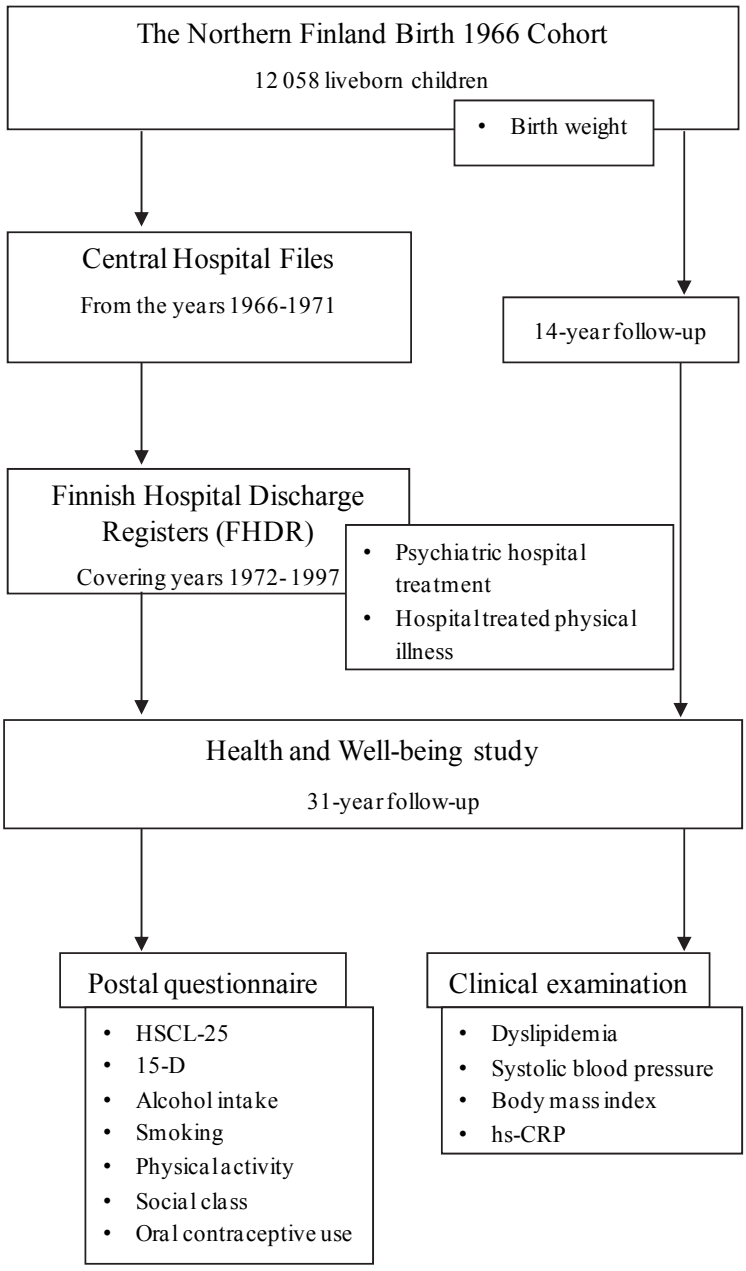

Fig. 3. The flowchart of the data collection procedure in the Northern Finland 1966 Birth Cohort Study and the main data used in the original publications I, II, and IV.

4.1.2 Pieksämäki study

The Pieksämäki study was conducted in order to determine the prevalence of risk factors and long-term course of metabolic syndrome. The study population consists of middle-aged, Caucasian subjects born in 1942, 1947, 1952, 1957, and 1962. All inhabitants in Pieksämäki, Finland within these age cohorts and included in the official population register were invited on three separate occasions during 1997–98 (n = 1,294) to participate in the clinical examination. (Vanhala *et al.* 2008, Koponen *et al.* 2008, Saltevo 2008) No exclusion criteria were applied. The follow-up took place in 2005–06. The participation rate at baseline in 1997–98 was 923/1,294 (71.3%). 512 of the eligible 730 women (70.1%) participated at baseline. The study population of the original publication III consists of those 512 women born in 1942 (n = 69), 1947 (n = 146), 1952 (n = 107), 1957 (n = 110) and 1962 (n = 80) who participated in the baseline investigations in 1997–98.

4.2 Methods

4.2.1 Assessment of inflammation (I–IV)

Inflammation was assessed by using high sensitivity C-reactive protein (hs-CRP). It is considered to be more stable than other measures of inflammation, such as IL-6 (Pearson *et al.* 2003).

In original publications I, II and IV, hs-CRP levels were measured from the sera collected during the clinical examination (the 31-year follow-up survey of the NHBC 1966) after 12-hour fasting by using a sensitive immunoenzymometric assay (Medix Biochemica, Helsinki, Finland). The sensitivity of the assay was 0.08 mg/L (Taponen *et al.* 2004). Hs-CRP levels of ≥ 1.0 mg/L were considered elevated (I, II and IV), and levels of > 3.0 mg/L highly elevated (I, IV) (Pearson *et al.* 2003).

In the Pieksämäki study (III), fasting blood samples were drawn after 12 hours of fasting. Plasma was separated by centrifugation, and samples frozen immediately at −20ºC, sent weekly from Pieksämäki to Kuopio and stored there at −70ºC until analyses were made in 2003. Hs-CRPs were measured with an Immulite 2000 High Sensitivity CRP assay (Diagnostic Product Corporation, Los Angeles, CA, USA). (Saltevo 2008)

4.2.2 Assessment of depression and anxiety (I–IV)

Hopkins Symptoms Checklist-25 ((I, II, IV)

The depressive symptoms were defined by using Hopkins Symptom Checklist-25 (HSCL-25; I, II, IV)). HSCL-25 was included in the postal questionnaires sent to cohort subjects in 1997 (Sorri & Järvelin 1998). HSCL-25 is a 25-item shortened version of an originally 90-item questionnaire designed by Derogatis and colleagues (1974), and has been found to be a valid instrument in a Nordic multicenter investigation, also in Finnish (Fink *et al.* 1995, Veijola *et al.* 2003). HSCL-25 includes both depression and anxiety subscales. A depression subscale (I, II, IV) containing 13 questions, and an anxiety subscale (IV) containing 10 questions were used to define depressive and anxiety symptoms (Mollica *et al.* 1987, Winokur *et al.* 1984).

The depression subscale covers items of 1) feeling low in energy or feeling slowed down, 2) blaming yourself for things, 3) crying easily, 4) loss of sexual interest or pleasure, 5) feeling hopeless about the future, 6) feeling blue, 7) feeling lonely, 8) thoughts of ending your life, 9) feeling of being trapped or caught, 10) worrying too much about things, 11) feeling no interest in things, 12) feeling everything is an effort, 13) feeling of worthlessness. The anxiety subscale covers items of 1) being suddenly scared for no reason, 2) feeling fearful, 3) feeling of faintness or dizziness or weakness, 4) nervousness or feeling of shaking inside, 5) heart pouncing or racing, 6) trembling, 7) feeling tense or keyed up, 8) headaches, 9) spells of terror or panic, 10) feeling restless or difficulty in sitting still (Winokur *et al.* 1984). HSCL-25 also contains two additional items, which were not used in this thesis, because the original article (Derogatis et al. 1974), which defined depression and anxiety subscales, had not included those questions.

In both subscales, cohort members rated their estimates of severity with their depressive/anxiety symptoms on a scale ranging from 1 ("not at all") to 4 ("extremely severe"). Responses from each question were summed and divided by the number of answered items to generate an anxiety mean score as well as a depression mean score ranging from 1.0 to 4.0. Cut-off points of depression subscale mean scores of ≥ 1.55 (I, II), ≥ 1.75 (I, IV) and ≥ 2.01 (I) were used to define depressive symptoms. In original publication I the cut-off points of depression subscale mean scores were used to determine subgroups with different severity of depressive symptoms; 1) 1.55–1.74, 2) 1.75–2.0 and 3) 2.01–4.00.

(Derogatis *et al.* 1974, Nettelbladt *et al.* 1993, Winokur *et al.* 1984, Sandanger *et al.* 1998) The presence of anxiety symptoms was defined when HSCL-25 anxiety subscale mean score was ≥ 1.75 (IV; Winokur *et al.* 1984).

