Depression and inflammation

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DEPRESSION & INFLAMMATION

A life perspective Hester E. Duivis

Depression and Inflammation

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Depression and Inflammation: a life perspective.

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A life perspective

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I want to live where soul meets body

Death Cab for Cutie, ‘Plans’ (2005)

Contents

CHAPTER 1

General introduction

Introduction

Ever since the beginning of modern medicine, the relationship between psychology and immune function has received considerable interest. In 1865, Claude Bernard, a French physiologist coined the concept *milieu interieur,* describing the relationship between the internal physiological state and functioning in life[1].

"The constancy of the internal environment is the condition for a free and independent life"

Over a century later, in 1981, Robert Ader founded the term Psychoneuroimmunology (PNI), describing the field that investigates the interaction between psychological factors and immune, and neuroendocrine systems and their influence on health and diseases, incorporating a broad range of disciplines such as psychology, medicine, immunology, biology, and neuroscience[2]. PNI still is a widely studied field covering a broad range of psychiatric disorders and physiological functioning in humans[2]. One of the major topics within PNI research is the association between *depression and inflammation.*

In the 1980's Dantzer and colleagues provided evidence for what is called cytokine induced sickness behavior, by injecting pro-inflammatory cytokines in healthy persons[3]. Sickness behavior is thought to be characterized by engaging in less physical activity, fatigue, and anorexia and can be viewed as a motivational state that is necessary to face the infection that causes the inflammation[3, 4]. In the years that followed up until now, the idea that there are similarities between this motivational behavior and (somatic symptoms of) depression, gained considerable interest[5] and research provided evidence for an association between *depression and inflammation* [for an overview[6]]. It has even been proposed that this association could serve as a possible physiological link between highly comorbid somatic diseases and psychiatric complaints such as coronary heart disease (CHD) and depression[7]. However, it is still unknown (1) how depression and inflammation are associated (uni-directional or bi-directional) and (2) what the specific characteristics of this relationship are (e.g. persistent depressive symptoms versus single episodes of depression and somatic versus cognitive symptoms of depression). The aim of this thesis is to provide a better understanding of this relationship.

Depression

Depression is characterized by feelings of depression or low mood and/or loss of interest or pleasure in normal activities[8]. In addition, these feelings of depressed mood are often accompanied by insomnia or hypersomnia, psychomotor retardation or hyperactivity, eating problems, major weight change, feelings of worthlessness, feelings of guilt, loss of energy, or suicidal ideation. When at least five of these symptoms are present during at least two weeks, the majority of the day, and they interfere with everyday life functioning, a major depressive episode (MDD) can be diagnosed[8]. According to the World Health Organization, MDD affects over 121 million people worldwide and will be the number one leading cause of disability in Western countries in 2020[9]. Nemesis-2, A large population based study conducted in the Netherlands showed that in 2009 642.800 people suffered from depression, which is reflected in a one year prevalence rate of about 6%[10]. Lifetime prevalence rates suggest that almost 19% of Dutch society will suffer from depression at one time point in their life. Moreover, depression is often a recurrent disorder; approximately 30% of depressed patients experiences a new episode and the risk of a new future episode increases by 16% with every recurrent episode[11].

Depression and cardiovascular health

In initially healthy people, depression has been associated with subsequent cardiovascular risk factors and even cardiovascular diseases[12, 13]. Also patients with already established CHD, and especially patients with myocardial infarction (MI), often experience depressive symptoms or a depression. Around 20% of all patients with CHD suffer from depression during the months following MI[14-16]. Another 20% report depressive symptoms that do not fulfil diagnostic criteria[17]. These rates are around three times higher than those in the general population[18]. Furthermore, suffering from MDD after a MI is associated with poor cardiac prognosis. Van Melle and colleagues[19] and Meijer and colleagues[20] have described the prognostic association between depression and coronary heart disease (CHD) in two meta-analyses, reporting that depressed patients with CHD were at higher risk of all-cause and cardiac mortality (OR: 1.6-2.7). Many explanations have been proposed to explain this adverse association between depression and CHD, among which the hypothalamic-pituitary-adrenal (HPA) axis, autonomic nervous system functioning, adverse health behaviors (smoking, physical inactivity, alcohol consumption, and body mass index (BMI)), and inflammation[7]. In this thesis the focus will be on the

associations between depression and inflammation in both healthy and CHD samples.

Inflammation

One of the physiological links that have been proposed to explain the adverse effects of depression on cardiovascular health is inflammation. Inflammation is a collective term describing the body's response to infection or injury (also see Box 1). It destroys or inactivates invaders and initializes tissue repair in the affected area. Inflammation plays an important role in the development and progression of atherosclerosis, a risk factor for coronary heart disease, by being involved in the development of plaques and destabilizing the plaque[21]. If inflammation and depression are associated, it could be hypothesized that through inflammation people can become depressed and that this depression is a reflection of a subclinical cardiovascular risk factors which could eventually lead to CHD. This might explain why patients with CHD are at greater risk for new cardiac events or mortality. However, it could also be that depression causes inflammation and that through this mechanism depression affects the development and progression of atherosclerosis. This could explain why initially CHD free people and patients with CHD suffering from depression have an increased risk for (new) cardiovascular events.

Depression and inflammation

Various hypotheses have been proposed to give an explanation for the association between depression and inflammation, i.e. the macrophage theory[22,23], the cytokine hypothesis of depression[24, 25], the immune response system (IRS) model of depression[5], and cytokine-induced sickness behaviour[26]. All these hypotheses share one common idea: pro-inflammatory cytokines are able to induce depression or depressive symptoms. In support of this hypothesis, research has shown that medical illnesses which are characterized by chronic inflammation are often accompanied by depression[24]. In addition, administration of pro-inflammatory cytokines in relation to cancer or multiple sclerosis treatment can cause depressive symptoms[27]. It also seems that inflammation is associated with neurotransmitter changes in several ways, e.g. a decrease in availability of tryptophan to the brain[27,28], which can result in serotonin shortage and thus in depression. Finally,

there is evidence suggesting that inflammation can cause HPA-axis hyperactivity[27,28], which is known to be associated with depression, and causes hypotrophy in the hypothalamus, hippocampus and pre-frontal cortex[29]. Epidemiological research has shown inconclusive results regarding the depression-inflammation relationship, with results ranging from a negative association[30] to positive associations. A meta-analysis conducted by Howren and colleagues however, showed that depression and inflammation were cross-sectionally associated in both healthy and CHD samples, with stronger effect sizes in patients with CHD[6].

Box 1. White blood cells, Cytokines, Acute Phase Reactants, and Viruses

White blood cells (also known as leukocytes) are cells of the immune system, involved in defending the body against pathogens and injury. White blood cells consist of different cell types: neutrophils, basophils, eosinophils, monocytes, and lymphocytes. The individual cells all have different functions in the immune system and are involved in the formation and progression of atherosclerosis.

Cytokines are released by activated immune cells and are often referred to as messenger molecules that arrange cross-talk between different immune cells in order to precisely time the functions of the immune system. Cytokines that are determined and used in this thesis are **interleukin 6** (IL-6) and **tumor necrosis factor-α** (TNF-α). IL-6 and TNF-α stimulate a systemic response to injury and infection and are both involved in the production of C-reactive protein (CRP).

Fibrinogen and **C-reactive protein** (CRP) are acute phase reactants, both produced in the liver under the influence of IL-6 and TNF-α. Fibrinogen and CRP levels are found to be higher in patients with CHD, with CRP levels being upregulated in the presence and progression of atherosclerosis and damage to the myocardium. Fibrinogen is involved in vessel damage repair.

In this thesis data on presence of antibodies to **herpes simplex virus 1** (HSV-1), **epstein barr virus** (EBV), and **cytomegalovirus** (CMV) were used. These viruses have been found in brain areas involved in depression and emotion processing, such as the hippocampus, the amygdala, and the orbitofrontal cortex. Furthermore, in response to a viral infection monocytes and macrophages secrete TNF-α and IL-6.

In contrast to the previous findings, there is some evidence suggesting that it is not inflammation that precedes depression but vice versa: depression precedes inflammation. Research in patients with CHD[31], healthy older adults[32], and adolescents[33] has shown that depression is associated with higher subsequent levels of inflammation, and not vice versa. Interestingly, recent studies have shown that especially recurrent episodes of depression are associated with subsequent inflammation[34,35], suggesting that not those who suffer from a single episode of

depression, but people who frequently experience depressive feelings are at risk for the adverse effects of depression on cardiac health.

Finally, some research revealed an association between the presence of viruses and depression[36-38] and this could be another possible way to look at depression and inflammation (also see Box 1). Presence of viruses are found in the limbic system, such as the hippocampus, amygdala, and orbitofrontal cortex[39,40], areas which are involved in depression and emotion processing[41]. Moreover, it has been shown that people with herpes simplex virus encephalitis present with volume reduction in the amygdala and hippocampus, as well as hypoperfusion in the pre-frontal cortex[42]. Furthermore, viral infections are also associated with an upregulation of systemic inflammation and endothelial adhesion molecules[38]. It could thus be argued that viruses promote depression by affecting important brain regions, with inflammation as a possible mediator or outcome of this depression.

Somatic and cognitive symptoms of depression and inflammation

As was explained in a previous paragraph, depression consists of a range of symptoms. These symptoms can be divided into two dimensions: somatic symptoms (sleeping problems, eating problems, weight change, and psychomotor problems) and cognitive symptoms (difficulty concentrating, loss of interest, depressed mood, suicidal thoughts, and feelings of guilt and worthlessness). Based on theories such as the sickness-behavior theory[26,43], it could be hypothesized that these symptom dimensions are differentially associated with inflammation, with mainly *somatic* symptoms of depression being associated with inflammation and not cognitive symptoms of depression. The sickness-behavior theory states that in the presence of upregulated levels of inflammation, feelings of fatigue, reduced appetite, malaise, and decreased motor activity are often experienced[26,43]. These symptoms show similarities with somatic symptoms of depression[43]. More evidence for this theory comes from a study on the treatment with immunotherapy in patients with cancer. They found that patients who underwent IFN-α therapy developed neurovegetative symptoms of depression i.e. fatigue, abnormal sleep, abnormal appetite and psychomotor retardation (somatic symptoms). In a subgroup of patients, immunotherapy induced emotional/affective symptoms or cognitive symptoms of depression[44,45]. Interestingly, when these patients were treated with an anti-depressant (Paroxetine) they were relieved from the cognitive symptoms, pain and disturbed mood, while the somatic symptoms were not effectively treated[44]. There is some epidemiological research underlining these

findings[46]. Elovainio and colleagues found in a large representative cohort that somatic and affective symptoms of the BDI were both associated with CRP in men and women. In men, the association between somatic symptoms of depression and CRP was robust for adjustment for demographics, health behaviors and cardiac risk factors but, in women the association diminished. This is further supported by Stewart and colleagues who reported that somatic symptoms were predictive of IL-6, whereas cognitive symptoms were not[32]. In contrast, there is also evidence that cognitive symptoms of depression are associated with inflammation[31,47,48]. Kupper and colleagues found in a sample of patients with heart failure that next to somatic symptoms of depression, cognitive symptoms were also associated with inflammation, both cross-sectionally and prospective[47]. Furthermore, Gimeno and colleagues reported that inflammation was associated with subsequent reports of cognitive symptoms of depression in healthy participants[48]. Thus far, results are conflicting which could be the result of the sample characteristics, such as age and disease status. However, examining the possible differential associations of somatic and cognitive symptoms of depression with inflammation may provide new insights in the depression-inflammation mechanism, possibly resulting in new treatment strategies.

Health behaviors as an explanation for the association between depression and inflammation

When the association between depression and inflammation is studied, the effects of (adverse) health behaviors on both depression and inflammation are complicating the interpretation of the result[49]. Health behaviors such as physical activity[50,51], smoking[52], alcohol use[53-55], and body mass index (BMI)[56] are found to be associated with both depression and inflammation. Relationships between depression and health behaviors are often found to be bi-directional. For instance, a meta-analysis on the prospective association between obesity and depression revealed that obesity served as a risk factor depression, but also that depression predicted obesity[56]. Similar findings have been reported for physical activity and depression[50,51], with physical activity being inversely associated with depressive symptoms, but also vice versa. Furthermore, health behaviors are found to be related to higher levels of inflammation. For instance, in overweight persons levels of CRP, IL-6, and TNF-α were higher than in normal weight controls, probably through the inflammatory properties of adipocytes [for an overview:[49]]. To further support this, research showed that by intervening in dietary intake and promoting

moderate physical exercise in somatic healthy people, levels of inflammation lowered[57]. It could therefore be expected that health behaviors explain (a substantial part of) the depression-inflammation link and should be considered as a possible mediator of this relationship. This is supported by findings from various studies on the depression-inflammation relationship in healthy participants[32,35,58]. For instance, Hamer and colleagues found that physical inactivity, weight gain, alcohol consumption, and smoking were important mediators in the association between depressive symptoms and subsequent inflammation, with physical inactivity explaining the largest proportion[35]. Furthermore, Miller and colleagues[58] and Stewart and colleagues[32] reported important effects of BMI on the depression-inflammation link. Taken together, there is sufficient evidence that suggests a mediating role of health behaviors and when these variables are not properly assessed in depression-inflammation research, results might not represent true effects. Furthermore, if these (adverse) health behaviors indeed have a mediating role in the depression-inflammation relationship, targeting on promoting healthy behavior when treating people with depression or depressive symptoms, could possibly lead to more favorable health outcomes.

General aim of this thesis

The general aim of this thesis is to thoroughly investigate the association of depressive symptoms and depression with inflammation across the lifespan, especially focusing on the nature of the depression (clinical vs. subclinical, type of symptoms, and recurrence) and the directionality of the relation between depression and inflammation. Furthermore, in this thesis the influence of adverse health behaviors on the depression-inflammation relationship will be examined. Finally, the association between viral infections and depression will be evaluated.