HSCL-25 anxiety and depression subscale mean scores were used to form variables of anxiety symptoms, depressive symptoms, and comorbid anxiety and depressive symptoms (IV). Anxiety symptoms (without depression) were defined to be present if cohort members had mean scores of ≥ 1.75 (Derogatis *et al.* 1973, Mollica *et al.* 1987, Winokur *et al.* 1984) on the HSCL-25 anxiety subscale and mean scores of < 1.75 on the HSCL-25 depression subscale. Depressive symptoms (without anxiety) were defined to be present if cohort members had mean scores of ≥ 1.75 on the HSCL-25 depression subscale and < 1.75 on the HSCL-25 anxiety subscale. Anxiety symptoms with co-occurring depressive symptoms were defined to be present if cohort members had a mean score of ≥ 1.75 on both the HSCL-25 anxiety and the depression subscales. (IV; Derogatis *et al.* 1973, Mollica *et al.* 1987, Winokur *et al.* 1984)

In original publication II, the HSCL-25 depression subscale mean score of ≥1.55 was used to define depressive participants in order to exclude them from the statistical analyses.

Self-reported doctor-diagnosed earlier depression (I)

The NFBC 1966 cohort members were also asked the following question: ”Have you ever been diagnosed by a doctor as having depression or have you ever been treated by a doctor because of depression (yes/no).” If the subject answered “yes” to the question about earlier doctor-diagnosed depression, and the mean score on the HSCL-25 depression subscale was < 1.55, this was defined as a previous depressive episode (I).

Current single depressive episode was defined with a mean score of ≥1.55 on the HSCL-25 depression subscale, and the absence of earlier doctor-diagnosed depression. The severity of a current single depressive episode was assessed with the mean scores of ≥ 1.55, ≥ 1.75, and ≥ 2.01 on the HSCL-25 depression subscale.

Recurrent depression was defined with an earlier doctor-diagnosed depression and a current mean score of ≥ 1.55 on the HSCL-25 depression subscale. The severity of the current period of recurrent depression was assessed similarly as with a single episode.

Beck's Depression Inventory -21 (III)

In the Pieksämäki study, Beck's Depression Inventory -21 (BDI-21) was included in the questionnaire. BDI-21 was developed in the 1960s to measure depression severity (Beck *et al.* 1961). BDI-21 includes 21 questions with increasing level of severity from 0 to 3. Total score is generated by summing up scores from each question. A sum score of 0 to 9 is considered to represent minimal symptoms, 10 to 16 mild, 17 to 29 moderate, and a sum score of ≥ 30 severe symptoms (Blacker 2009). Thus, in original publication III, sum score ≥ 10 was considered a depressive state.

4.2.3 Assessment of sleep disturbances (II)

The postal questionnaires of the 31-year follow-up survey of the Northern Finland Birth Cohort 1966 also included the 15D-questionnaire (Sintonen 2001). 15D is a self-administered quality of life scale, which includes 15 dimensions: mobility, vision, hearing, breathing, sleeping, eating, speech, elimination, usual activities, mental function, discomfort and symptoms, depression, distress, vitality, and sexual activity. Each question is rated from 1 to 5 with increasing severity of problem. In original publication III, question number 5 defining the quality of sleep was used to measure sleep disturbances. Alternative 1 indicated no sleep problem, 2 slight, 3 moderate, 4 considerable, and alternative 5 severe sleep disturbances. For the purpose of this study sleep disturbances were categorized as 1 no sleep disturbances, 2 slight sleep disturbances and 3 pooled moderate, considerable, or severe sleep disturbances (III).

4.2.4 Confounding variables

The confounding variables taken into account in statistical analyses in the original publications I-IV are presented in Table 2.

Table 2. Confounding variables taken into account in the original publications (I–IV).

Confounding variables	Original publication
Alcohol intake	I,III,IV
Body mass index	I,II,III,IV
Smoking	I,II,III,IV
Systolic blood pressure	I,II,IV
Physical activity	I,II,III,IV
Social class	I,II,IV
Oral contraceptive use	I,II,III,IV
Dyslipidemia	II
Psychiatric hospital treatment	II
Triglycerides	III
High-density lipoprotein	III
Education	III
Hypertension	III
Birth weight	IV
Hospital-treated physical illness	IV

Alcohol intake

Alcohol use is known to be associated with depression and anxiety, and also with CRP levels (Roy *et al.* 1991, Thiussen *et al.* 2011, Koenig *et al.* 1999).

The data regarding alcohol consumption were obtained through self-reported questionnaires included in the 31-year follow-up survey of the NFBC 1966 (I, IV). This information was converted into grams of absolute alcohol used per day, and dichotomized further into non-drinkers/slight drinkers (≤ 40g/d) and heavy drinkers (> 40 g/d) (Sillanaukee *et al.* 2003).

In the original publication III, alcohol intake was asked as drinks consumed per week during the last 12 months and grouped further as non-users and light drinkers (maximum of 5 drinks per week), medium drinkers (6–14 drinks per week), and heavy drinkers (more than 15 drinks per week). One drink was defined as one bottle (33cl) of beer, 4cl of spirits, 12cl of wine or 8cl of liquor.

Body Mass Index

Since body mass index (BMI) is known to have an association with depression, anxiety, sleep disturbances and also with CRP, it was taken into account as a

confounding variable in all four original publications (Roberts *et al.* 2000, Koenig *et al.* 1999, Knutson 2005).

Body weight (kg, in underwear) and height (cm, without shoes) were measured at clinical examination of the 31-year follow-up survey NFBC 1966, and if the measurements were not available, reported weight and height were used instead (I, II, IV). Weight and height were measured in 70% of the subjects. The self-reported and measured weight and height were almost identical (Pearson's correlation 0.98). (Herva 2007) BMI was classified into three categories (WHO 1998): normal weight (BMI < 25 kg/m2), overweight (BMI 25–29.9 kg/m2) and obese (BMI ≥ 30 kg/m2)

In the Pieksämäki study, height and weight were measured during the clinical examination and information saved to the nearest 0.5 cm and 0.1 kg, respectively.

Smoking

Smoking has been found to associate with depression, anxiety, and sleep disorders, as well as with CRP levels (Breslau *et al.* 1993, McLeish *et al.* 2009, Kaneita *et al.* 2005, Eklund 2009). In the NFBC 1966 31-year follow-up survey questionnaire the current smoking of the subjects was asked as: "Do you smoke now?" The response categories were: 1) 7 days a week, 2) 5–6 days a week, 3) 2–4 days a week, 4) one day a week, 5) occasionally, and 6) never. Answers regarding smoking were grouped into current regular smoking at least once a week (answer 1–4) and non-smoking (no smoking or smoking less than once a week, answers 5–6) (I, II, IV). In the Pieksämäki study, information about smoking was based on questionnaires and classified into two groups as no/light (persons who smoked less often than every day) versus current (persons who smoked every day) smokers (III).

Systolic blood pressure and hypertension

Systolic blood pressure and hypertension have been revealed to associate with depression, anxiety and sleep disturbances, as well as CRP (Panagiotakos *et al.* 2004, Pitsavos *et al.* 2005, Kato *et al.* 2000, Williams *et al.* 2004). Systolic blood pressure (SBP) was taken into account as a confounding variable in original publications I, II and IV. The data of SBP (mmHg) were collected during the clinical examination of the 31-year follow-up survey of the NFBC 1966. SBP was

measured twice with a mercury sphygmomanometer in a sitting position from the right forearm after at least 15 minutes of rest, and the average value of SBP was used (Järvelin *et al.* 2004).

Hypertension was taken into account as a confounding variable in original publication III. It was defined as diastolic blood pressure being ≥90 mmHg and/or systolic blood pressure being ≥140 mmHg. In the Pieksämäki study data collection, the blood pressure was measured in mmHg twice at 5-minute intervals after fifteen minutes' rest. The average of those two measurements was used as the blood pressure value.

Physical activity

Physical activity has been shown to associate with depression, anxiety, sleep disorder, and CRP (Paluska & Schwenk 2000, Paparrigopoulos *et al.* 2010, Ladwig *et al.* 2003). The information on physical activity was gathered in the 31-year follow-up of NFBC 1966 (I, II, IV). The subjects were asked: "How often do you do physical exercise in your leisure time?" Exercise was defined as becoming breathless and sweating at least mildly. The response categories were: 1) once a month or less often, 2) 2–3 times a month, 3) once a week, 4) 2–3 times a week, 5) 4–6 times a week, and 6) daily. Categories 4 to 6 were considered to represent regular physical activity, which was taken into account as a confounding variable in original publication II. In original publications I and IV, physical inactivity was taken into account as a confounding variable and defined as corresponding to answers 1 to 3 on the questionnaire.

In original publication III based on the Pieksämäki data, the information regarding physical activity was collected from questionnaires. Physical activity was defined as activity resulting in becoming breathless and sweating at least mildly at least twice a week.

Social class and education

Social class and education are known to associate with various psychiatric disorders, and also with depression, anxiety, and sleep disorders, as well as with CRP (Lehtinen & Joukamaa 1994, Panagiotakos *et al.* 2004, Pitsavos *et al.* 2005, Paparrigopoulos *et al.* 2010, Eklund 2009). Information regarding social class was gathered from the 31-year follow-up questionnaire of the NFBC 1966. Social class was categorized into three classes: 1) higher professional, 2) lower

professional/manual worker/student, 3) unemployed/retired/unknown, and taken into account as a confounding variable in original publications I, II and IV (Tilastokeskus 2001).

In original publication III, the information about education was collected with a questionnaire and categorized into three classes: 1) primary school education, no later vocational training, 2) vocational training/school, 3) college level education/university degree.

Oral contraceptive use and hormone therapy

Due to the fact that female sex hormones may play a role in the mechanisms associated with depression, anxiety and CRP levels (Freeman *et al.* 2006, Joffe & Cohen 1998, Solomon & Herman 2009, Dreon *et al.* 2003), the use of oral contraceptives (yes/no) was also entered as a confounding variable in females (I, II, IV). The information on oral contraceptive use was gathered through questionnaire of the 31-year follow-up of the NFBC 1966.

In the Pieksämäki study, all participants filled in a standard questionnaire including questions about the use of exogenous hormones (hormonal therapy or use of contraceptives) together with a research nurse. The information was categorized as use of exogenous hormones (hormonal therapy or use of contraceptives; yes/no).

Dyslipidemia, triglycerides and high density lipoprotein

High-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglyceride levels were measured from the sera collected during the clinical examination of the 31-year follow-up of the NFBC 1966. The blood sample was collected after an overnight fast. Dyslipidemia was considered if the serum total cholesterol was $\geq$ 5.0 mmol/L, LDL level was $\geq$ 3.0 mmol/L, triglyceride levels was $\geq$ 2.0 mmol/L, or HDL was $\geq$ 1.0 mmol/L (II; Dyslipidemias 2004).

In the Pieksämäki study (III), blood samples were collected after an overnight fast the morning after the interview, and serum triglycerides and HDL cholesterol were measured by enzymatic colorimetric methods (CHOD-PAP (cholesterol oxidase peroxidase-amidopyrine), GPO-PAP (glycerophosphate oxidase-peroxidase-4 aminophenazone), Boehringer Mannheim GmbH, Germany) (Saltevo 2008).

Psychiatric hospital treatment and lifetime hospital treated physical illness

Psychiatric hospital treatment was taken into account as a confounding variable because sleep disturbance is known to occur in severe mental illnesses (II). The information on hospital-treated physical disorders was entered as a confounding variable in order to control the effect of possible poor physical health or physical disease as confounding factors (IV). The information was gathered from the national Finnish hospital discharge register covering the years 1982–1997 (Poikolainen 1983). Both psychiatric hospital treatment and lifetime hospital-treated physical illness were dichotomized.

Birth weight

Birth weight was used in original publication IV (measured in grams immediately after birth; Rantakallio 1969) in order to control the effect of possible poor physical health as a confounding factor, since birth weight is known to be associated with physical health and also with CRP later in life (Tzoulaki *et al.* 2008).

4.3 Statistical analyses

In original publication I, analyses were based on 2,641 males and 2,628 females, whose hs-CRP levels, the information on doctor-diagnosed depression as well as mean HSCL-scores, and complete data on confounding factors were available. Due to the fact that female sex hormones may play a role in the mechanisms associated with both depression (Freeman *et al.* 2001, Joffe & Cohen 1998) and CRP levels (Dreon *et al.* 2003), pregnant females (n = 207) were excluded from the analyses.

Multiple multivariate logistic regression analyses were performed firstly for three different categories of single current depressive episode and recurrent depression, and secondly for previous depressive episode. After adjusting for the potential confounders alcohol intake, body mass index, smoking, systolic blood pressure, physical activity, and social class, odds ratios (OR) with 95% confidence intervals (CI) were calculated for hs-CRP levels of ≥1.0 mg/L to estimate the increased risk of previous depressive episode, current depressive episode, and recurrent depression.

To determine whether the association between depressive symptoms of current single depressive episode or recurrent depression and hs-CRP exhibits a "gradient effect" (Penninx *et al.* 2003) with higher levels of hs-CRP associating more strongly with depressive symptoms, corresponding analyses were also conducted with regard to CRP levels of 1.0mg/L to 3.0 mg/L, and > 3.0 mg/L (Pearson *et al.* 2003). The reference category for the above-mentioned hs-CRP cut-offs was the hs-CRP level of < 1.0 mg/L. Non-depressive subjects in the outcome variable comprised the subjects who had neither self-reported doctor-diagnosed earlier depression nor depressive symptoms in the HSCL-25 depression subscale (mean score < 1.55) (N = 2,180). The differences of the median values (inter-quartile ranges in parenthesis) between depressive and non-depressive cases were tested with the Wilcoxon Rank-Sum test. In multiple comparisons, the observed p-values were adjusted using the Bonferroni method. P-values < 0.05 were considered statistically significant. The statistical analyses were performed with SAS, version 8.02 (SAS Institute, Cary, NC).

In original publication II, the distributions of continuous variables were expressed as means ± standard deviations (SD) and as medians with interquartile range, and with categorical variables as numbers and percentages of proportions. Comparisons of continuous variables across categories of sleep disturbances (no, slight, moderate, considerable or severe) and hs-CRP levels were based on the Kruskal-Wallis test, and pairwise testing was carried out using the Wilcoxon Rank-Sum test.

The main analyses to investigate the impact of sleep disturbance on hs-CRP levels were carried out in two ways. Primarily, a robust regression analysis was conducted to minimize the effect of outliers and influential data points in multivariate analyses, given that the results of robust regression are much less sensitive to outliers than those of the more familiar ordinary least squares regression. In multivariate analyses, a logarithmic transformation of hs-CRP value was used (log [hs CRP +1]) (Huber 1964, Li 1985). Multivariate models were adjusted for body mass index, current smoking, physical inactivity, socioeconomic status, systolic blood pressure, psychiatric hospital treatment, dyslipidemia and oral contraceptive use in women. Secondly, the multivariate log-binomial regression analysis (a generalized linear model with a logarithmic link function and binomial distribution of the residual) was used to examine the association between elevated hs-CRP levels and sleep disturbances after adjusting for the above-mentioned confounders. Logistic regression analysis, which is

commonly used to model binary outcome, was rejected because of its substantial overestimation of the risk ratio in situations of common outcomes (McNutt *et al.* 2003). The subgroup of pregnant women was excluded from this analysis. P-values < 0.05 were considered statistically significant. The statistical analyses were performed with SAS, version 9.1 (SAS Institute, Cary, NC).

In original publication III, the results are shown as means and SD, except for variables with a skewed distribution (hs-CRP, triglycerides, BDI-21), which are given as medians and interquartile ranges, unless stated differently. The normality of the variables was tested using Kolmogorov-Smirnov test. The comparison between age groups for continuous variables was performed using analysis of variance (ANOVA) for normally distributed variables and Kruskal-Wallis test for non-normal variables or the χ^2 test for categorical variables.

In the analyses of original publication III, subjects with hs-CRP level ≥ 10 (n = 11, including one subject with diabetes) were excluded to avoid somatic illnesses known to affect CRP level, such as an acute state of infection (Aziz *et al.* 2003). Subjects with diabetes (n = 4), and those using statin medication or having CHD (n = 6) were also excluded because of the known effects on CRP levels of these diseases or medications (Picardi *et al.* 2006, Koenig *et al.* 1999, Pearson *et al.* 2003, Lesperance *et al.* 2004, Mora & Ridker 2006). Thus, a total of 20 participants were excluded.