Studies used in this thesis

For this thesis data from the following three prospective cohort studies were used:

The Heart and Soul study

Data from patients with stable CHD come from the Heart and Soul study. The Heart and Soul study is a study that started in 2000 and enrolled 1024 patients with stable coronary heart disease in the San Francisco Bay area. The main goal was to

determine why depression is associated with an increased risk of cardiovascular events in stable CHD outpatients. Administrative databases were used to identify potential participants with documented coronary artery disease at the Department of Veteran Affairs Medical Centers in San Francisco and Palo Alto (N = 438), the university medical center (university of California, San Francisco) (N = 346), and 9 public health clinics in the Community Health Network of San Francisco (N = 240). Patients were eligible to participate if they had at least one of the following: history of myocardial infarction, angiographic evidence of at least 50% stenosis in one or more coronary vessels, prior evidence of exercise-induced ischemia by treadmill or nuclear testing, a history of coronary revascularization, or a diagnosis of coronary artery disease.

The Netherlands Study of Depression and Anxiety (NESDA)

The NESDA study is a prospective ongoing cohort study which investigates 1) the long-term course of depression and anxiety, 2) the clinical, psychosocial, biological and genetic determinants of depression and anxiety on the long-term course, and 3) the expectations, evaluation, and provision of (mental) health care by patients and their association with the long-term course and consequences of depressive and anxiety disorders[59]. Participants were recruited from two population studies (ARIADNE N = 261; and NEMESIS N = 303), 65 general practices (N = 1610) and from mental health care institutes (807) in the area of Amsterdam, Leiden, and Groningen.

Tracking Adolescents' Individual Lives Survey (TRAILS)

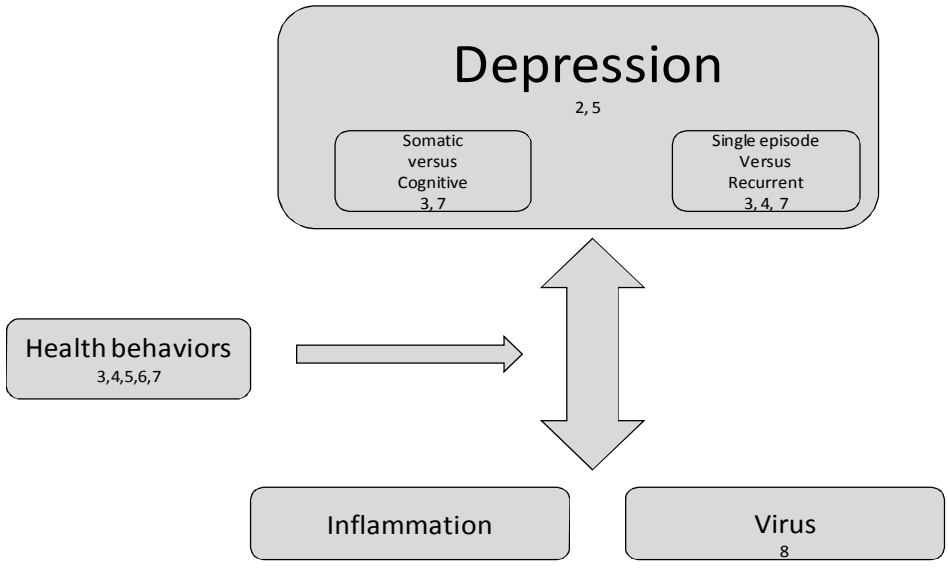
TRAILS is an ongoing cohort study from the north of the Netherlands aiming to contribute in a better understanding of the determinants of adolescents mental health and social development and the mechanisms underlying the association between these determinants and outcomes[60]. In short, selected municipalities in the north of The Netherlands were asked to provide names and addresses of all inhabitants born between October 1, 1989 (first two) and September 30, 1990 or October 1, 1990 and September 30, 1991 (last three). This yielded 3483 names. In addition, primary schools within these municipalities were asked to participate in TRAILS. The participation of schools was a prerequisite for eligible children and their parents to be approached for participation in TRAILS. Of all children

approached for inclusion in the study (N = 3145), 2230 (76.0%) were enrolled in the study.

Outline of this thesis

This thesis consists of three parts and will discuss the association between depression and inflammation in a reversed chronological order. First, because depression and higher levels of inflammation are most prevalent in cardiac patients, the depression-inflammation link will be evaluated in a sample of older patients with stable CHD. In part 2, a community sample consisting of middle aged participants will be used to investigate if depression and inflammation are associated in people free of cardiac disease. Finally, in part 3 depression, inflammation, and viral infections will be discussed in an adolescent cohort to evaluate if the association is already present at a young age.

Figure 1. Schematic representation of studies in this thesis. Numbers represent chapters.



The first part will start with a meta-analysis of the cross-sectional unadjusted association between depression and inflammation in patients with acute and stable CHD (**Chapter 2**). In this chapter, data from carefully selected studies on the cross-sectional association between depression and inflammation will be used to calculate a combined effect size. Chapter 3 and 4 will examine the bi-directional relationship between depressive symptoms and cytokines (**Chapter 3**) and white blood cell count (**Chapter 4**) in patients with stable CHD focusing on single episode and recurrent depressive symptoms.

In the second part, **chapter 5** will discuss the association between major depressive disorder and hsCRP, IL-6 and TNF-a in the NESDA cohort, considering depression characteristics as depression severity and onset and antidepressant medication use. **Chapter 6** will then, also in the NESDA cohort evaluate if depressive symptoms and anxiety symptoms, and more specific, somatic and cognitive symptoms of depression and anxiety are differentially associated with IL-6, hsCRP and TNF-α.

Part 3 will evaluate data from the TRAILS study; **chapter 7** discusses the prospective association between trajectories of symptoms of depression over a 5 year follow-up period and subsequent hsCRP using a latent class approach. Furthermore, differences between the trajectories of somatic and cognitive symptoms of depression will be analyzed. **Chapter 8** will examine the association between virus infection and depression in the TRAILS sample and the possible effects of inflammation on this relationship.

Chapter 9 will summarize and discuss the results from chapter 2 through chapter 8 in the light of existing literature and implications for research and clinic are discussed.

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PART 1

Depression and inflammation in patients with coronary heart disease

CHAPTER 2

Depression and inflammation in patients with acute and stable coronary heart disease:

a meta-analysis

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Abstract

Background: Around 20% of all CHD patients are suffering from major depression, representing a threefold increased risk compared to the general population. Inflammation has been suggested as a possible physiological link between depression and CHD. Several studies have investigated the association between depression and inflammation, with conflicting results. We therefore conducted a meta-analysis on the association between depression and inflammation in patients with acute and stable coronary heart disease.

Methods: A literature search was performed in Medline, Embase and Psycinfo for studies reporting on the association between depression and inflammatory markers in patients with stable and acute coronary heart disease.

Results: Only with respect to CRP and IL-6, we found sufficient numbers of studies to perform a meta-analysis. The pooled effect size combining data from 12 articles showed that the association between depression and CRP was d=0.30, p<0.001. For IL-6 the pooled effect size was d=0.005, p=0.92, based on 9 articles.

Conclusions: We conclude that depression in patients with stable or acute coronary heart disease is associated with elevated levels of CRP, but not IL-6. For CRP, the effect size was small to moderate. CRP could be a more general and stable reflection of different pro- and anti-inflammatory processes. This possibly explains why depression was associated with CRP, but not with IL-6.

Introduction

Patients with coronary heart disease (CHD), and in particular myocardial infarction (MI) frequently suffer from depression. Around 20% of all patients with CHD are found to be clinically depressed during the months following MI[1-3], while another 20% present with elevated depressive symptoms that do not fulfil diagnostic criteria[4]. These rates are around three times higher than those in the general population[5]. Depression in cardiac patients seems to negatively affect cardiac prognosis[6]. Meijer and colleagues concluded in a meta-analysis that post-MI depression is associated with a 2.7 time increased risk of cardiac mortality and a 1.6 times increased risk of new cardiac events[7].

Several physiological mechanisms have been proposed to explain the association between depression and CHD outcomes, e.g., heart rate variability[8], hypothalamus pituitary adrenal (HPA) axis hyperactivity[9] and inflammation[10]. Several markers of inflammation, such as C-reactive protein (CRP), interleukin(IL)-6 or tumor necrosis factor(TNF)-α have indeed been found to be higher in depressed people compared to people without depression[11,12]. Various researchers have proposed hypotheses in which the association between depression and inflammation is explained, i.e. the macrophage theory[13,14], the cytokine hypothesis of depression[15,16], the immune response system (IRS) model of depression[17], and cytokine-induced sickness behaviour[18]. All hypotheses have in common that pro-inflammatory cytokines are able to induce depression or depressive symptoms. In addition, inflammation is proposed as a causal mechanism in the formation of atherosclerosis[19,20], which in turn plays a role in the aetiology and progression of CHD[21-24]. Pai and colleagues reported that in initially cardiovascular disease free participants, CRP was associated with non-fatal MI or fatal cardiovascular events over six to eight years of follow up[25].

Several studies have investigated the association between depression and inflammation in cardiac patients. Hekler and colleagues showed a positive association between depression and IL-6 in MI patients, but only at seven months after the MI had occurred. At two weeks post-MI there was no association[26]. A Chinese study among MI patients reported a positive association between depression and CRP. However, the drawback of that study is the small sample size (n=35)[27]. In the largest study to date, Whooley and colleagues found no evidence for an association between depression and elevated levels of CRP, fibrinogen and IL-6 in stable CHD patients. Instead, they reported an association between *lower*

levels of inflammation and depression[28]. Results of other individual studies on the association between depression and inflammation have been conflicting.

A large-scale meta-analysis[29] was undertaken by Howren and colleagues in which the association between depression and inflammatory markers was evaluated in several populations, including patients with heart disease. They concluded that in heart disease patients, depression is associated with increased levels of CRP (d = .18, p = .02) and IL-6 (d = .10, p = .05). However, there were some limitations to this specific subgroup analysis, namely that non-English studies were not included, patients with congestive heart failure[30-32] (in which arguably inflammation plays a different role than in ACS patients) were included, as well as a study without a clinically overt patient sample consisting of subjects with atherosclerosis[33].

We therefore set out to conduct a meta-analysis of the association between depression and inflammatory markers in patients with CHD, to combine and summarize the results of individual studies and decrease the effects of their limitations. We hypothesized there would be a positive association between depression and inflammatory markers, most notably CRP and IL-6.

Methods

Literature search

An exhaustive literature search was conducted in MEDLINE, EMBASE and PSYCHINFO between 1987 and November 2010 using pre-specified search terms and inclusion criteria. In addition, a search of the reference sections of eligible studies was performed.

Study selection

Three independent raters (H.D., A.M. and J.R) identified studies that met the inclusion criteria. Disagreements were resolved through group discussion.

Studies were reviewed using the following criteria:

- Studies should be observational or intervention trials containing a control group.
- Patients should have either stable or acute coronary ischemia, specified as myocardial infarction (MI), severe angina, atypical chest pain or stenosis in coronary vessels at some point in their adult lives.

- Animal studies were excluded.
- Depression had to be measured with a validated self-rating instrument or a structured interview designed to assess depression, or a psychiatric diagnosis had to be made by a psychiatrist based on established diagnostic criteria.
- Patients had to be rated on a continuous scale of depression severity or divided into groups with different levels of depression.
- Levels of inflammation had to be measured by one of the following inflammatory markers: IL-1ra, TNF-α, soluble receptors 1 and 2 of TNF- α (sTNFR1, sTNFR2), IL-6 or CRP.
- A statistical measure of the association between depression and inflammation had to be given, including the significance of the effect or a 95% confidence interval.
- Inflammatory markers and depression had to be measured within two weeks of each other.
- Case studies, reviews, meta-analyses, editorials and commentaries were excluded.

The authors of six studies were contacted for additional information (mean + SD) of CRP and IL-6 values for depressive symptoms group and no depressive symptoms group, number of participants per subgroup and the amount of time between blood collection and depressive symptoms assessment[26,34-38]. They provided us kindly with sufficient information to include their studies in our meta-analyses. For two studies[27, 39], information on percentage of male patients or depressed participants were not reported and could not be obtained from the authors.

Quantitative data synthesis

Data from all studies concerning the association between depression and inflammation were first converted into Cohen's d effect sizes, and thereafter pooled using MIX version 1.7[40,41]. Assuming that the included studies consisted of different samples, pooled effect sizes were calculated using a random effects model[42]. Because not all the included studies adjust for covariates or adjust for various different covariates we did not conduct an adjusted meta-analysis.

Results

Literature search

Figure 1 shows the flow diagram from the literature search. A total of 13 studies were selected for the analyses. Because only one study reported on IL-1ra[39], sTNFR1 and sTNFR2[43] and TNF-α was reported on in only two articles[34,36,43], the association between depression and these markers were not evaluated any further and final analyses were restricted to CRP and IL-6.

CRP and depressive symptoms

We included 12 articles on the association between CRP and depression in cardiac patients[27,28,34-39,43-46] (Table 1). These articles together comprised a total of 2429 patients. All participants were diagnosed with either MI, unstable angina, stenosis, exercise-induced ischemia, or had undergone coronary revascularization, bypass surgery or coronary angioplasty. Time of the measurements after the cardiac event differed per study, varying from 1 week up to any point in their adult lives (Table 1).

Depressive symptoms were measured with the BDI in nine studies[27,34,36,37,39,43,45,46], but were analyzed in different ways. When studies used multiple questionnaires including the BDI, we chose to include data using the BDI, in order to increase comparability across the studies. Two studies did not use a cut-off score[39,46], three studies used a cut-off score of 10[35,36,43], and two studies used a cut-off score of 14[27,45]. Shimbo and colleagues reported on associations at two different timelines, at baseline and after three months follow up[35]. Because all studies were cross-sectional, we used the baseline depression measurement for analyses. Kronish and colleagues applied a cut-off score of ≤4 for no depressive symptoms and ≥10 for depressive symptoms[37]. Miller and colleagues divided the patients into three evenly sized groups having either no depression, mildly severe depression or moderately severe depression[34]. We merged the latter two groups for our analyses. Whooley and colleagues evaluated depression with the CDIS-IV and PHQ-9[28]. Bankier and colleagues used the SCID to assess major depressive disorder[44] (table 1). Von Känel and colleagues assessed depressive symptoms using the validated German version of the depression scale from the HADS[38]. They divided participants into 'no depression' (score range 0-7), 'mild depression' (score range 8-10), 'moderate depression' (score range 11-14), and 'severe depression' (score range 15-21).