High-sensitivity CRP level and BDI-21 score were log transformed in further analysis. Pearson correlation coefficients were used to examine the association between hs-CRP levels and depressive symptoms. Correlation coefficients in different age categories were calculated separately for those who used exogenous hormones and for those who did not. Linear regression models were used to explore the strength of the association between hs-CRP levels and BDI-21 score (the latter being the dependent variable) in peri- and postmenopausal women ($n = 304$). Two of the subjects were dropped out from the analysis due to missing data in confounding variables. The possible interaction between hs-CRP levels and exogenous hormone therapy (oral contraceptive or postmenopausal hormone therapy) was added to the regression model to investigate whether the hs-CRP levels and depressive symptoms (measured by BDI-21) have a different relationship when taking into account the exogenous hormone use or without this effect. The confounding variables including body mass index, current smoking, physical inactivity, socioeconomic status, systolic blood pressure, psychiatric hospital treatment, dyslipidemia and oral contraceptive use were controlled in linear regression model. Statistical significance was considered to be $P < 0.05$.

The statistical analyses were performed using the SAS system version 9.1 for Windows (SAS Institute, Cary, NC).

In original publication IV, the analyses were based on 2,688 males and 2,837 females whose hs-CRP levels, information on mean HSCL-scores and complete data on confounding factors were available. Participants having hs-CRP > 10 mg/L (n = 229) were excluded in order to avoid possible effects of acute somatic illnesses. Pregnant females (n = 207) were also excluded from the analyses. Multiple multivariate logistic regression analyses were performed for categories of hs-CRP levels of 1.0mg/L to 3.0 mg/L, and of > 3.0 mg/L. After controlling for potential confounders, which included alcohol intake, body mass index (BMI), smoking, systolic blood pressure, and physical inactivity, as well as social class, odds ratios (OR) with 95% confidence intervals (CI) were calculated for anxiety symptoms, depressive symptoms, as well as comorbid anxiety and depressive symptoms to estimate the increased risk of elevated hs-CRP levels of 1.0mg/L to 3.0 mg/L and > 3.0 mg/L The statistical analyses were performed with SAS software package, version 9.2 (SAS Institute, Cary, NC).

4.4 Ethical considerations and investigator's personal involvement in the study

Permission for gathering data for the entire Northern Finland Birth Cohort was obtained from the Ministry of Social Welfare and Health Affairs in 1993. The research plan for the 31-year follow-up study design of the Cohort named the Northern Finland Health and Well-being Study (Sorri & Järvelin 1998) was reviewed by the Ethics Committee of the Faculty of Medicine, University of Oulu on June 17, 1996. This permission covers the present study as well. During the 31-year follow-up, the cohort subjects have been given a complete description of the study and they have had the chance to refuse further participation in the study. A written informed consent was obtained from all participants.

This study was approved by the Postgraduate Research Committee of the Faculty of Medicine, University of Oulu, on 31 March 2009 as part of the Northern Finland Health and Well-being Study. The author of this thesis has participated in the Northern Finland Birth Cohort as a researcher since 2006.

The Pieksämäki study protocol was approved by the Ethics Committee of Kuopio University Hospital and the University of Kuopio in 1996. All participants gave a written informed consent.

The author has participated in study design, data analysis, and reporting the results in all original studies I–IV. The contribution of the author in all original studies has been central; the author has had original ideas for the studies and has written the first and last versions of original publications I–IV.

5 Results

5.1 Depression and low-grade inflammation (I)

The adjusted ORs for a current single depressive episode defined by HSCL-25 cut-off points of ≥1.55, ≥1.75, and ≥2.01 in male cohort members with elevated hs-CRP levels (≥1.0 mg/L) were 1.2 (95% CI, 0.9–1.5), 1.4 (95% CI, 1.03–1.9), and 1.7 (95% CI, 1.1–2.9), respectively (I; Table 3).

The hs-CRP levels of ≥1.0 mg/L increased the probability for recurrent depression in the highest HSCL-25 subgroup (mean score ≥2.01) 3-fold (adjusted OR 3.1, 95% CI 1.1–8.8) I; Table 4). Regarding the gradient effect, there was a tendency towards statistical significance concerning only recurrent depression in males (N = 20) as defined by the highest HSCL-25 subgroup (p-value for linear trend = 0.085, when CRP levels were categorized as follows: < 1 mg/L, 1.0–3.0 mg/L, and > 3.0 mg/L). In comparison with hs-CRP level of < 1 mg/L, the OR for the hs-CRP level of 1.0–3.0 mg/L was 2.7, (adjusted OR = 2.7, 95% CI = 0.9–8.4). A hs-CRP level of > 3.0 mg/L increased the probability of recurrent depression 4.1-fold (adjusted OR, 4.1, 95% CI, 1.1–14.6).

There were no statistically significant associations between hs-CRP levels of ≥ 1.0 mg/L (adjusted OR = 1.4; 95% CI, 0.7–2.9) and previous depression of cohort males. In females, no statistically significant association was found between any of the measurements of depression and hs-CRP levels.

Table 3. Associations between current single depressive episode and elevated hs-CRP level as assessed with three logistic regression models according to different cut-off points for HCSL-25 depression subscale mean scores after adjusting for confounding variablesa in the Northern Finland 1966 Birth Cohort. (I).

Category for hs-CRPc	Total N	HSCL-25 depression subscale mean score			Analyses		
		1.55–1.74	1.75–2.0	2.01–4.0	HSCL mean score ≥ 1.55 (vs. no depression[b])	HSCL mean score ≥ 1.75 (vs. no depression[b])	HSCL mean score ≥ 2.01 (vs. no depression[b])
		N (%)	N (%)	N (%)	Adjusted OR[a] (95% CI)	Adjusted OR[a] (95% CI)	Adjusted OR[a] (95% CI)
Males[d]							
< 1 mg/L	1,615	93 (5.8)	85 (5.3)	38 (2.4)	Reference	Reference	Reference
≥ 1.0 mg/L	944	57 (6.0)	69 (7.3)	37 (3.9)	1.2 (0.9–1.5)	1.4 (1.03–1.9)	1.7 (1.1–2.9)
Females[e]							
< 1 mg/L	1,381	124 (9.0)	117 (8.5)	80 (5.8)	Reference	Reference	Reference
≥ 1.0 mg/L	1,119	94 (8.4)	106 (9.5)	62 (5.5)	1.0 (0.8–1.2)	0.9 (0.7–1.2)	0.8 (0.6–1.2)

Abbreviations: hs-CRP, high sensitivity C-reactive protein; HSCL, Hopkins Symptom Checklist.

[a] Odds ratios from logistic regression; controlled for socioeconomic situation, alcohol intake, body mass index, smoking, systolic blood pressure, and physical inactivity. Further, in females the use of oral contraceptives was also controlled.

[b] Control subjects were those whose HSCL-25 depression subscale mean score was <1.55, and who had not reported a doctor-diagnosed depression.

[c] Category for hs-CRP level to asses the risk for cardiovascular disease.

[d] Male cohort members with current single depressive episode (n = 379) and non-depressive males control subjects (n = 2,180).

e. Female cohort members with current single depressive episode (n = 583) and non-depressive females (n = 1,917).

Table 4. Associations between recurrent depression and elevated hs-CRP level as assessed with three logistic regression models according to different cut-off points for HCSL-25 depression subscale mean scores after adjusting for confounding variables[a] in the Northern Finland 1966 Birth Cohort. (I).

Category for hs-CRP[c]	Total N	HSCL-25 depression subscale mean score			Analyses		
		1.55–1.74	1.75–2.0	2.01–4.0	HSCL mean score ≥ 1.55 (vs. no depression[b])	HSCL mean score ≥ 1.75 (vs. no depression[b])	HSCL mean score ≥ 2.01 (vs. no depression[b])
		Proportions%	Proportions %	Proportions %	Adjusted OR† (95% CI)	Adjusted OR† (95% CI)	Adjusted OR† (95% CI)
Males[d]							
< 1 mg/L	1,420	5 (0.35)	10 (0.70)	6 (0.42)	Reference	Reference	Reference
≥ 1.0 mg/L	805	4 (0.50)	6 (0.75)	14 (1.74)	1.6 (0.8–3.0)	1.9 (0.9–3.9)	3.1 (1.1–8.8)
Females[e]							
< 1 mg/L	1,102	9 (0.82)	13 (1.18)	20 (1.81)	Reference	Reference	Reference
≥ 1.0 mg/L	889	6 (0.67)	11 (1.24)	15 (1.69)	1.2 (0.7–2.0)	1.2 (0.6–2.1)	0.9 (0.4–2.0)

Abbreviations: hs-CRP, high sensitivity C-reactive protein; HSCL, Hopkins Symptom Checklist.

[a] Odds ratios from logistic regression; controlled for socioeconomic situation, alcohol intake, body mass index, smoking, systolic blood pressure, and physical inactivity. Further, in females the use of oral contraceptives was also used as confounding variable.

[b] Control subjects were those whose HSCL-25 depression subscale mean score was < 1.55, and who had not self-reported doctor-diagnosed lifetime depression.

[c] Category for hs-CRP level to asses the risk for cardiovascular disease.

[d] Male cohort members with recurrent depression (n = 45) and non-depressive males (n = 2,180).

e Female cohort members with recurrent depression (n = 74) and non-depressive females (n = 1,917).

5.2 Low-grade inflammation and sleep disturbance (II)

Compared with men with no sleep disturbances, those with moderate, considerable or severe sleep disturbances had an increased chance of having elevated hs-CRP values (≥ 1.0 mg/L), as high as 1.6-fold (RR 1.60, 95% CI 1.22–2.11). Slight sleep disturbances in men and any level of sleep disturbance in women did not result in significant increases of elevated hs-CRP levels. After adjusting for potential confounding variables the corresponding risk ratio for moderate, considerable and severe sleep disturbances remained significant in men (adjusted RR 1.30, 95% CI 1.05–1.61).

5.3 Effect of menopause and use of contraceptives/ hormone therapy on association of low-grade inflammation and depression (III)

A statistically significant positive correlation was found between hs-CRP levels and depressive symptoms in perimenopausal women not using exogenous hormones (Pearson correlation coefficient, $r = 0.248$, $p < 0.001$), and a trend towards significance was also noted in the subgroup of postmenopausal women not using exogenous hormones (Pearson correlation coefficient, $r = 0.343$, $p = 0.059$). On the contrary, no significant correlations were found in peri- and postmenopausal women using exogenous hormones, or in premenopausal women irrespective of their exogenous hormone use (Table 5).

Table 5. Pearson correlation coefficients between log transformed hs-CRP and Beck's Depression Inventory (BDI-21) levels in pre-, peri- and postmenopausal women. (III).

Exogenous hormones (hormonal therapy or use of contraceptives	Premenopausal (34–36/39–41 years old)			Perimenopausal (44–46/49–51 years old)			Postmenopausal (54–56 years old)		
	N	r	p	N	r	p	N	r	p
Yes	23	−0.111	0.613	51	−0.042	0.770	33	−0.018	0.922
No	165	0.069	0.381	189	0.248	< 0.001	31	0.343	0.059

5.4 Anxiety and low-grade inflammation (IV)

The adjusted ORs for elevated (1.0–3.0mg/L) and highly elevated (> 3.0 mg/L) hs-CRP levels in male cohort members with anxiety symptoms were 1.35 (95% CI, 0.73–2.51) and 2.19 (95% CI, 1.08–4.46), respectively (Table 6). With

comorbid anxiety and depressive symptoms adjusted ORs for elevated (1.0–3.0mg/L) and highly elevated (> 3.0 mg/L) hs-CRP levels in male cohort members were 1.76 (95% CI, 1.13–2.74) and 1.70 (95% CI, 0.94–3.09), respectively. Regarding pure depressive symptoms, no significant association was apparent between depressive symptoms and hs-CRP levels. (Table 6)

In women, no significant associations were found between anxiety, depressive, or co-morbid anxiety and depressive symptoms with elevated hs-CRP levels of 1.0–3.0 mg/L or > 3.0 mg/L (Table 6).

Table 6. Associations between depression, anxiety, comorbid anxiety and depressive symptoms and elevated hs-CRP levels with regard to HSCL-25 mean score cut-off point of 1.75 (IV).

HSCL-25 depression and anxiety subscale mean score ≥ 1.75	Total N	Category for hs-CRP		Analyses	
		≥ 1.0–3.0 mg/L	> 3.0 mg/L	≥ 1.0–3.0 mg/L	> 3.0 mg/L
		%	%	OR (95% CI)[b]	OR (95% CI)[b]
Males	2,688				
No anxiety or depressive symptoms	2,345	25.5	9.5	Reference	Reference
Depressive symptoms[a]	162	30.9	11.1	1.12 (0.83–1.82)	1.27 (0.75–2.12)
Anxiety symptoms[a]	64	29.7	17.2	1.35 (0.73–2.51)	2.19 (1.08–4.46)
Comorbid anxiety and depressive symptoms[a]	117	37.6	13.7	1.76 (1.13–2.74)	1.70 (0.94–3.09)
Females	2,837				
No anxiety or depressive symptoms	2,303	27.4	16.5	Reference	Reference
Depressive symptoms[a]	274	29.6	17.5	1.12 (0.81–1.53)	0.95 (0.66–1.37)
Anxiety symptoms[a]	75	16.0	20.0	0.54 (0.27–1.07)	1.08 (0.57–2.05)
Comorbid anxiety and depressive symptoms[a]	185	27.0	14.1	0.92 (0.62–1.35)	0.64 (0.62–1.35)

Abbreviations: hs-CRP, high sensitivity C-reactive protein; HSCL, Hopkins Symptom Checklist.

[a] Odds ratios from logistic regression; controlled for socioeconomic situation, alcohol intake, body mass index, smoking, physical inactivity, and hospital-treated physical disease (hospital discharge register years 1982–1997) as categorized variables, and for systolic blood pressure and birth weight as continuous variables. Furthermore, in females the use of oral contraceptives was also controlled as a confounding variable.

6 Discussion

6.1 Overview of the results

The results of this study revealed that at epidemiological level, elevated hs-CRP levels of ≥ 1.0 mg/L increased the probability of current depressive symptoms of single depressive episode in the two highest subgroups (*i.e.*, HSCL-25 mean scores ≥ 1.75 and ≥ 2.01) 1.4- and 1.7- fold in males, respectively. Furthermore, elevated hs-CRP levels of ≥ 1.0 mg/L increased the probability of current depressive symptoms of recurrent depression over 3-fold in the highest depression subgroup (*i.e.*, HSCL-25 mean score ≥ 2.01) in males.

Second, when investigating whether sleep disturbances associate with increased levels of hs-CRP as a marker of low-grade inflammation, the risk ratio of 1.3 was found for males with moderate to severe sleep disturbances and elevated hs-CRP levels (≥ 1.0 mg/L).

Third, while investigating an effect of menopausal status and exogenous hormone use on the association between elevated CRP levels and depressive symptoms, a positive correlation between these was found only among peri- and postmenopausal women not using exogenous hormones. On the contrary, no correlation was found among peri- and postmenopausal women using exogenous hormones, or in premenopausal women irrespective of their exogenous hormone use.

Finally, an association between anxiety symptoms and elevated hs-CRP levels was shown in young males at epidemiological level. After adjusting for potential confounding variables, anxiety symptoms (HSCL-25 anxiety scale mean score ≥ 1.75) increased independently the probability of elevated hs-CRP levels (> 3.0 mg/L) in males over 2-fold. It is also worth noting that in the present study there was an increased probability (OR 1.76) in male subjects to have elevated levels of hs-CRP (1.0–3.0 mg/L) together with comorbid anxiety and depressive symptoms.