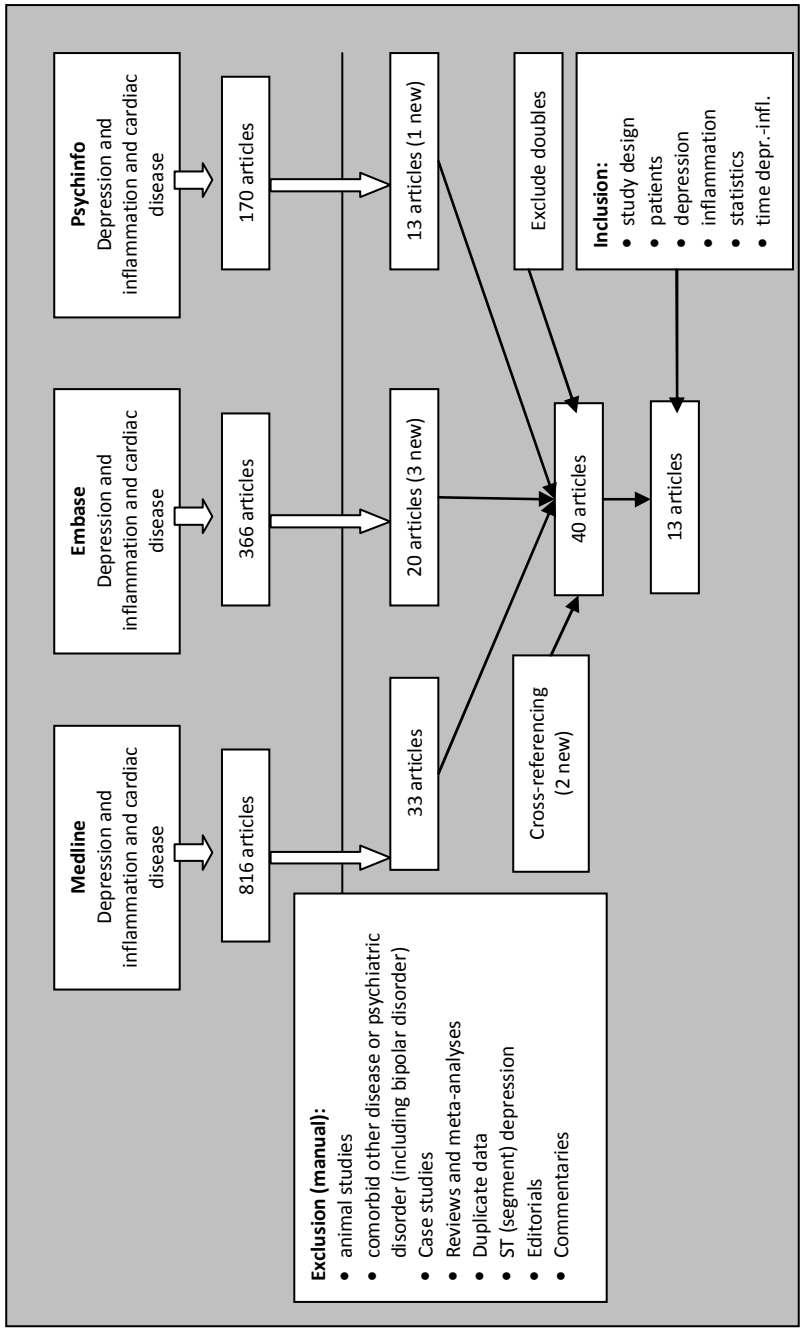


Figure 1. Flow chart for literature search

Data from these participants with a score of ≥8 were merged in order to obtain two groups consisting participants with and participants without depressive symptoms. Out of 12 articles, five studies[27,34,35,44,45] reported significant associations between CRP and depression. Three studies[39,43,46] did not find an association, while one study[28] reported a non-significant trend towards a negative association. Three additional studies did not report on the association between depression and CRP, but provided us with the data upon request, so we were able to calculate Cohen's d for these studies[36-38]. Cohen's d for each individual study is presented in figure 2.

Data-analyses were conducted using a random effects model and showed a pooled Cohen's d of 0.30 (95%CI=0.13-0.46, p<0.001) (Figure 2). In order to evaluate potential publication bias, the dissemination bias was calculated. The fail safe N was relatively high[a] and a trim-and-fill plot revealed a fill of 1 study[b]. The Egger regression test was non-significant (p=0.79). All these results suggest that there is no publication bias. A fixed effect model showed similar results; Cohen's d=0.30 (95%CI=0.13-0.46, p<0.001). Heterogeneity tests revealed no heterogeneity[c]. Because the depression and inflammation measurements in the studies by Whooley and colleagues[28] and Janszky and colleagues[39] were administered a considerable amount of time after the cardiac event took place, we performed sensitivity analyses without their data.

A random effects model showed that Cohen's d increased to 0.42 (95%CI=0.20-0.64, p<0.001). Fixed effect model analyses showed comparable results (Cohen's d=0.41;95%CI=0.19-0.64, p<0.001).

IL-6 and depressive symptoms

A total of nine articles were included for analyses on depression and IL-6[26-28, 34, 36, 38, 39, 43, 45]. These studies described data on 2080 participants with either MI or stable or acute CHD. Two articles did not report on number of depressed participants[26,39], and one article[27] did not explain the characteristics of the participants excluded from analysis. As the authors could not provide this information upon request, so for those we were not able to report on total

[a] 16 unpublished studies would be needed to lower the p-value to a non-significant level. This is relatively high as it equals the number of studies included in the present analyses, suggesting that the effect size presented in this meta-analysis is a good reflection of the true effect size.

[b] This did not decrease the effect size (Cohen's d=0.30, 95%CI=0.13-0.46).

[c] (Q=9.54, p =0.57 and I^2=0%)

numbers of depressed participants and sex. Depression and inflammation level measurements took place varying from 2 weeks after the cardiac event up until anywhere in the participants' adult life after a cardiac event (Table 2). One study assessed depression at baseline and seven months post discharge of the hospital[26]. In order to increase comparability with the remaining studies we used baseline data for the analyses.

Six studies used the BDI to evaluate depressive symptoms[27, 34, 36, 39, 44, 45], two of them applying a cut-off score of 14[27, 45], and one using a cut-off score of 10[43]. One study used the continuous total BDI score[39]. One study provided us with additional data on IL-6 and BDI using a cut-off of 10[36]. Another study used a combination of the BDI and the 17-HRSD to divide patients into three groups[34]. One study used the validated German version of the HADS, and divided participants into 'no depression' (score range 0-7), 'mild depression' (score range 8-10), 'moderate depression' (score range 11-14), and 'severe depression' (score range 15-21)[38]. For more information on how we used the data from Miller and colleagues, and Von Känel and colleagues for analyses, see the section on CRP. Furthermore, two studies used either the CES-D[26] or the CDIS-IV[28] to assess depression (Table 2). None of the studies reported a significantly positive association between depression and IL-6. One study reported a significantly negative association between IL-6 and depression[28], and another study found a non-significant negative association[26]. Five studies found no association at all[27, 34, 39, 43, 45]. Two studies[36, 38] did not report on the association between depressive symptoms and IL-6, but the authors provided us with additional data upon request, so we were able to calculate Cohen's d for these studies. Cohen's d was calculated for each study separately (figure 3) and then merged into a pooled effect size. A random effects model revealed an overall effect size of 0.04 (95%CI=-0.11-0.18, p=0.59). To analyze a potential publication bias, a trim-and-fill plot was used. This did not lead to a major difference in effect size, and the association remained non-significant (Cohen's d=0.06, 95%CI=-0.08-0.19). The fail-safe N test was low, but the effect size for depressive symptoms and IL-6 already was non-significant[d]. Similar results were obtained using a fixed effect model (Cohen's d=0.04; 95%CI=-0.11-0.18, p=0.59).

With respect to IL-6, we performed sensitivity analyses without the data from studies in which depression and inflammation status was assessed a relatively long

[d] The fail-safe-N test indicates how many unpublished non-significant studies are needed to lower the pooled effect size to non-significant (37).

Table 1. Overview of studies investigating the association between depressive symptoms and CRP in patients with stable and acute CHD

Study	Patients	n	Mean age (yr)	Male (%)	Instrument	Depression assessment post event	Depression n (%)	Cohen's *d*
Cui et al.[27]	MI	31	56	-	BDI	2-11 months post MI	32	0.95
Whooley et al.[23]	CHD	984	Depressed: 62 Non-depressed: 68	81	CDIS-IV PHQ	> 6 months post cardiac event, no limitation	22	-0.03
Miller et al.[34]	ACS	65	61	62	BDI HRSD	≥ 3 months	67	0.56
Shimbo et al.[35]	ACS	100	-	51	BDI	1 week	45	0.70
Carney et al.[36]	CHD	44	Depressed: 56 Non-depressed: 62	59	BDI	3 months post MI	55	0.28
Kronish et al.[37]	ACS	105	Depressed: 59 Non-depressed: 60	55	BDI	1 week	45	-0.049
Von Känel et al.[38]	MI	44	Depressed: 59 Non-depressed: 57	80	HADS	4.5 months	23	0.02
Janszky et al.[39]	CHD	156	-	0	BDI	17 months	-	0.16
Schins et al.[43]	MI	103	Depressed: 57 Non-depressed: 56	87	BDI CIDI-auto	3-12 months post MI	55	0.13
Bankier et al.[44]	CHD	72	Depressed: 67 Non-depressed: 68	78	SCID	-		0.49
Frasure-Smith et al.[45]	ACS	682	Depressed:58 Non-depressed:60	81	BDI	2 months	28	0.19
McGlory[46]	ACS	43	57	0	BDI	At admission	49	-0.41
Pooled Cohen's d								**0.30**

CHD = Coronary Heart Disease; ACS = Acute Coronary Syndrome; MI = Myocardial Infarction; CDIS-IV = Computerized Diagnostic Interview Schedule;PHQ = Patient Health Questionnaire;BDI = Beck Depression Inventory; HRSD = Hamilton Rating Scale for Depression; SCID = Structured Clinical Interview for DSM; CIDI-auto = Composite International Diagnostic Interview.

Table 2. Overview of studies investigating the association between depressive symptoms and IL-6 in patients with stable and acute CHD

Study	Patients	n	Mean age (yr)	Male (%)	Instrument	Depression assessment post event	depression (%)	Cohen's *d*
Hekler et al.[26]	MI	50	56.8	78	CES-D	≤ 9 days	-	-0.25
Cui et al.[27]	MI	31	56	-	BDI	2-11 months post MI	32	0.03
Whooley et al.[28]	CHD	984	Depressed: 62 Non-depressed:68	81	CDIS-IV PHQ	> 6 months post cardiac event, no limitation	22	-0.03
Miller et al.[34]	ACS	65	61	62	BDI HRSD	≥ 3 months	67	0.10
Carney et al.[36]	CHD	44	Depressed: 56 Non-depressed: 62	59	BDI	3 months post MI	55	0.06
Von Känel et al.[38]	MI	44	Depressed: 59 Non-depressed: 57	80	HADS	4.5 months	23	0.11
Janszky et al.[39]	CHD	157	-	0	BDI	17 months	-	0.18
Schins et al.[43]	MI	103	Depressed: 57 Non-depressed: 56	87	BDI CIDI-auto	3-12 months post MI	55	0.11
Frasure-Smith et al.[45]	ACS	682	Depressed:58 Non-depressed:60	81	BDI	2 months	28	0.15
Pooled Cohen's d								**0.004**

CHD = Coronary Heart Disease; ACS = Acute Coronary Syndrome; MI = Myocardial Infarction; CDIS-IV = Computerized Diagnostic Interview Schedule;PHQ = Patient Health Questionnaire;BDI = Beck Depression Inventory; HRSD = Hamilton Rating Scale for Depression; SCID = Structured Clinical Interview for DSM; CIDI-auto = Composite International Diagnostic Interview;CES-D = The Center for Epidemiologic Studies-D

Figure 2. Forest plot for CRP included papers

Study	Year	Cohen's d	SE
McGlory (46)	2009	-0.41	3.17
Kronish et al. (37)	2010	-0.05	4.25
von Känel et al. (38)	2010	0.02	0.29
Whooley et al. (28)	2007	0.03	0.27
Schins et al. (43)	2005	0.13	0.76
Janszky et al. (39)	2005	0.16	0.15
Frasure-Smith et al. (45)	2009	0.19	0.21
Carney et al. (36)	2007	0.28	0.79
Bankier et al. (45)	2009	0.49	1.66
Miller et al. (34)	2005	0.56	0.27
Shimbo et al. (35)	2006	0.72	0.23
Cui et al. (27)	2006	0.82	0.36
Pooled:		0.30	