6.2 Discussion of the results

6.2.1 The association between low-grade inflammation and depression

Studies published prior to this study were largely based on data consisting of elderly participants (Kop *et al.* 2002, Tiemeier at al. 2003, Penninx *et al.* 2003, Panagiotakos 2004). Earlier studies regarding young adults showed inconsistent results, revealing in some studies only an association among obese men (Ladwig *et al.* 2003), or an association attenuating to nonsignificant after adjusting triglycerides and obesity (Elovainio *et al.* 2006). It is noteworthy that obesity did not explain the association in this study, and supported the hypothesis of the association under discussion in young adult men as well. In accordance with the studies of Danner *et al.* (2003) as well as Ford and Erlinger (2004), the present study showed a corresponding association among young men. Contrary to finding of a positive association between low-grade inflammation and a previous depressive episode, which was reported by Danner *et al.* (2003), a similar association between low-grade inflammation and previous depressive episode was not found in this study. In the study by Danner *et al.* (2003) previous depressive episode was defined by a structured interview, the Diagnostic Interview Schedule, compared to a questionnaire used in this study asking whether the participant had earlier doctor-diagnosed depression, which may have resulted in more false negative cases in this study.

Recently, several studies have revealed a positive association between low-grade inflammation and depression (Howren *et al.* 2010, Dressler *et al.* 2006, Pikhart *et al.* 2008, Elovainio *et al.* 2009, Hamer *et al.* 2009a), while some studies have not found the association under discussion (Almeida *et al.* 2007, Bremmer *et al.* 2008, Pan *et al.* 2008, Steward *et al.* 2009). It should be noted that the direction of association cannot be investigated in either this study or in the above-mentioned studies with a cross-sectional design. Recently, some studies with longitudinal designs have reported elevated CRP levels prior to an episode of depression (Hamer *et al.* 2009b, Luukinen *et al.* 2010, Matthews *et al.* 2010, Pasco *et al.* 2010). However, one study has shown the opposite temporal direction, namely depression preceding elevated CRP levels (Deverts *et al.* 2010).

6.2.2 The association between sleep disturbances and low-grade inflammation

Most of the earlier evidence supporting the association between sleep disturbances and low-grade inflammation comes from experimental and small population-based studies suggesting an association between sleep disturbances and elevated levels of CRP (Meier-Ewert *et al.* 2004, McDade *et al.* 2006, Okun *et al.* 2009, van Leeuwen *et al.* 2009). In the present study, moderate to severe sleep disturbances were found to associate with elevated CRP levels in men, which is in line with the study of McDade *et al.* (2006). In that study sleep latency was found to associate with elevated levels of CRP in a sample of 188 subjects, including men and women. Contrary to the findings of Okun *et al.* (2009), the present study did not reveal any association between self-reported sleep disturbances and hs-CRP levels in young adult women. Regarding the gender differences, the findings of this study are opposite to the study of Miller *et al.* (2009) based on the Whitehall II cohort of 4,677 participants. In that study, sleep duration was found to associate with elevated CRP levels in women, but not in men. One possible explanation for this different result could come from the fact that sleep duration as a measure of sleep disturbances measures only the absolute length of sleep rather than the experience of subjective sleep disturbance.

6.2.3 Effect of menopause and use of contraceptives/hormone therapy on association of C-reactive protein and depression

The association between CRP and depression was not found in either the NFBC 1966 database (I, IV) when controlling oral contraceptive use among young women, or in the Pieksämäki data among premenopausal women, regardless of whether they used exogenous hormones or not. These findings are in general in line with other studies published, since in the meta-analysis of Howren *et al.* (2010) no significant relationship was found between depression and CRP levels in women. Furthermore, the greater proportion of females in the study sample did not relate significantly to the magnitude of the association (Howren *et al.* 2010). There are only few cross-sectional studies with young adults showing results separately for women. Danner *et al.* (2004), and also Ford and Erlinger (2004) found no association among women using a database of young adults aged 17 to 39 years. In contrast, Panagiotakos *et al.* (2004) showed a positive association using data from the ATTICA-study in women aged 18 to 84 (44 ± 18). On the

other hand, Dressler *et al.* (2006) showed some evidence for a possible inverse association between depression and CRP with a close-to-significant result among women with a mean age of 40.8 (± 11.7). When interpreting these results in the context of examining the effect of female hormones on the association between depression and CRP levels, there are some points to be taken into account. First, Danner *et al.* (2004) as well as Ford and Erlinger (2004) controlled hormonal contraceptive use and hormone replacement therapy as confounding factors whereas Panagiotakos *et al.* (2004) and Dressler *et al.* (2004) did not. Second, part of the subjects in the ATTICA study may have been perimenopausal or even postmenopausal women, since the mean age of menopause in Greek women is given as 48.7 years (Adamopoulos *et al.* 2002), and perimenopausal hormonal changes (*i.e.*, lower level of estrogen) are known to be present for some years before the actual menopause.

Among peri- and postmenopausal women, the finding of this study in the Pieksämäki data is that women not using exogenous hormones had a positive correlation between elevated CRP levels and depressive symptoms. Unfortunately, earlier literature offers only few relevant studies for comparison. Loucks *et al.* (2006) showed a negative association in a cross-sectional study with 425 women aged 70 to 79. At this age the use of hormone replacement therapy can be supposed to be rare. In another study Elovaino *et al.* (2009) revealed no association using the Health 2000 Survey data including female participants with a mean age of 53. The study included participants > 30 years of age, and therefore these female participants can be supposed to have been pre-, peri-, and postmenopausal women.

In summary, the present study supports the hypothesis that female hormone replacement therapy has a modulating effect on the association between elevated CRP levels and depressive symptoms in peri- and postmenopausal women. A novel finding is that women not using exogenous hormones at peri- and postmenopause had a positive correlation between elevated CRP levels and depressive symptoms, whereas the association was absent when exogenous hormones were used. In addition, this association could not be found in premenopause regardless of the use of exogenous hormones.

6.2.4 The association between anxiety and C-reactive protein levels

The major finding in this study was that at epidemiological level, anxiety symptoms associate with elevated hs-CRP levels in young males. Anxiety symptoms (HSCL-25 anxiety scale mean score ≥ 1.75) increased independently the probability for elevated hs-CRP levels of > 3.0 mg/L in males over 2-fold after adjusting for potential confounding variables. This finding is in line with results from the ATTICA study by Pitsavos *et al.* (2006) reporting a positive correlation between anxiety and hs-CRP levels in males.

Among females, contrary to the results by Pitsavos *et al.* (2006), this study revealed no association between anxiety symptoms with or without depression and elevated hs-CRP levels. One possible explanation for this could be the age of the women studied. In earlier studies no association between elevated hs-CRP and depression has been found in young, *i.e.*, premenopausal women (Danner *et al.* 2004, Ford & Erlinger 2004). In the other part of this study the use of exogenous female hormones showed an effect on the association between depression and CRP in peri- and postmenopausal women, but not in premenopausal women. Thus, it is reasonable to assume that hormonal environment related to age might have an effect on the association between anxiety symptoms and CRP levels. It is noteworthy that participants in the present study were 31 years of age, but in the ATTICA study most of the female participants were perimenopausal (46 ± 11 years) (Pitsavos *et al.* 2006), since the mean age of menopause is given as 48.7 years of age in Greek women (Adamopoulos *et al.* 2002). Perimenopausal hormonal changes (*i.e.*, lower level of estrogen) are known to be present for some years before the actual menopause.

In the present study there was an increased probability in male subjects to have elevated levels of hs-CRP (1.0–3.0 mg/L) together with comorbid anxiety and depressive symptoms. However, contrary to expectation, pure depressive symptoms were not associated independently with elevated hs-CRP levels (IV). Interpreting these findings is difficult, since other similar studies are not available. According to the findings it seems evident that anxiety symptoms have a significant role in the association between psychiatric symptoms and inflammation. Whether comorbid anxiety symptoms have had an effect on the association between depression and low-grade inflammation in previous studies remains unclear, since these studies have not taken into account the possible comorbid anxiety symptoms (Howren *et al.* 2010).

6.3 Theoretical background

The findings of this study can be explained according to the following theoretical background, which might serve as a link between low-grade inflammation and psychiatric symptomatology, namely depression, anxiety and sleep disturbances. In the paragraphs below, each theoretical hypothesis will be briefly and critically discussed.

6.3.1 Cytokines

Immune functions are proposed to be involved in the pathophysiology of depression. Depressed patients are found to have elevated levels of proinflammatory cytokines, such as interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) in the blood (Miller *et al.* 2009, Dowlati *et al.* 2010). Elevations of cytokines have been suggested to precede depression, based on findings in clinical and animal studies (Capuron & Miller 2004): acute and chronic administration of cytokines can cause symptoms known as sickness behavior, overlapping with those found in depression, including anhedonia, decreased activity, cognitive dysfunction, and altered sleep (Miller *et al.* 2009a). These findings may indicate that there is a pathway in the development of depression via sickness behavior. In fact, 30–50% of patients treated with interferon alpha (innate immune cytokine) are known to develop clinical depression (Raison *et al.* 2009). Cytokines are supposed to have an impact on the monoamine system, HPA axis (stimulating its function), and also on GR functioning, which have all been found to be dysfunctional in depression. Besides somatic infectious diseases, psychosocial stress can also activate inflammatory response resulting in elevation of cytokines. (Miller *et al.* 2009b) Childhood maltreatment has been proposed to have a major effect on inflammatory functions, with elevated levels of inflammatory markers in adults with a history of childhood maltreatment (Danese *et al.* 2007). Furthermore, individuals with adverse childhood experiences have been found to be at elevated risk for depression and high inflammation level (Danese *et al.* 2009).

Administered inflammatory cytokines, *e.g.* IFN-alpha and endotoxines, causing immune challenge increasing cytokines, are found to cause anxiety symptoms (Reichenberg *et al.* 2001, Miller *et al.* 2009a, Capuron & Miller 2004). Since anxiety is also found to be associated with infectious and autoimmune

diseases, it has also been hypothesized that immune functions are involved in the pathophysiology of anxiety (Garcia-Bueno *et al.* 2008).

6.3.2 HPA axis

Hyperactivity of the HPA axis is one of the most consistent biological findings in major depression (Pariante & Lightman 2008). One part of that mechanism is supposed to be an impaired glucocorticoid-mediated feedback inhibition, *i.e.*, GR resistance (Bao *et al.* 2008). Adverse experiences during childhood, such as abuse, neglect or loss, are found to be accompanied by permanent hyperactivity of the HPA axis, as well as an increased risk of developing depression in adulthood (Bao *et al.* 2008, Heim *et al.* 2008). Adverse childhood experiences are supposed to lead to sensitization of stress response system (namely HPA axis) and to an enhanced stress reaction and development of depression following a stressful life event during adulthood. Cortisol is one of the most potent anti-inflammatory hormones, but in case of depression, the immune system can become glucocorticoid-resistant (Bao *et al.* 2008).

Hippocampal volume reduction is a relatively common finding in patients with depression. Stress-induced HPA axis activation, especially glucocorticoid neurotoxic effect on the hippocampus has been proposed as a possible explanation for hippocampal volume reduction, and also for impaired function of the hippocampus. In addition to hippocampal volume loss, chronic stress causes dendritic retraction and a suppressed rate of adult neurogenesis. Adult neurogenesis refers to the production of new neurons in the adult brain. Hippocampal neurogenesis is regulated by various factors including stress, disturbed sleep, exercise, and inflammation. Lasting reduction in neurogenesis may represent impaired hippocampal plasticity and can contribute to the cognitive symptoms of depression. (Lucassen *et al.* 2010)

Dysfunction of the HPA axis has also been proposed to play a role in the pathophysiology of anxiety disorders (Greaves-Lord *et al.* 2009). Individuals who develop anxiety symptoms are supposed to have a lower threshold for HPA axis activity following stimuli, and therefore be more prone to developing a stress reaction, which is followed by anxious and withdrawing behavior when trying to avoid stressful situations. As a result of the activation of the HPA axis, higher levels of cortisol or higher cortisol awakening response are found in patients with different anxiety disorders (Mantella *et al.* 2008, Vreeburg *et al.* 2010a), but there are conflicting findings (Kloet *et al.* 2008, Greaves-Lord *et al.* 2009). The finding

of elevated cortisol awakening response in individuals without anxiety disorder and with parental history of anxiety possibly reflects a trait marker indicating a biological vulnerability for developing anxiety (Vreeburg 2010b). HPA axis activation is also known to cause activation of the immunity system, which in turn is found to be associated with anxiety as mentioned earlier (Pitsavos *et al.* 2006).

6.3.3 Estrogen

The gonadal hormones in women, especially estrogen, have been thought to at least moderate the pathophysiology of depression, since both postpartum and perimenopause, the times characterized with lowering of estrogen, associate with high incidences of depression. Lowering of estrogen levels is also present at prementruum, when prementrual symptoms, overlapping with depression are seen. (Young & Korszun 2010, Bao *et al.* 2008, Cohen *et al.* 2006, Freeman *et al.* 2006). Estrogen has been found to have an effect on the serotonin system according to the findings of animal studies (Bao *et al.* 2008, Cohen *et al.* 2006, Freeman *et al.* 2006). In addition, estrogen receptor β has been associated with effects on the serotonergic system and emotional processing (Aloysi *et al.* 2007), and the use of an estrogen receptor-β blocker, tamoxifen, is associated with an increased risk for developing depression (Lee *et al.* 2007). Exposure to monthly changes of steroid milieu during reproductive years has been proposed to sensitize women to stress, and in vulnerable individuals, lead to depression (Young & Korszun 2010). On the other hand, estrogen has been proposed to stabilize the HPA axis (DeNicola *et al.* 2006), which could theoretically explain the positive effects on mood in some clinical trials of hormone replace therapy (HRT) use in postmenopausal women (Joffe & Cohen 1998).

There is a clear gender difference in the association between low-grade inflammation and depression among young men and women in this study. The association between low-grade inflammation and depression was found in subgroups of young men and peri- and postmenopausal women not using HRT, *i.e.*, in subgroups supposedly characterized by low level of estrogen. In the context of interaction between estrogen and HPA axis, the results could be understood so that estrogen levels may stabilize the HPA axis (DeNicola *et al.* 2006), resulting in lower inflammatory activation in a stressful situation.

6.3.4 Sleep disorders, cytokines and HPA axis

Stress in known to cause sleep disturbances, possibly through activation of the stress system, *i.e.*, the HPA axis and sympathetic nervous system, causing arousal and sleeplessness (Basta *et al.* 2007, Mullington *et al.* 2010). Activation of the HPA axis may result in elevation of cytokines and eventually CRP levels. Vice versa, sleep has an inhibitory effect on the stress system including the HPA axis and sympathetic nervous system (Basta *et al.* 2007). On the other hand, during infection cytokine production is increased, causing so-called sickness behavior including fatigue and tiredness. Furthermore, IL-6 has been shown to significantly alter sleep architecture (Späth-Schwalbe *et al.* 1998). To conclude, insomnia is probably not a state of sleep loss, but rather a disorder of hyperarousal present during daytime and night. Similarly, fatigue and poor sleep associated with insomnia might be due to cytokine hypersecretion associated with HPA axis hyperactivation (Basta *et al.* 2010).

6.4 Methodological considerations

6.4.1 Strengths of the study

Major strengths of this study are from the databases used, namely the Northern Finland Birth Cohort 1966 and the Pieksämäki study databases. First, NFBC 1966 is a large, unbiased, genetically homogeneous birth cohort. A variety of biological, sosioeconomical and health conditions as well as living habits and family characteristics of the cohort members have been collected prospectively from the prenatal stages up to the age of 31. (Rantakallio 1969, 1988)

The Pieksämäki study was conducted in order to determine the prevalence of risk factors and long-term course of metabolic syndrome. The study population comprised a representative sample of middle-aged Caucasian subjects invited to participate in the study through official registers from a specified geographic area, namely the town of Pieksämäki. The Pieksämäki data allowed studying associations between depression and low-grade inflammation in three different categories of hormonal stages of women's life span, pre-, peri-, and postmenopause, in the same database.

Secondly, a highly sensitive measure of CRP in both databases, NFBC 1966 and the Pieksämäki study, allowed to accurately specify the cut-off points of elevated and highly elevated CRP levels to represent risk assessment cut-off

points used in another inflammation related disease, namely CHD (Pearson *et al.* 2003).

Finally, HSCL-25 has been shown to be a reasonable screening tool identifying psychiatric symptoms with specificity of 73–91% and sensitivity of 69–84% (mean cut-off point of ≥ 1.75) in general population (Nettelbladt *et al.* 1993, Sandanger *et al.* 1998). In addition, HSCL-25 has been found to be an acceptable tool in screening psychiatric cases with DSM-III-R axis I disorders in the same NFBC 1966 database (Veijola *et al.* 2003). Furthermore, BDI-21 (Beck & Beamesderfer 1974) used in the Pieksämäki study has been widely used, and it has also been found to be a valid screening tool in Finnish (Honkalampi *et al.* 2000, Pesonen *et al.* 2007).