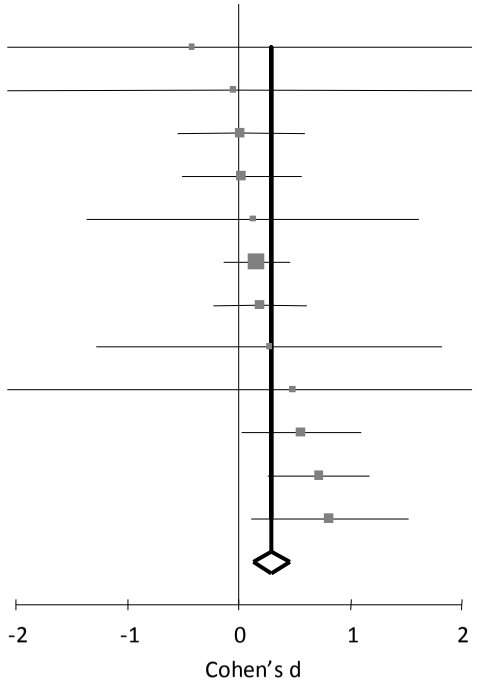
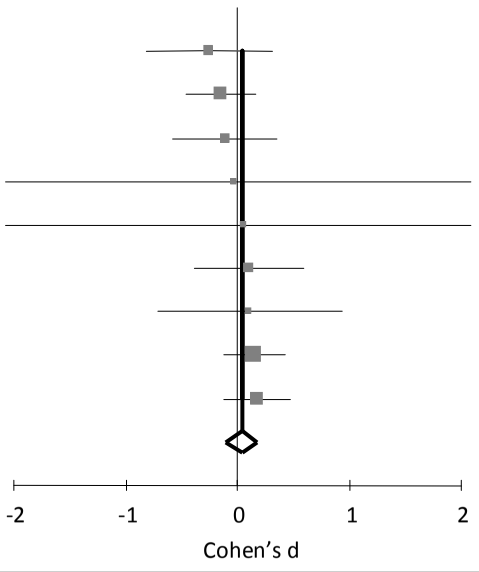


Figure 3. Forest plot for IL-6 included papers

Study	Year	Cohen's d	SE
Hekler et al. (26)	2007	-0.25	0.29
Whooley et al. (28)	2007	-0.15	0.16
von Känel et al. (38)	2010	-0.11	0.24
Cui et al. (27)	2006	-0.03	14.77
Carney et al. (36)	2007	0.06	3.18
Miller et al. (34)	2005	0.11	0.25
Schins et al. (43)	2005	0.11	0.42
Frasure-Smith et al. (45)	2009	0.15	0.14
Janszky et al. (39)	2005	0.18	0.15
Pooled:		0.04	



time after a cardiac event[28, 39]. The effect size did not change and remained non-significant (*d*=0.05; 95%CI=-0.14-0.24,*p*=0.61).

Discussion

This meta-analysis revealed that depression and CRP are significantly associated in patients with stable or acute CHD, with a moderate effect size of 0.30. IL-6 was not associated with depression. Sensitivity analyses showed that the association between depression and CRP was stronger when CRP and depression were measured more closely after the cardiac event (Cohen's d = 0.42 vs. Cohen's d = 0.30).

The finding that CRP was associated with depressive symptoms, whereas IL-6 was not could be explained by the differences in their physiological background. CRP is produced in the liver during a systemic response to infection or injury under the influence of IL-6, but also in the presence of other inflammatory markers such as TNF-α, IL-1 and activated macrophages[47]. CRP secretion is thus elicited by various inflammatory cytokines and it could therefore be argued that CRP is a more general and overall reflection of systemic low grade inflammation than IL-6.

Our results are partly in contrast with those found in a recent meta-analysis on depression and inflammation conducted by Howren and colleagues[29]. In their meta-analysis, the association between depression and inflammation, i.e. CRP, IL-1 and IL-6 was evaluated in different samples i.e. clinical samples (amongst others coronary artery disease [CAD]-related diseases) and community samples. Their reported overall effect sizes for the link between depression with CRP and IL-6 among patients with CHD-related diseases were rather small but statistically significant, 0.18 (p = 0.02) and 0.10 (p = 0.05) respectively. We did not find a significant association between depression and IL-6. In concordance with our present findings, Howren and colleagues found stronger associations between depression and CRP than with IL-6[29]. Still, their results differ from our current results, for which multiple explanations are suitable. First, Howren and colleagues included CAD related papers, also encompassing heart failure[30-32, 48, 49] and atherosclerosis[33]. We excluded these studies because heart failure is a more advanced stage than CHD, often a consequence of MI, with a different underlying pathophysiology. Similarly, adding patients with atherosclerosis makes the overall results for persons with clinically overt CHD debatable. Atherosclerosis is an inflammatory process and mainly involved in the aetiology of CHD. However, it

does not always lead to CHD and is less severe than CHD. We identified six corresponding studies for both CRP and IL-6[26,28,34,35,39,43] and we additionally included five papers that were published after January 2008 (when Howren and colleagues finished their search)[37,38,44,46] of which one was a duplicate[45] of a study[50] included by Howren and colleagues. Finally, we included one non-English paper[27] and one paper of which the authors for additional information upon our request[36]. Both studies were not identified by Howren and colleagues[29]. By excluding papers on heart failure and atherosclerosis, and including six more papers concerning CHD, we believe that the results found in the present study are more representative of CHD patients specifically. The fact that Howren and colleagues found less pronounced associations for hsCRP may be due to the fact that they included a wider range of both less severe and more severe cardiovascular pathology in which the role of inflammation may also be different.

There is a variety of evidence and hypotheses explaining the association between pro-inflammatory markers and depression in heart disease. First, there may be a common genetic factor affecting both inflammation and depression, as was found for depressive symptoms and IL-6, suggesting that depressive symptoms and inflammation may be markers of the expression of a common, genetically modulated pathway[51].

A second explanation could be that inflammation induces sickness behaviour, which is closely related to or may induce symptoms of depression. It has previously been found that pro-inflammatory cytokines are able to cause depressive disorder[18]. A possible biological explanation for this is immune-to-brain communication, in which pro-inflammatory cytokines affect the brain causing feelings of depression[18]. Other sources of evidence for this theory come from immunotherapy in cancer patients in whom IFN-α therapy was associated with the development of neurovegetative symptoms of depression such as fatigue, abnormal sleep, abnormal appetite and psychomotor retardation. In a subgroup of patients, immunotherapy induced emotional/affective symptoms or cognitive symptoms of depression[52, 53]. Treating these cancer patients with an antidepressant (Paroxetine) relieved cognitive symptoms, pain and disturbed mood, while neurovegetative symptoms remained present[52]. In patients with heart disease, such a distinction is rarely made. Elovainio and colleagues found in a large representative cohort that somatic and affective symptoms of the BDI were both associated with CRP in men and women[54]. In men, somatic symptoms of depression and CRP remained associated after adjustment for demographics, health behaviors and cardiac risk factors but, in women, the association was not robust for full

adjustment. Moreover, previous studies reported that somatic symptoms were more predictive than cognitive symptoms of new cardiac events[55, 56], and associated with disease severity and all-cause mortality[57] in patients with cardiac disease. This could first of all explain the inconclusive results found in this area of research, but also how somatic symptoms can contribute to worse cardiac prognosis. Therefore, we emphasize that in future research on the association between depression and inflammation a distinction should be made between the different types of symptoms of depression.

In contrast to the sickness behaviour theory, there is some preliminary evidence suggesting that it is not inflammation causing depressive symptoms, but depressive symptoms causing inflammation[58,59]. Stewart and colleagues found that in an otherwise healthy sample, depression was prospectively associated with levels IL-6, but not vice versa. Another prospective cohort study found that significant depressive symptoms were associated with subsequent levels of CRP and IL-6 after 5 years of follow-up, whereas again, CRP and IL-6 were not prospectively associated with significant depressive symptoms. In addition to this, it appeared that subsequent levels of inflammation were highest in the participants who reported depressive symptoms at two or more time points, suggesting that particularly recurrent chronic depressive symptoms are associated with inflammation[58]. Furthermore, the prospective association between depressive symptoms and inflammation was mainly explained by health behaviors, e.g., smoking, physical inactivity, and obesity. Taken together, this suggests that future research should not only be prospective, but should also evaluate whether a depression is chronic, recurrent or experienced during a single episode. Finally, it would be informative to adjust the association between inflammation and depression for the confounding or mediating effects of health behaviors.

Study limitations

In this meta-analysis we only reviewed articles using cross-sectional data. There is hardly any longitudinal data on the association between depression and pro-inflammatory cytokines, though this is necessary to be able to investigate causality in this association. Furthermore, we were not able to adjust for potential confounding or mediating effect of health behaviors and demographic variables which could possibly influence the effect sizes.

Conclusion

We conclude that in patients with CHD, CRP appears to be moderately associated with depression, whereas IL-6 does not. CRP is an end product of an inflammatory sequence, produced in the presence of IL-6, but also in the presence of for instance TNF-α. CRP could therefore be a more general and stable reflection of different pro- and anti-inflammatory processes. This could explain why depression was associated with CRP, but not with IL-6. We emphasize that in future research more attention needs to be given to the relation between individual symptoms of depression and inflammation. In addition, prospective research needs to be conducted to investigate causality in the association between depression and inflammation.

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CHAPTER 3

Depressive symptoms, health behaviors, and subsequent inflammation in patients with coronary heart disease

Prospective findings from the Heart and Soul Study

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Abstract

Background: Depression has been associated with inflammation in patients with coronary heart disease. However, it is uncertain whether depressive symptoms lead to inflammation or vice versa.

Methods: The authors evaluated 667 outpatients with established coronary heart disease from the Heart and Soul Study. Depressive symptoms were assessed annually with the 9-item Patient Health Questionnaire. Participants were categorized as having no significant depressive symptoms (score below 10 at all interviews), depressive symptoms (score of 10 or higher) at one interview, or depressive symptoms at two or more interviews. At baseline and 5-year follow-up, fasting blood samples were collected to measure three inflammatory biomarkers: fibrinogen, interleukin-6 (IL-6), and high-sensitivity C-reactive protein (hsCRP).

Results: Of the 667 participants, 443 had no depressive symptoms, 86 had depressive symptoms at one assessment, and 138 had depressive symptoms at two or more annual assessments. Across the three groups, greater depressive symptoms were associated with higher subsequent log-transformed levels of IL-6 and hsCRP, and the association with higher fibrinogen levels approached significance. Baseline inflammation did not predict subsequent depressive symptoms. The association of depressive symptoms with subsequent inflammation levels was eliminated after adjustment for health behaviors associated with depression - physical inactivity, smoking, and higher body mass index.

Conclusions: Depressive symptoms predicted higher IL-6 and hsCRP levels among outpatients with coronary heart disease, but higher inflammation levels did not predict subsequent depressive symptoms. The association between depressive symptoms and inflammation was no longer significant after adjustment for health behaviors, which suggests these behaviors may mediate depressive effects.

Introduction

Depression is common in patients with cardiac disease, with prevalence rates nearly three times as high as in the general population[1, 2]. Depression is also associated with worse cardiac prognosis and greater mortality[3]. However, there is still considerable debate regarding how
depression might contribute to a worse cardiac prognosis or mortality[4]. Inflammation is associated with both cardiac disease and depression and is a plausible physiological link between depression and coronary heart disease. Cross-sectional studies have demonstrated an association between depression and inflammation in healthy subjects[5-9] and in cardiac patients[10, 11], but relatively little is known about the directionality of the association (i.e., whether depression causes inflammation or vice versa). Previous studies on the direction of the association between depression and inflammation have yielded conflicting results. Stewart and colleagues[12] examined the directionality of the relationship in otherwise healthy subjects. They found that depressive symptoms as measured with the Beck Depression Inventory-II significantly predicted interleukin-6 (IL-6) levels after 6 years of follow-up, whereas inflammation did not predict depressive symptom scores at follow-up, suggesting that depression leads to an up-regulation of IL-6 levels. However, the Whitehall study came to the opposite conclusion. In over 3,000 British civil servants followed for almost 12 years, Gimeno and colleagues found that inflammation preceded depressive symptoms, whereas depressive symptoms did not predict inflammation[13]. To our knowledge, whether depressive symptoms are the cause or result of inflammation has not been evaluated in patients with cardiovascular disease. We therefore set out to evaluate the prospective association between depressive symptoms and inflammation using repeated measurements of depressive symptoms and inflammation in patients with stable coronary heart disease. We evaluated whether depressive symptoms (assessed annually for 6 years) were associated with higher levels of subsequent inflammation and vice versa. In addition, we evaluated the role of health behaviors in mediating the association between depression and inflammation.

Method

Design and participants

The Heart and Soul Study is an ongoing prospective cohort study of psychosocial factors and health outcomes in patients with coronary heart disease. The methods

have been described previously[14]. Briefly, 1,024 outpatients with stable coronary heart disease were recruited and completed a baseline examination between September 2000 and December 2002. Following the baseline examination, patients received annual telephone calls for assessment of depressive symptoms. Between September 2005 and December 2007, 667 participants (80% of the 829 survivors) completed a 5-year follow-up examination that included measures of inflammation. The study protocol was approved by the appropriate institutional review boards, and all participants provided written informed consent.

Depressive symptoms

Depressive symptoms were assessed annually for 6 years by using the 9-item Patient Health Questionnaire, a self-report instrument that measures the frequency of depressive symptoms corresponding to the nine DSM-IV criteria for depression[15]. A paper-and-pencil version of the questionnaire was administered at the baseline examination (year 0), telephone versions were administered after 1, 2, 3, and 4 years of follow-up, and a paper-and-pencil version was again administered after 5 years of follow-up. Of the 667 participants who were examined after 5 years, 640 completed five or more interviews, 23 (3.4%) completed four interviews, three (0.4%) completed three interviews, and one (0.1%) completed two interviews.

At each assessment, participants were asked to indicate the frequency of experiencing each depressive symptom during the last two weeks. Each of the nine symptoms was scored as 0 (not at all), 1 (on several days), 2 (more than half the days), or 3 (nearly every day), with total scores ranging from 0 to 27[16]. The Patient Health Questionnaire has demonstrated excellent validity when compared with a mental health interview for depression in patients with coronary heart disease[17, 18]. Telephone and in-person assessments yield similar results[19]. As a summary measure of depressive symptoms, we calculated the mean score for each participant as the sum of the annual questionnaire scores divided by the number of interviews completed. We also created a categorical variable by defining “depressive symptoms” as a score of 10 or higher on the Patient Health Questionnaire. We used this to group the participants into three categories: depressive symptoms at two or more interviews, depressive symptoms at one interview, or no depressive symptoms. We chose these groups for analysis because further divisions would have yielded too few participants in each category.