6.4.2 Limitations of the study

When interpreting the results of this study there are some limitations to be noted. First, measures of depression and anxiety were based on self-rating questionnaires (HSCL-25 and BDI-21), which do not provide clinical diagnosis as structured interviews do. Second, self-reported doctor-diagnosed depression was used to define previous depression (I). This information was based only on a single question provided retrospectively in a self-administered questionnaire. Therefore this method can be sensitive to recall bias, leading to false negative answers.

Third, it is possible that some other unknown factor, such as somatic illness or medication, could have had an effect on the results of this study. However, this possibility is seen as extremely unlikely due to the relatively young study population, and consequently a low incidence of somatic diseases and use of medication in the NFBC 1966 data. In the Pieksämäki study data, by excluding known diabetes, CHD, use of statins, and participants having hs-CRP ≥ 10 mg/L, the corresponding possibility should be minimal (III). It is also noteworthy that a number of confounders have been taken into account in the analyses.

Fourth, the definition of sleep disturbances was based on a single question in the 15D scale, although the 15D has been proved to be a valid measure of health-related aspects of the quality of life (II, Sintonen 2001).

Fifth, participants were divided into pre-, peri- and postmenopausal groups by age, which is not fully comparable with hormonal status, and, therefore, the study ought to be considered as a preliminary study, and the findings should be repeated with studies assessing menopausal status by female hormone measurements from

the blood (III). The results can, however, be considered important, given that hormonal levels between premenopausal and peri- and postmenopausal women inevitably differ clearly from each other (Luoto *et al.* 1994).

Finally, the measurements of hs-CRP were based on one-time measurements, although short-term fluctuations of CRP levels are known to be infrequent (Macy *et al.* 1997). Nevertheless, another measure or additional markers of inflammation, such as IL-6, could have been useful in further validation of the CRP finding.

7 Conclusions

7.1 Main conclusions of the results

The main novel finding is the shown association between low-grade inflammation and anxiety in young adult men. Furthermore, comorbid anxiety and depression was also revealed to be associated with low-grade inflammation, but not with depression alone, when anxiety was taken into account. These findings add an important consideration to the earlier literature by showing the important role of anxiety in the association between psychiatric disorders and low-grade inflammation.

Low-grade inflammation showed to associate with depression among young adult men. This finding has given an important motivation for further research investigating directionality between low-grade inflammation and depression in longitudinal settings.

In this study, the possible explanation of the effect of hormonal milieu between pre-, and peri- and postmenopausal women on the association of depression and low-grade inflammation was shown. The effect of exogenous hormone use on that putative association was also revealed. These findings clarify at least in part the possible reasons why the association under discussion has not been found in young women in earlier studies (Howren *et al.* 2010).

Sleep disturbances were revealed to associate with low-grade inflammation, which is still a unique finding when using large population-based data. This result from epidemiological level supports the earlier laboratory and clinical studies. This result also points out the possible importance of sleep disturbances in the context of pathology of inflammation-related diseases and depression and anxiety disorders.

7.2 Research implications

Depression has been established to associate with low-grade inflammation, but studies investigating the association between anxiety as well as comorbid anxiety and depression with low-grade inflammation are limited. Therefore, the findings of this study should be repeated in other large databases. If these results are confirmed, future depression investigations should include assessments of anxiety

symptoms to determine further the role of anxiety disorders in the context of the association between depressive disorders and low-grade inflammation.

The results of this study also bring up the biological mechanisms behind psychopathology of anxiety and depression. The results reflect the hyperactivity of the HPA axis as part of the pathophysiology of anxiety and depression. Depression, on the other hand, is a heterogeneous diagnosis, and therefore it would be interesting to focus in the future on whether some clinical subcategory of depression, such as melancholic depression, would associate differently with low-grade inflammation.

Studies on the association between sleep disturbances and low-grade inflammation at epidemiological level are rare, and therefore the results need to be repeated in other large databases. Since sleep disturbances are also symptoms of depression, it is very challenging to take into account in which degree sleep disturbances or comorbid sleep disorders are responsible for low-grade inflammation. It should also be noted that there are many other different kinds of specific sleep disturbances.

Earlier studies and the results of this study show clear gender differences in the association of low-grade inflammation with anxiety, depression, and sleep disturbances at epidemiological level. Female hormonal milieu has been proposed to be responsible for these gender differences, but studies are still limited and large epidemiological studies are called for.

7.3 Clinical implications

Treating anxiety and depression in clinical practice is challenging, although several types of treatments are available. This study and earlier literature have shown low-grade inflammation to be associated with anxiety and depression. If, in future studies, the inflammatory functions are shown as a relevant pathway in depression and anxiety, medication could be developed to target that pathway relieving depression and anxiety. In the future, it is possible that medications acting specifically on that part of pathophysiological mechanisms could be available. Furthermore, it would be helpful to detect depressive or anxious patients whose disorder is related to low-grade inflammation, and be able to use medications specifically according to this inflammatory process.

Hormonal environment has been shown to have an effect on the association between low-grade inflammation and depression. If low-grade inflammation is

shown as a causal factor for depression in the future, it would be reasonable to consider using HRT at peri- and postmenopause to prevent depression in the subpopulation of women with elevated CRP. It would also to be reasonable to consider using HRT at peri- and postmenopause in the subgroup of women with depression to prevent inflammatory process associated with disorders such as CHD, if causality between depression and low-grade inflammation is established.

Sleep disorders were found to be related to low-grade inflammation, and if the hypothesis that sleep disorder promotes inflammation would turn out to be confirmed by future research, it would be beneficial to pay attention to detection and treatment sleep disorders earlier and more effectively in order to prevent further development of inflammation-related disorders like depression.

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I Liukkonen T, Silvennoinen-Kassinen S, Jokelainen J, Räsänen, P, Leinonen M, Meyer-Rochow VB & Timonen M (2006) The association Between C-Reactive Protein Levels and Depression: Results from the Northern Finland 1966 Birth Cohort Study. Biol Psychiatry 60: 825–830.

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III Liukkonen T, Vanhala M, Jokelainen J, Keinänen-Kiukaanniemi S, Koponen H & Timonen M (2010) Effect of Menopause and use of contraceptives/ hormone therapy on association of C-reactive protein and depression: A population-based study. Journal of Psychosomatic Research 68(6): 573–579.

IV Liukkonen T, Räsänen, P, Jokelainen J, Leinonen M, Järvelin M-J, Meyer-Rochow VB & Timonen M (2011) The Association between anxiety and C-reactive protein (CRP) levels: Results from the Northern Finland 1966 Birth Cohort Study. European Psychiatry 26(6): 363–369.

Original publications are not included in the electronic version of the dissertation.

1119. Mikkola, Ilona (2011) Prevalence of metabolic syndrome and changes in body composition, physical fitness and cardiovascular risk factors during military service

1120. Venhola, Mika (2011) Vesicoureteral reflux in children

1121. Naillat, Florence (2011) Roles of *Wnt4*/*5a* in germ cell differentiation and gonad development & *ErbB4* in polarity of kidney epithelium

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1127. Pirilä-Parkkinen, Kirsi (2011) Childhood sleep-disordered breathing – dentofacial and pharyngeal characteristics

1128. Thevenot, Jérôme (2011) Biomechanical assessment of hip fracture : development of finite element models to predict fractures

1129. Moilanen, Kristiina (2011) Diagnostics and determinants of schizophrenia : The Northern Finland 1966 Birth Cohort Study

1130. Haapsamo, Mervi (2011) Low-dose aspirin therapy in IVF and ICSI patients

1131. Hyttinen, Laura (2011) Long-term effects of the cholesterol level and its drug treatment

1132. Hannula, Samuli (2011) Hearing among older adults–an epidemiological study

1133. Paananen, Markus (2011) Multi-site musculoskeletal pain in adolescence: occurrence, determinants, and consequences

1134. Kaustinen, Teija (2011) Oulu-hoitoisuusluokitus ja hoitohenkilökunnan ajankäyttö hoitotyön laatuvaatimusten näkökulmasta

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