Inflammatory biomarkers

Fasting blood samples were obtained at baseline and after 5 years of follow-up. Levels of high-sensitivity C-reactive protein (hsCRP), IL-6, and fibrinogen were determined from plasma and serum samples. The laboratory technicians were blinded to the depression status of the participants.

A highly sensitive CRP assay was performed with a BN II nephelometer (Dade-Behring, Newark, Del.). Interassay coefficients of variation were 1.66%–5.32%. IL-6 was measured by using the Millipore Milliplex Map kit (Millipore, Billerica, Mass.), with interassay coefficients of variation from 6.3% to 11.6%. Concentrations of serum fibrinogen were determined by the Clauss assay with coefficients of variation less than 3%. We log-transformed the hsCRP and IL-6 levels because they were not normally distributed, and we verified that the transformation resulted in normal distributions. We also created three separate dichotomous variables for IL-6, hsCRP, and fibrinogen, defining a priori a high level as one in the highest quartile. For IL-6 a high level was defined as above 5.40 pg/ml, for hsCRP a high level was one above 3.48 mg/liter, and a high fibrinogen level was defined as above 420 mg/dl.

Other assessments

We recorded self-reported age, gender, ethnicity, education, and medical history at baseline and at the 5-year examination. In addition, we assessed health behaviors known to be associated with inflammation, such as smoking status, physical activity, and body mass index (BMI)[20,21]. To assess physical activity, we asked, "Which of the following statements best describes how physically active you have been during the last month, that is, done activities such as 15–20 minutes of brisk walking, swimming, general conditioning, or recreational sports?" Participants responded by choosing one of the six following categories: not at all active, a little active (one or two times per month), fairly active (three or four times per month), quite active (one or two times per week), very active (three or four times per week), or extremely active (five or more times per week). Self-report of physical activity has been shown to be valid, accurate, and reliable[22-25]. Physical inactivity was defined as "not at all active" or "a little active." Height and weight were measured and used to calculate BMI (weight in kilograms divided by the square of height in meters). Alcohol consumption was measured with the AUDIT-C self report questionnaire[26]. Left ventricular ejection fraction was assessed by means of two-

dimensional echocardiography. High density lipoprotein (HDL) levels were measured from fasting venous blood samples, and non-HDL cholesterol was calculated as total cholesterol minus HDL cholesterol. Participants were asked to bring all of their medication bottles to the study appointment, and all current medications were recorded. Medications were categorized by using Epocrates Rx (Epocrates, San Mateo, Calif.).

Statistical analyses

The goal of this study was to determine the directionality of the depression-inflammation relationship. We used general linear models to compare mean levels of each marker across three groups: participants who were not depressed at any interview (having a score below 10 on the Patient Health Questionnaire at all interviews), participants who reported depressive symptoms (a score of 10 or higher) at one interview, and those who reported significant depressive symptoms at two or more interviews. To further evaluate the association between Patient Health Questionnaire score and levels of inflammation, we used multivariate analysis of variance adjusted for other patient characteristics associated with depressive symptoms (age, gender, education, race, aspirin use, history of diabetes, myocardial infarction, and congestive heart failure) and health behaviors (physical activity, smoking, and BMI). In addition, we evaluated change in inflammation over 5 years across the three subgroups. Finally, we used multivariate analysis of covariance (MANCOVA) to evaluate the effect of depressive symptoms on all three measures of inflammation as a single dependent variable, adjusted for the baseline level of inflammation. We also used MANCOVA to evaluate the effect of inflammation on subsequent depressive symptoms (as a single dependent variable), adjusted for the baseline level of depressive symptoms. Analyses were performed by using SAS 9.2 (SAS Institute, Cary, N.C.).

Results

Characteristics of participants

As compared with the 162 patients who were alive and did not complete the 5-year follow-up examination, the 667 participants who completed the exam were older

(mean age=66 years, SD=10, versus mean=64 years, SD=12; t=–2.09, df=827, p=0.04) and had fewer baseline depressive symptoms as indicated by scores on the Patient Health Questionnaire (mean=4.75, SD=5.26, versus mean=6.77, SD=5.84; t=4.29, df=827, p<0.01). Participants who completed the exam also had lower log-transformed baseline hsCRP levels (mg/liter) than those who did not complete the 5-year exam (mean=0.57, SD=1.29, versus mean=0.85, SD=1.32; t=2.48, df=795, p=0.01). Baseline levels of IL-6 and fibrinogen were similar in the patients who did and did not complete the 5-year examination (p>0.10 in both cases).

Of the 667 participants who completed the 5-year examination, 138 (21%) had depressive symptoms (i.e., a score of 10 or higher on the Patient Health Questionnaire) at two or more interviews, 86 (13%) had depressive symptoms at one interview, and 443 (66%) had no significant depressive symptoms (a score less than 10 on the Patient Health Questionnaire at all interviews). As compared to participants without significant depressive symptoms, those with depressive symptoms at any interview were younger and less likely to be male, to be white, or to have graduated from high school (Table 1). They were more likely to have a history of diabetes, myocardial infarction, or congestive heart failure and had higher BMI values. Participants with depressive symptoms were less likely to use aspirin and more likely to smoke and to be physically inactive. The three groups had similar levels of hypertension, cardiac disease severity, cardiac risk factors, and use of cardioprotective medications.

Presence of depressive symptoms as predictor of subsequent inflammation

MANCOVAs evaluating the effect of depression on subsequent inflammation showed that depressive symptoms predicted subsequent inflammation in an unadjusted analysis (F=2.56, df=6, 1304, p=0.02) and after adjustment for age, gender, education, race, history of diabetes, myocardial infarction, congestive heart failure, aspirin use, and baseline levels of inflammatory markers (F=2.15, df=6, 1182, p<0.05). This association was no longer significant after adjustment for health behaviors (F=1.74, df=6, 1128, p=0.11). Across the three groups, greater depressive symptoms were associated with higher subsequent log-transformed levels of IL-6 and hsCRP, and they were nonsignificantly associated with higher levels of fibrinogen (Table 2). Depressive symptoms remained associated with subsequent levels of IL-6 and were marginally related to levels of fibrinogen but were not associated with hsCRP, after adjustment for age, gender, education, race,

Table 1. Characteristics of 667 participants with CHD by presence of depressive symptoms during the previous 5 years

	No depressive symptoms (n=443)		Depressive symptoms at one interview (N=86)		Depressive symptoms at 2 or more interviews (n=138)		Analysis		
Variable	Mean	SD	Mean	SD	Mean	SD	*F*	df	*p*
Demographic characteristics									
Age (years)	72.8	9.2	68.6	12.0	66.3	11.0	24.26	2, 661	<0.01
	N	%	N	%	N	%	χ^2	df	*p*
Male	381	86%	62	72%	106	77%	13.18	2	0.01
White	277	63%	42	49%	79	57%	6.03	2	0.05
High school graduate	402	91%	70	81%	112	81%	13.63	2	0.01
Comorbid conditions									
Hypertension	324	74%	65	77%	110	80%	2.55	2	0.28
Myocardial infarction	205	47%	53	63%	70	51%	7.66	2	0.02
Congestive heart failure	68	16%	17	20%	42	31%	15.56	2	<0.01
Diabetes mellitus	122	28%	35	41%	35	26%	7.12	2	0.03
Medication use									
Aspirin	338	76%	58	67%	86	62%	11.40	2	<0.01
Beta blocker	300	68%	62	72%	91	66%	0.94	2	0.62
Renin-angiotensin system inhibitor	298	67%	55	64%	85	62%	1.63	2	0.44
Statin	123	28%	20	23%	26	19%	4.66	2	0.10

Health behaviors									
Regular alcohol use	132	30%	19	23%	32	24%	3.24	2	0.20
Current smoking	42	10%	13	16%	37	27%	25.81	2	<0.01
Physically inactive	140	32%	41	49%	82	60%	38.43	2	<0.01
	Mean	SD	Mean	SD	Mean	SD	*F*	df	*p*
Body mass index (kg/m^2)	28.1	5.03	29.8	6.01	29.6	6.97	5.59	2, 663	<0.01
Cardiac disease severity and risk factors									
Left ventricular ejection fraction	0.62	0.11	0.63	0.11	0.60	0.13	1.43	2, 654	0.24
LDL cholesterol (mg/dl)	93.7	34.2	98.0	28.6	95.0	32.9	0.62	2, 653	0.54
HDL cholesterol (mg/dl)	47.5	14.8	47.3	18.6	45.6	14.3	0.75	2, 663	0.47
Non-HDL cholesterol	115.7	38.9	124.0	34.1	120.8	37.0	2.28	2, 663	0.10

LDL = low-density lipoprotein, HDL = high-density lipoprotein

Table 2. Mean ± SE levels of inflammatory markers at 5-year follow-up examination, by depressive symptoms (sum score of ≥10 for all 9 items on Patient Health Questionnaire)

5-year inflammatory marker levels	No depressive symptoms at any interview N=443		Depressive symptoms at one interview N=86		Depressive symptoms at 2 or more interviews N=138		Analysis		
	Mean	SD/SE*	Mean	SD/SE*	Mean	SD/SE*	*F*	df	*P*
Log interleukin 6									
Unadjusted	1.19	0.66	1.19	0.71	1.38	0.71	4.36	2, 658	0.01
Model 1	1.13	0.06	1.34	0.08	1.51	0.07	4.46	2, 631	0.01
Model 2	1.40	0.06	1.38	0.08	1.52	0.07	1.91	2, 602	0.15
Log hsCRP									
Unadjusted	0.34	1.14	0.58	1.30	0.70	1.30	5.12	2, 658	<0.01
Model 1	0.70	0.11	0.88	0.15	0.90	0.12	1.78	2, 631	0.17
Model 2	0.80	0.12	0.90	0.16	0.91	0.13	0.45	2, 602	0.64
Fibrinogen									
Unadjusted	371	79	370	87	391	102	2.87	2, 659	0.06
Model 1	385	8	381	11	404	9	2.50	2, 632	0.08
Model 2	393	9	386	12	408	10	1.80	2, 603	0.17

*Unadjusted values use SD, adjusted values use SE

Model 1: adjusted for age, gender, education, race, history of diabetes, MI, CHF and aspirin use

Model 2: adjusted for all of the above variables plus physical activity, smoking and BMI

Table 3. Mean ± SE change in inflammatory markers (5-year minus baseline levels), by depressive symptoms (sum score of ≥10 on Patient Health Questionnaire).

5-year change	No depressive symptoms at any interview N=443		Depressive symptoms at one interview N=86		Depressive symptoms at 2 or more interviews N=138		Analysis		
	Mean	SD/SE*	Mean	SD/SE*	Mean	SD/SE*	*F*	df	*P*
Log interleukin 6									
Unadjusted	1.24	2.83	1.30	2.82	1.80	3.14	1.87	2, 628	0.16
Model 1	1.45	0.26	1.60	0.35	1.95	0.30	1.33	2, 602	0.26
Model 2	1.43	0.30	1.54	0.39	1.92	0.33	1.11	2, 574	0.33
Log hsCRP									
Unadjusted	-0.54	7.74	0.98	12.38	0.26	13.28	1.00	2, 629	0.37
Model 1	-0.85	0.89	0.85	1.23	0.10	1.03	1.12	2, 603	0.33
Model 2	-1.48	1.04	0.57	1.36	-0.18	1.14	1.54	2, 575	0.22
Fibrinogen									
Unadjusted	-13.36	81.72	-16.68	80.52	7.06	102.64	3.10	2, 626	0.05
Model 1	-14.22	7.85	-19.35	11.05	5.07	9.00	2.63	2, 600	0.07
Model 2	-13.67	9.04	-17.13	12.07	7.21	9.85	2.66	2, 573	0.07

*Unadjusted values use SD, adjusted values use SE

Model 1: adjusted for age, gender, education, race, history of diabetes, MI, CHF and aspirin use

Table 4. Mean ± SE levels of inflammatory markers at baseline examination and subsequent depressive symptoms

Baseline inflammatory marker levels	No depressive symptoms at any interview N=443		Depressive symptoms at one interview N=86		Depressive symptoms at 2 or more interviews N=138		Analysis		
	Mean	SD/SE*	Mean	SD/SE*	Mean	SD/SE*	*F*	df	*P*
Log interleukin 6									
Unadjusted	0.84	0.66	0.85	0.66	0.92	0.73	0.66	2, 633	0.52
Model 1	0.91	0.06	0.94	0.08	1.06	0.07	2.15	2, 618	0.12
Model 2	1.05	0.07	1.04	0.08	1.05	0.07	0.02	2, 612	0.98
Log hsCRP									
Unadjusted	0.49	1.26	0.66	1.28	0.76	1.37	2.45	2, 634	0.09
Model 1	0.94	0.12	1.00	0.16	1.13	0.14	0.98	2, 619	0.37
Model 2	1.14	0.13	1.12	0.16	1.09	0.14	0.05	2, 613	0.95
Fibrinogen									
Unadjusted	384	82	387	78	383	92	0.05	2, 630	0.95
Model 1	400	8	399	11	401	9	0.02	2, 615	0.99
Model 2	414	9	409	11	402	9	0.91	2, 609	0.40

*Unadjusted values use SD, adjusted values use SE

Model 1: adjusted for age, gender, education, race, history of diabetes, MI, CHF and aspirin use

Model 2: adjusted for all of the above variables plus physical activity, smoking and BMI

history of diabetes, myocardial infarction, congestive heart failure, and aspirin use (Table 2). However, after further adjustment for health behaviors (physical activity, smoking, and BMI), depressive symptoms were no longer associated with any inflammation index. When we evaluated the multivariable-adjusted mean change in levels of inflammatory markers, depressive symptoms did not predict statistically significant changes in IL-6, hsCRP, or fibrinogen (Table 3). When we analyzed depressive symptoms as a continuous variable, the average score on the Patient Health Questionnaire across the annual assessments predicted subsequent log-transformed levels of IL-6 (β=0.102, p=0.009) and hsCRP (β=0.121, p=0.002) but not the level of fibrinogen (β=0.058, p=0.13)Again, this association was no longer significant after adjustment for health behaviors (log IL-6: β=0.038, p=0.36; hsCRP: β=0.018, p=0.67; fibrinogen: β=0.018, p=0.68). When inflammation was evaluated as a dichotomous variable, the proportions of participants with levels of inflammatory markers in the highest quartile were 22% to 24% in those without depression at any interview, 23% to 28% in those with depressive symptoms at one interview, and 30% to 35% in patients with depressive symptoms at two or more interviews (Figure 1).

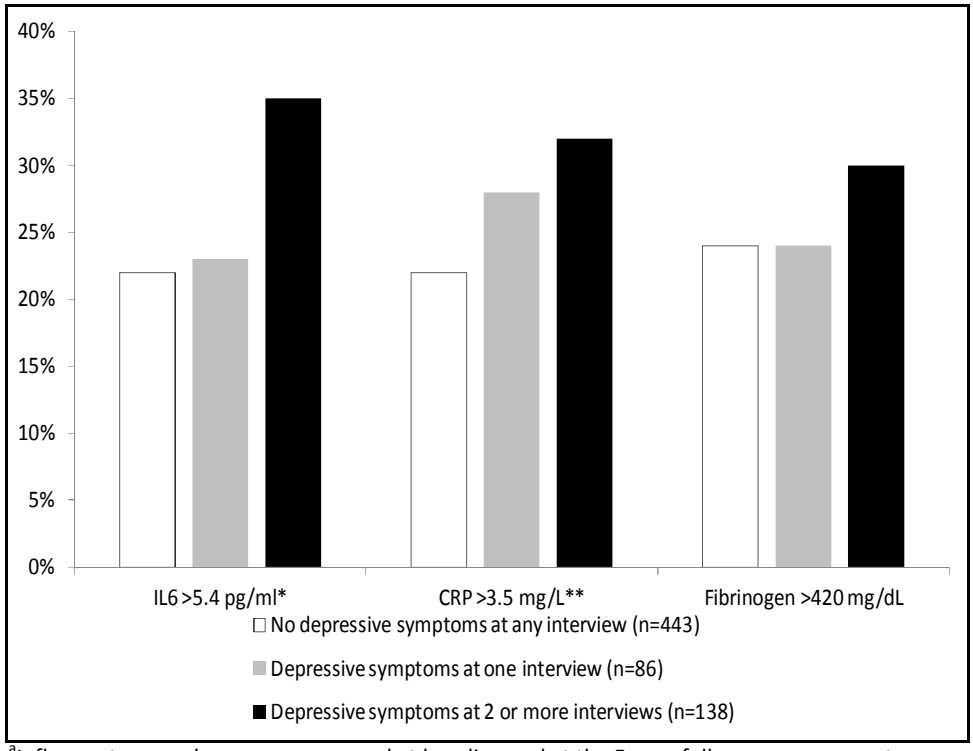
Inflammation as predictor of subsequent depressive symptoms

MANCOVAs revealed no effect of inflammation on subsequent depression in unadjusted analyses (F=1.37, df=6, 1256, p=0.22) or after adjustment for age, gender, education, race, history of diabetes, myocardial infarction, congestive heart failure, aspirin use, and baseline depressive symptoms (F=0.65, df=6, 1224, p=0.69). In addition, baseline levels of inflammatory markers were not associated with subsequent depressive symptoms (Table 4). As compared with participants who had significant depressive symptoms at two or more annual assessments, those without depressive symptoms had similar baseline levels of log IL-6, log hsCRP, and fibrinogen.

Discussion

The aim of this study was to examine the directionality of the depression-inflammation relationship in a group of patients with stable coronary heart disease. The rate of depressive symptoms was consistent with the rate in a previous study[27]:

Figure 1. Proportion of patients with coronary heart disease who had high levels of inflammatory markers at 5 –Year follow-up, by depressive symptoms over 5 years[a]



[a]Inflammatory markers were measured at baseline and at the 5-year follow-up assessment. Depressive symptoms were assessed with the nine-item Patient Health Questionnaire at baseline and annually for the next 5 years.

*Significant difference among groups IL-6 (χ^2=8.45, df=2, p=0.01).

**Significant difference among groups hsCRP (χ^2=5.96, df=2, p=0.05).

34% of the participants had a score of 10 or higher on the Patient Health Questionnaire at one or more time points. We found that depressive symptoms were associated with higher subsequent levels of inflammation, whereas baseline levels of inflammation did not predict subsequent depressive symptoms. The association between depressive symptoms and subsequent inflammation was eliminated after adjustment for health behaviors associated with inflammation—physical inactivity, smoking, and higher BMI.

Taken together, these findings suggest that depression may lead to inflammation through poor health behaviors, but inflammation does not lead to depression in

patients with coronary heart disease. There have been only a few studies regarding the directionality of the depression-inflammation relationship, and to our knowledge, this is the first study on this subject in a cardiac population. We also believe this to be the first study evaluating the association of depressive symptoms over time with subsequent inflammation. Our results extend the findings of Stewart and colleagues[12], who found that depression predicted high levels of IL-6, whereas high levels of IL-6 did not predict depression. However, our results differ from those of Gimeno and colleagues, who reported that CRP and IL-6 levels were predictive of subsequent depressive symptoms, but not vice versa, in the Whitehall II study[13]. One possible explanation for this discrepancy is that the Whitehall II study used a different measure of depressive symptoms. Their assessment was based on four items from the General Health Questionnaire (thinking of yourself as a worthless person, feeling that life is entirely hopeless, feeling that life is not worth living, and finding times when you could not do anything because your nerves were too bad) and did not include measures of sleep, appetite, concentration, energy, suicidal thoughts, or depressed mood. In contrast, we administered the Patient Health Questionnaire, which was specifically designed to assess the nine symptoms of depression. Another possible explanation for the difference in findings is the difference between study populations. The association between depressive symptoms and inflammation in the general population may differ from the relationship in patients with established coronary heart disease.

It is interesting that our results show that depressive symptoms predicted high levels of hsCRP and IL-6 but not fibrinogen. A possible explanation could be that hsCRP and IL-6 are more specific for the underlying inflammation process in stable patients with coronary heart disease. In contrast to hsCRP and IL-6, which are both markers of inflammation, fibrinogen not only reflects inflammation but also is a blood coagulation factor[28], which could explain why we did not find a prospective association between depression and fibrinogen.

We also found that patients with depressive symptoms at two or more interviews had higher levels of inflammation at follow-up than patients with depressive symptoms at one interview. To the extent that depressive symptoms at two or more interviews represented chronic or recurrent depression, our findings suggest that persistent depression may have greater effects on inflammation than a single episode of depression. Consistent with these findings are the results reported by Hamer and colleagues[29], who found that in a group of 3,609 aging subjects, participants with depressive symptoms at two timepoints had higher levels of CRP

and fibrinogen than those with depressive symptoms at one timepoint. In addition, Kaptein and colleagues[30] showed that patients with chronic depressive symptoms following myocardial infarction were at higher risk for having new cardiovascular events than those whose depressive symptoms resolved. Taken together, the findings suggest that the duration of depressive symptoms may influence cardiac health.

The prospective association of depression with inflammation was no longer significant after adjustment for health behaviors (physical inactivity, BMI, and smoking). Although our results cannot determine the direction of the association between depression and health behaviors, this raises the possibility that helping depressed cardiac patients improve these behaviors could potentially reduce inflammation. These findings are further supported by a longitudinal community-based study in which Matthews and colleagues[31] investigated the directionality of the depression-inflammation relationship among 1,781 premenopausal and early perimenopausal women who were free of cardiac disease at baseline. They found that depression, as measured with the Center for Epidemiologic Studies Depression Scale, was associated with higher CRP levels at follow-up. Similar to our results, this association was no longer significant after adjustment for a range of covariates including health behaviors. In addition, Hamer and colleagues[29] found that weight change, waist circumference, current smoking, alcohol use, and especially physical activity were significant mediators in the depression-inflammation relationship. Finally, Dod and colleagues[32] showed in a group of nonsmoking patients with stable coronary heart disease that a combination of intensive exercise and dietary changes significantly lowered levels of IL-6 and CRP, suggesting that physical inactivity and BMI may influence inflammation levels.

Several strengths can be attributed to this study. First, the annual assessments of depressive symptoms and medical health status presented us with the opportunity to assess the direction of this association. In addition, biological and behavioral mediators were carefully assessed at baseline and after 5 years of follow-up. However, there are also several limitations. This study focused on outpatients with stable coronary heart disease. Our results may therefore not apply to healthy participants or to patients with acute coronary syndromes. Furthermore, the study group mostly consisted of older men, so these results may not be generalizable to either women or younger men. Finally, 20% (162 of 829) of the surviving participants did not complete the 5-year follow-up examination. However, these participants were younger and had worse depression scores than those who

completed the examination, so including them would probably have strengthened the association between depression and inflammation.

In conclusion, we found no evidence of a bidirectional relationship between depression and inflammation. Depression was prospectively associated with IL-6 and hsCRP, but not vice versa. This prospective association was no longer significant after adjustment for physical inactivity, BMI, and smoking. These findings raise the possibility that helping depressed cardiac patients improve health behaviors could reduce inflammation.

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CHAPTER 4

Depressive symptoms and white blood cell count in coronary heart disease patients

Prospective findings from the Heart and Soul Study

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Abstract

Background: Depression has been associated with elevated white blood cell (WBC) count –indicative of systemic inflammation- in cross-sectional studies, but no longitudinal study has evaluated whether depressive symptoms predict subsequent WBC count or vice versa. We sought to evaluate the bidirectional association between depressive symptoms and WBC count in patients with coronary heart disease (CHD).

Methods: Depressive symptoms were assessed at baseline and annually during 5 consecutive years of follow-up in 667 outpatients with stable CHD from the Heart and Soul Study. The presence of significant depressive symptoms was defined as a score of ≥10 on the Patient Health Questionnaire (PHQ-9) at one or more assessments. WBC count was measured in blood samples collected at baseline and after 5 years of follow-up.

Results: Of the 667 participants, 443 (66%) had no depressive symptoms (PHQ-9 <10), 86 (13%) had depressive symptoms (PHQ-9≥10) at 1 assessment, and 138 (21%) had depressive symptoms at 2 or more annual assessments. Across the three groups, participants with recurrent depressive symptoms had higher WBC levels after 5 years of follow-up ($p<.001$). This relationship was essentially unchanged after adjustment for demographics, traditional cardiovascular risk factors, cardiac disease severity, inflammatory cytokine levels, and health behaviors ($p=.009$). Baseline WBC count was not associated with subsequent depressive symptoms ($p = .18$).

Conclusions: Depressive symptoms independently predicted higher subsequent WBC count in patients with stable CHD, but baseline WBC count did not predict subsequent depressive symptoms. These findings support a unidirectional relationship in which depression is a risk-factor for inflammation.

Introduction

Depression is common in patients with cardiac disease, with prevalence rates nearly three times as high as in the general population[1-3]. Depression has been found to have a negative influence on cardiac prognosis[4], but the mechanisms of this association remain unclear[5]. Several studies have evaluated the association between depression and inflammatory markers, including interleukin (IL)6, high sensitive C-reactive protein (hsCRP), tumor necrosis factor (TNF)-α and its soluble receptors[6-9]. A meta-analysis reported small to moderate cross-sectional associations between depression and these cytokines, both in healthy subjects and in cardiac patients[10]. Currently, several prospective studies have been performed[8, 11-14]. In a recent study in heart failure patients, depressive symptoms at baseline were associated with current and future inflammation after one year follow-up, independent of classic cardiovascular risk factors, disease severity and adverse health behaviors[8]. In line with this, we found in a previous study that depressive symptoms predicted subsequent levels of hsCRP and IL-6 over a period of five years in patients with coronary heart disease (CHD), but not vice versa[11]. However, the association of depressive symptoms with subsequent inflammation in this study was mainly explained by the presence of adverse health behaviors[11].

A relatively understudied inflammatory marker in the depression-inflammation relationship is white blood cell (WBC) count. Like cytokines, WBCs (or leukocytes) are part of the immune system, but they come from different sources. WBCs are formed in the bone marrow from hematopoietic stem cells, whereas cytokines are protein messengers produced by mature immune cells, e.g. monocytes[15]. Although WBCs and cytokines have different physiological roles in the immune response, they interact in a complex way. Thus, it is unclear whether depression increases the production of WBC in the bone marrow, if it increases the secretion of inflammatory cytokines from mature WBC in peripheral vessels, or both.

Earlier research has shown that higher WBC count is associated with increased risk of atherosclerosis[16,17] and cardiac mortality[18, 19]. Furthermore, decreased lymphocyte percentage is associated acute coronary syndrome and major adverse cardiac events in CHD patients[20], whereas higher monocyte and neutrophil count are associated with a history of cardiovascular disease[21]. Moreover, several studies have reported a cross-sectional association between high WBC count and depression[22-24] in participants free of cardiac disease. In contrast, depressive symptoms have also been found to be associated with lower levels of WBC count in elderly patients with acute hospital admission[25]. Whether depressive symptoms are

associated with WBCs, and if so, whether depressive symptoms predict higher WBC levels or vice versa, has not been evaluated in patients with cardiovascular disease. We therefore sought to investigate the temporal, bidirectional associations between depressive symptoms and WBC count, while adjusting for socio-demographic factors, traditional risk factors, cardiac disease severity, and inflammatory cytokines.

Methods

Design and Participants

The Heart and Soul study is an ongoing prospective cohort study of psychosocial factors and health outcomes in patients with CHD. Methods have been described previously[26]. Briefly, 1024 outpatients with stable CHD were recruited and completed a baseline examination between September 2000 and December 2002. Following the baseline examination, patients received annual telephone calls for assessment of depressive symptoms. Between September 2005 and December 2007, 667 participants (80% of the 829 survivors) completed a 5-year follow-up examination that included measures of inflammation. The study protocol was approved by the appropriate institutional review boards, and the study was performed in accordance with the standards of the most recent Helsinki declaration (2008). All participants provided written informed consent.

Depressive symptoms

Depressive symptoms were assessed at baseline and annually during 5 consecutive years using the 9-item Patient Health Questionnaire (PHQ-9), a self-report instrument that measures the frequency of depressive symptoms corresponding to the 9 Diagnostic and Statistical Manual-IV criteria for depression[27]. A paper and pencil version of the PHQ was administered at the baseline examination (year 0), telephone versions were administered annually (after 1, 2, 3 and 4 years of follow-up), and a paper and pencil version was again administered after the 5th year of follow-up (year 5). Of the 667 participants who completed the 5-year examination, 640 (96%) completed 5 or more interviews, 23 (3.4%) completed 4 interviews, 3 (0.4%) completed 3 interviews, and 1 (0.1%) completed 2 interviews.

At each assessment, participants were asked to indicate the frequency of experiencing each depressive symptom during the last two weeks. Every one of the 9 symptoms was scored as not at all (0), several days (1), more than half the days (2), or nearly every day (3), with a total score range of 0 to 27[28]. The PHQ-9 has demonstrated excellent validity when compared with a mental health interview for depression in patients with CHD[29,30]. Telephone and in-person PHQ assessments yield similar results[31]. As a summary measure of mean depressive symptoms over time, we calculated the sum of the annual PHQ scores divided by the number of interviews completed. We also created a 3-category variable indicating significant depressive symptoms at (1) 2 or more interviews (N = 138), (2) at one interview (N = 86), or (3) at no interview (N = 443), where significant depressive symptoms were defined as PHQ ≥ 10. We chose these groups for analysis because further divisions would have yielded too few participants in each category.

White blood cell count

Fasting blood samples were obtained at baseline and after 5 years of follow-up to determine WBC count. Prior to each study appointment, participants completed an overnight fast except for taking their regularly prescribed medications. A 21G butterfly needle was inserted intravenously in the forearm, and blood samples were drawn into EDTA tubes. WBC was measured using a Beckman Coulter analyzer (Beckman Coulter, Inc., California). Laboratory technicians were blinded to the depression status of the participants.

Cytokines

Cytokines, amongst others, are involved in attracting white blood cells to the arterial wall and thus affects levels of WBC count[32]. In order to determine if depressive symptoms are associated with WBC count independent of cytokines, analyses were adjusted for IL-6, hsCRP and fibrinogen. High sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6) and fibrinogen were determined from plasma and serum samples at baseline and after 5 years of follow-up. Laboratory technicians were blinded to the depression status of the participants.
High-sensitivity C-reactive protein (hsCRP) levels were measured using the Roche Integra assay or the Beckman Extended Range assay. Interassay coefficients of variation were 1.66-5.32%. IL-6 was determined using the Millipore Milliplex Map

kit, with interassay coefficients of variation from 6.3 to 11.6%. Concentrations of serum fibrinogen were determined by the Clauss assay with coefficients of variation <3%.

The individual inflammatory markers showed small, but significant correlations with WBC at baseline (loghsCRP: r = .28, *p* <.0001; IL-6: r = .26, *p* <.0001; fibrinogen: r = .26, *p* <.0001) and at the year 5 assessment (loghsCRP: r = .26, *p* <.0001; IL-6: r = .25, *p* <.0001; fibrinogen: r = .26, *p* <.0001).

Cardiac disease severity

The presence of congestive heart failure (CHF) and myocardial infarction (MI) were determined by self-report at baseline. Year 5 CHF and MI were determined by reviewing medical records. CHF was defined as hospitalization for a clinical syndrome involving at least 2 of the following: paroxysmal nocturnal dyspnea, orthopnea, elevated jugular venous pressure, pulmonary rates, third heart sound, and cardiomegaly or pulmonary edema on chest radiography. These signs and symptoms must have represented a clear change from the usual clinical status[33]. MI was defined using standard criteria[34]. High-density lipoprotein levels were measured from fasting venous blood samples, and non-HDL cholesterol was calculated as total minus HDL cholesterol. To assess cardiac function, left ventricular ejection fraction was measured using 2-dimensional echocardiography. To assess exercise capacity, participants underwent a symptom-limited, graded exercise treadmill test based on a standard Bruce protocol with continuous 12-lead ECG monitoring. Participants were asked to exercise until they experienced dyspnea, symptom-limited fatigue, chest discomfort or ECG changes suggestive of ischemia[34]. Exercise capacity was calculated as the total number of metabolic equivalents (1MET = 3.5 mL of oxygen uptake/kg/min) achieved at peak exercise.

Health behaviors

At baseline and at the 5-year examination, smoking status was determined by self-report. Participants were asked to rate their level of physical activity by answering the following question: “Which of the following statements best describes how physically active you have been during the last month, that is, done activities such as 15-20 minutes of brisk walking, swimming, general conditioning, or recreational sports?” Self-report of physical activity has been shown to be a valid, accurate and

reliable[35-39]. Physical inactivity was defined as not at all or a little active vs. fairly, quite, very or extremely active. Height and weight were measured, and used to calculate body mass index (BMI: weight in kilograms divided by the square of height in meters). Waist and hip circumference were measured to calculate waist-hip ratio. The correlation between BMI and waist and hip circumference was low (baseline: r = .35, p <.0001; year 5: r = .34, p <.0001). Alcohol consumption was assessed with the Audit-C self-report questionnaire[40].

Other patient characteristics

Age, gender, ethnicity, education, and medical history were determined by self-report both at baseline and at the 5-year examination. Participants were asked to bring all of their medication bottles to the study appointment, and all current medications were recorded. Medications were categorized using Epocrates Rx (San Mateo, California) and were defined as a dichotomous variable (yes or no).

Statistical analyses

We compared characteristics of participants across the three depression subgroups using ANOVA for continuous variables and chi-square tests for dichotomous variables. General Linear Models were used to compare mean levels of WBC at baseline and at 5-year follow-up across the three subgroups of participants. We used standardized βs to determine the magnitude of the associations between depressive symptoms (entered as both a continuous and categorical variable) and WBC count. To further evaluate the association between depressive symptoms and WBC count, we used multivariate analysis of variance and created four models which additionally adjusted for (model 1) other patient characteristics associated with depressive symptoms i.e. age, gender, education, race, aspirin and corticosteroid use, history of diabetes, myocardial infarction (MI), congestive heart failure (CHF), and exercise capacity, (model 2) model 1 + log hsCRP, log IL-6, and fibrinogen, (model 3) model 2 + BMI and waist hip ratio, and (model 4) model 3 + physical activity and smoking. BMI and waist hip ratio were analysed in a separate model to investigate the effects of overweight or abdominal fat separately from physical activity and smoking. For analyses of baseline WBC levels, we adjusted for characteristics measured at the baseline exam. For analyses of 5-year WBC levels,

we adjusted for characteristics measured at the 5-year exam. Analyses were performed using SAS 9.2.

Results

Characteristics of participants

Of the 667 participants with stable coronary heart disease who completed both the baseline and 5-year examinations, 443 (66%) reported no significant depressive symptoms (PHQ-9 score ≥ 10) at any of the annual interviews, 86 (13%) had depressive symptoms at one interview only, and 138 participants (21%) had depressive symptoms at 2 or more annual interviews. As compared with the 162 participants who were alive after 5-years of follow-up but did not complete the follow-up examination, the 667 participants who completed the exam were older (mean age 66 ± 10 vs. 64 ± 12; p = 0.04) and had lower baseline depressive symptom scores (mean PHQ-9 scores 4.75 ± 5.26 vs. 6.77 ± 5.84; p < 0.001) as well as lower baseline WBC count (6.36 ± 1.76 vs. 6.95 ± 2.21; p <.001).

Baseline patient characteristics stratified by depression are presented in Table 1. As compared to participants without depressive symptoms, those with depressive symptoms were younger and less likely to be male, white, or high school educated. They were more likely to have a history of diabetes, MI, and CHF. They had worse exercise capacity and higher BMI. Greater depressive symptoms were associated with lower hemoglobin (Hb) levels. Participants with significant depressive symptoms were less likely to use aspirin and more likely to smoke and to be physically inactive. The 3 groups had similar levels of hypertension, left ventricular ejection fraction, cholesterol levels, use of cardio-protective medications, and waist-hip ratio.

Depressive symptoms as a predictor of white blood cell count

Patients who reported significant recurrent depressive symptoms at 2 or more interviews had higher WBC count levels after 5 years of follow-up (p < 0.001) than patients who were not depressed or reported depressive symptoms at 1 interview only. Recurrent depressive symptoms remained significantly associated with 5-year levels of WBC count after adjustment for age, gender, education, race, history of diabetes, MI, CHF, cardiac disease severity, aspirin use, and baseline WBC count

(p = .001) (Table 2). Adjustment for cytokine levels (hsCRP, IL-6 and fibrinogen) attenuated but did not eliminate this association (p = .002) (Table 2). Even after adjusting for health behaviors (BMI, waist to hip ratio, physical activity, and smoking), significant depressive symptoms remained associated with higher WBC count (p = .009) (Table 2). When we analyzed both depressive symptoms and WBC as continuous variables, the average of PHQ scores across the annual assessments predicted subsequent levels of WBC count in both unadjusted and fully adjusted models (Table 3).

White blood cell count as a predictor of subsequent depressive symptoms

We found no evidence that baseline WBC was associated with subsequent depressive symptoms. Baseline WBC count was not significantly different in participants who reported significant depressive symptoms at 2 or more interviews compared to participants without significant depressive symptoms or those who reported significant depressive symptoms at 1 interview only (Table 4).

Discussion

The current study showed that participants with recurrent depressive symptoms had higher WBC levels after 5 years of follow-up than those without or only a single episode of depressive symptoms. These findings persisted after adjustment for demographic characteristics, baseline WBC count, cardiac disease severity, medication use, and health behaviors. In contrast, baseline WBC levels were not predictive of subsequent depressive symptoms. These findings suggest that depressive symptoms are predictive of inflammation, but inflammation does predict depressive symptoms in patients with coronary heart disease.

The potential mechanisms by which depressive symptoms lead to elevations in WBC are unclear. In our previous study, we found that poor health behaviors explained the major part of the prospective relation between depressive symptoms and subsequent elevated levels of inflammatory cytokines[11]. In contrast, the present study demonstrated that depressive symptoms remained strongly predictive of WBC even after adjustment for hsCRP, IL-6, fibrinogen, BMI, waist hip ratio, physical activity and smoking. One possible explanation could be that our findings are a reflection of chronic stress or stress-related physiological dysfunction. The results showed that mainly the recurrent depressive symptoms

Table 1. Characteristics of 667 participants with coronary heart disease, by presence of depressive symptoms during the previous 5 years.

	No depressive symptoms (N=443)		Depressive symptoms at one interview (N=86)		Depressive symptoms at 2 or more interviews (N=138)		
Variable	N or mean	% or SD	N or mean	% or SD	N or mean	% or SD	*p*
Demographic characteristics							
Age (years)	72.8	9.2	68.6	12.0	66.3	11.0	<.01
Male (%)	381	86%	62	72%	106	77%	<.01
White (%)	277	63%	42	49%	79	57%	.05
High school graduate (%)	402	91%	70	81%	112	81%	<.01
Comorbid conditions							
Hypertension (%)	324	74%	65	77%	110	80%	.28
Myocardial infarction (%)	205	47%	53	63%	70	51%	.02
Congestive heart failure (%)	68	16%	17	20%	42	31%	<.01
Diabetes mellitus (%)	122	28%	35	41%	35	26%	.03
Cardiac disease severity and risk factors							
LVEF	0.62	0.11	0.63	0.11	0.60	0.13	.24
Exercise capacity (METS)	6.98	2.98	6.22	3.01	6.32	3.15	.03
LDL cholesterol (mg/dl)	93.7	34.2	98.0	28.6	95.0	32.9	.54
HDL cholesterol (mg/dl)	47.5	14.8	47.3	18.6	45.6	14.3	.47

Non-HDL cholesterol	115.7	38.9	124.0	34.1	120.8	37.0	.10
Hemoglobin (g/DL)	14.03	1.54	13.46	1.72	13.89	1.81	.01
Medication use							
Aspirin (%)	338	76%	58	67%	86	62%	<.01
Beta blocker (%)	300	68%	62	72%	91	66%	.62
RAS inhibitor (%)	298	67%	55	64%	85	62%	.44
Statin (%)	123	28%	20	23%	26	19%	.10
Corticosteroids (%)	14	3%	6	7%	6	4%	.24
NSAID (%)	58	13%	11	13%	23	17%	.54
Cytokine levels							
Log hsCRP	0.34	1.14	0.58	1.30	0.7	1.30	<.01
Log IL-6	1.19	0.66	1.19	0.71	1.38	0.71	.01
Fibrinogen	371.26	78.65	370.25	86.62	390.66	101.98	.06
Health behaviors							
Regular alcohol use (%)	132	30%	19	23%	32	24%	.20
Body mass index (kg/m^2)	28.13	5.03	29.76	6.01	29.6	6.97	<.01
Current smoking (%)	42	10%	13	16%	37	27%	<.01
Physically inactive (%)	140	32%	41	49%	82	60%	<.01
Waist-Hip Ratio	0.97	0.09	0.97	0.09	0.98	0.07	.60

LVEF = left ventricular ejection fraction, LDL = low-density lipoprotein, HDL = high-density lipoprotein, METS = metabolic equivalents (1MET = 3.5 mL of oxygen uptake/kg/min), RAS inhibitor = renin-angiotensin system inhibitor, NSAID = Non-steroidal anti-inflammatory drugs**,** hsCRP = high sensitive C-reactive protein, IL-6 = interleuking 6.

Table 2. Mean ± SE White blood cell count at 5-year follow-up examination, by presence of depressive symptoms during previous 5 years

	Baseline White blood cell count (K/cmm)						
	No depressive symptoms at any interview N=443		Depressive symptoms at one interview N=86		Depressive symptoms at 2 or more interviews N=138		
	Mean	SD/SE*	Mean	SD/SE*	Mean	SD/SE*	*p*
Unadjusted	6.49	1.91	6.54	2.04	7.32	2.49	<.001
Model 1	6.71	0.20	6.69	0.24	7.34	0.23	0.001
Model 2	6.60	0.20	6.57	0.24	7.20	0.22	0.002
Model 3	6.61	0.21	6.57	0.25	7.14	0.23	0.007
Model 4	6.69	0.23	6.64	0.26	7.21	0.25	0.009

Model 1: adjusted for year 5 age, gender, education, race, history of diabetes, myocardial infarction, heart failure, use of aspirin, corticosteroids, exercise capacity, and baseline white blood cell count.

Model 2: adjusted for model 1 variables + year 5 log high sensitive C-reactive protein, log interleukin 6, and fibrinogen.

Model 3: adjusted for model 2 variables + year 5 body mass index and waist to hip ratio.

Model 4:adjusted for model 3 variables + year 5 physical inactivity and smoking.

*Unadjusted values use standard deviation, adjusted values use standard error.

Table 3. Summary Patient Health Questionnaire 9 score (average score from 6 annual assessments) as a predictor of white blood cell count at follow up exam.

	Standardized β	*p* value
Unadjusted	0.17	<.001
Model 1	0.11	0.002
Model 2	0.11	0.001
Model 3	0.10	0.003
Model 4	0.09	0.007

Model 1: adjusted for 5-year age, gender, education, race, history of diabetes, myocardial infarction, heart failure, use of aspirin, corticosteroids, exercise capacity, and baseline white blood cell count.
Model 2: adjusted for model 1 variables+ year 5 log high sensitive C-reactive protein, log interleukin 6, and fibrinogen.
Model 3: adjusted for model 2 variables + year 5 body mass index and waist hip ratio.
Model 4: adjusted for model 3 variables + year 5 physical activity and smoking.

were associated with higher WBC count. It could be hypothesized that these recurrent symptoms of depression are a reflection of chronic stress, which may stimulate hematopoietic stem cells in the bone marrow to produce WBCs[15]. In this cascade, the role of health behaviors might be less important than in the more downstream depression-cytokine relationship. Another possible explanation could be that it in the case of chronic depression or stress, hypercortisolemia is present, as well as reduced vagal activity, both which may lead to higher inflammation, possibly manifested by more WBC[41].

We found that the group of participants reporting depressive symptoms at two or more interviews had the highest levels of WBC count. Two retrospective studies conducted by Liukkonen and colleagues, and by Hamer and colleagues respectively reported that patients with recurrent depressive symptoms had higher levels of inflammation[42, 43] whereas Matthews and colleagues[44] showed that in a group of middle-aged women, those who experienced recurrent depressive symptoms had greater progression of coronary artery calcification. Taken together, these findings

Table 4. Mean ± SE baseline white blood cell count at baseline, by subsequent depressive symptoms.

	Baseline White blood cell count (K/cmm)						
	No depressive symptoms at any interview N=443		Depressive symptoms at one interview N=86		Depressive symptoms at 2 or more interviews N=138		
	Mean	SD/SE*	Mean	SD/SE*	Mean	SD/SE*	*P*
Unadjusted	6.28	1.66	6.40	2.03	6.60	1.90	0.18
Model 1	6.53	0.29	6.44	0.31	6.59	0.32	0.83
Model 2	6.32	0.28	6.27	0.30	6.45	0.31	0.72
Model 3	6.32	0.29	6.27	0.31	6.41	0.32	0.81
Model 4	6.70	0.30	6.64	0.32	6.63	0.32	0.91

Model 1: adjusted for baseline age, gender, education, race, history of diabetes, myocardial infarction, heart failure, use of aspirin, corticosteroids, and exercise capacity.

Model 2: adjusted for model 1 variables + baseline log high sensitive C-reactive protein, log interleukin 6, and fibrinogen.

Model 3: adjusted for model 2 variables + baseline body mass index and waist hip ratio.

Model 4: adjusted for model 3 variables + baseline physical activity and smoking.

*Unadjusted values use standard deviation, adjusted values use standard error.

suggest that recurrent or chronic depressive symptoms may be more strongly associated with inflammation (and adverse health outcomes) than a single episode of depressive symptoms.

The results of this study could be an important step in better understanding the underlying physiological mechanisms of the adverse cardiac effects of depression. Previous studies have shown that WBC is a predictor of new cardiac events or even cardiac death[21]. As reported in this study, WBC count is higher in patients with cardiac disease reporting recurrent significant depressive symptoms. It could be hypothesized that depression might contribute to new cardiac events through a higher WBC count. However, it should be kept in mind that more factors could play a role in the relationship between depression and CHD. For instance, Kop and colleagues[24] found in a healthy sample that autonomic nervous system (ANS) dysfunction and elevated levels of inflammatory cytokines explained a small proportion of the mortality risk associated with depression. We have also found[26] that inflammatory cytokines explain a small part of the association between depressive symptoms and adverse cardiovascular events, but most of this association is explained by poor health behaviors. Future research is needed to provide more insight on the mechanisms underlying the depression-CHD relationship. Repeated assessments of depression, as well as simultaneous assessment of multiple physiological mechanisms and health behaviors, could help better define the joint pathophysiology.

The results of the current study should also be viewed in light of several limitations. The present study determined the complete WBC count, even though there are five different white blood cell types included in the complete count, all having different functions[15]. For example, monocytes can activate cytokine production in the blood or differentiate into macrophages which on their turn activate cytokine production[15]. To elaborate further on this, especially monocytes are associated with atherogenesis[45] and in addition to this, monocyte and neutrophil counts were associated with a history of cardiac disease, whereas lymphocyte, eosinophil, and basophil counts were not[21]. Furthermore, psychological factors, such as emotional support and perceived control have been found to be associated with % monocytes in acute coronary syndrome patients, but not total WBC count[46]. Differentiating between these cells could possibly provide more insight into the relationship between depression, inflammation and cardiac disease. In addition, WBC count was only determined at baseline and at the year 5 follow-up. Furthermore, this study focuses on outpatients with stable coronary heart disease. Our results may therefore not apply to healthy participants or to patients with acute coronary

syndromes. Also, the study population mostly consists of older men, so therefore might not be generalized to either women or younger men. Finally, 20% (162/829) of surviving participants did not complete the 5-year follow up examination. These participants had worse depression scores than those who completed the examination, so including them would probably have strengthened the association between depression and WBC levels.

In conclusion, we found that recurrent depressive symptoms were prospectively associated with subsequent levels of WBC count, independent of inflammatory cytokine levels and health behaviors or baseline WBC. These findings raise the possibility that WBC count is a potential mediator in the relationship between depression and adverse cardiac outcomes.

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PART 2

Depression and inflammation in depressed and non-depressed adults

CHAPTER 5

Association of depressive disorders, depression characteristics and antidepressant medication with inflammation

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Abstract

Background: Growing evidence suggests that immune dysregulation may be involved in depressive disorders, but the exact nature of this association is still unknown and may be restricted to specific subgroups.

Methods: This study examines the association between depressive disorders, depression characteristics and antidepressant medication with inflammation in a large cohort of controls and depressed persons, taking possible sex differences and important confounding factors into account. Persons (18-65 years) with a current (N=1132) or remitted (N=789) depressive disorder according to DSM-IV criteria and healthy controls (N=494) were selected from the Netherlands Study of Depression and Anxiety. Assessments included clinical characteristics (severity, duration, age of onset), use of antidepressant medication and inflammatory markers (C-reactive protein [CRP], interleukin-6 [IL-6], tumor-necrosis factor-alpha [TNF-α]).

Results: After adjustment for sociodemographics, currently depressed men, but not women, had higher levels of CRP (1.33 versus 0.92 mg/l, p<.001, Cohen's d=0.32) and IL-6 (0.88 versus 0.72 pg/ml, p=.01, Cohen's d=0.23) than non-depressed peers. Associations reduced after considering lifestyle and disease indicators - especially body mass index - but remained significant for CRP. After full adjustment, highest inflammation levels were found in depressed men with an older age of depression onset (CRP, TNF-α). Furthermore, inflammation was increased in men using serotonin-norepinephrine reuptake inhibitors (CRP, IL-6) and in men and women using tri- or tetracyclic antidepressants (CRP), but decreased among men using selective serotonin reuptake inhibitors (IL-6).

Conclusion: In conclusion, elevated inflammation was confirmed in depressed men, especially those with a late-onset depression. Specific antidepressants may differ in their effects on inflammation.

Introduction

Depression is a complex heterogeneous disorder, which may need a similarly heterogeneous offer of treatment possibilities. Currently available antidepressant medications largely target monoamine pathways, but treatment of depression is only effective in about a third to a half of patients[1, 2]. Identification of additional pathophysiological pathways involved in depression (subtypes) is needed to guide the development of alternative treatment strategies. Increasing interest has been directed to immune dysregulation in depression. Recently, two meta-analyses have shown that inflammatory marker levels such as C-reactive protein (CRP), interleukin (IL)-6 and tumor-necrosis factor (TNF)-α are increased in depressed persons compared with non-depressed subjects[3, 4].

Although the results of these meta-analyses are promising, the evidence for immune dysregulation in depression is not conclusive. A substantial portion of studies included in these meta-analyses did not adequately adjust for possible confounding factors. The association between depression and inflammation appeared much smaller, although still present, in studies adjusting for body mass index[4]. The effect estimate after a more complete adjustment (including several lifestyle and disease factors) is not entirely clear. Also, most of the larger studies examining depression and inflammation have used depressive symptoms questionnaires instead of assessing psychiatric diagnoses by means of clinical interviews. The former is much more prone to confounding by somatic health as a person can score high on these questionnaires based on having many physical complaints. Furthermore, a large part of previous studies has been conducted within older populations. More studies within younger age samples are therefore needed.

Next to these general limitations, meta-analyses have found very large heterogeneity across studies[3, 4]. It is plausible that immune dysregulation is not generally present in depression, but restricted to particular subgroups of depressed persons. Several factors that could influence the depression-inflammation relationship need further investigation to help delineate the depressed person with immune dysregulation. This is important to give direction to whom new treatment strategies could be targeted.

First, sex differences might exist, but results are thus far inconsistent. Stronger, weaker or similar effects have been found for women compared with men for different inflammatory markers[4, 5]. Inflammation levels fluctuate throughout female life according to hormonal changes due to phase of menstrual cycle, use of

hormonal contraceptives, menopause and use of estrogens[6-8], which might influence the relationship between depression and inflammation.

Second, it is largely unknown whether specific depression characteristics indicate immune dysregulation[3]. In line with a dose-response assumption, more severe and/or more chronic disorders can be hypothesized to show the most inflammation[9, 10]. In addition, late-onset depression has been associated with family history of vascular disease[7] and atherosclerosis[11, 12]. As immune dysregulation is critically involved in vascular disease[13], it can be hypothesized that increased inflammation is specifically present in those with late-onset depressive disorders.

Third, antidepressant medication might influence inflammation levels and this effect might differ across type of medication. As summarized by Miller et al.[14], several studies showed that antidepressant treatment, mainly selective serotonin reuptake inhibitors, was associated with decreases in inflammatory markers. In contrast, recent results of two large studies suggest that use of antidepressants, mainly tricyclic antidepressants, is associated with elevated inflammation levels[15].

To address the issues raised above, the first aim of the present study was to examine the association between diagnosed depressive disorders and inflammatory markers (CRP, IL-6, TNF-α), using a large and relatively young cohort of depressed persons and controls, taking possible sex differences and important confounding factors into account. The second aim was to investigate whether specific depression characteristics (severity, duration, age of onset) could further delineate the depressed person with increased inflammation. Lastly, possible effects of (specific types of) antidepressant medication on inflammation levels were examined.

Subjects and methods

Sample

The Netherlands Study of Depression and Anxiety (NESDA) is an ongoing cohort study designed to investigate the long-term course and consequences of depressive and anxiety disorders. Participants were 18 to 65 years old at baseline assessment in 2004-2007 and were recruited from the community (19%), general practice (54%) and secondary mental health care (27%). A total of 2981 persons were included, consisting of persons with a current or past depressive and/or

anxiety disorder and healthy controls. A detailed description of the NESDA study design and sampling procedures can be found elsewhere[16]. The research protocol was approved by the Ethical Committee of participating universities and after complete description of the study all respondents provided written informed consent.

During the baseline interview, presence of depressive disorder (major depressive disorder, dysthymia) and anxiety disorder (social phobia, generalized anxiety disorder, panic disorder, agoraphobia) was established using the Composite Interview Diagnostic Instrument (CIDI) according to DSM-IV criteria[17]. The CIDI is a highly reliable and valid instrument for assessing depressive and anxiety disorders[18] and was administered by specially trained research staff. In addition, the severity of depression was measured in all participants using the 28-item self-report Inventory of Depressive Symptoms (IDS)[19]. For the present analyses we selected persons with a current (i.e. past 6 months; N=1158) or remitted (lifetime, but not current; N=815) depressive disorder and healthy controls without any lifetime depressive or anxiety disorder and an IDS score below 14 (N=506). Of these 2479 persons, 64 were excluded due to missing information on inflammatory markers, leaving a sample of 2415 persons for the present study. Persons with missing data on inflammation did not differ from included persons in terms of sex, age, years of education and depressive disorder status.

Inflammatory markers

Markers of inflammation were assessed at the baseline NESDA measurement and included CRP, IL-6 and TNF-α. Fasting blood samples of NESDA participants were obtained in the morning around 8 am and kept frozen at -70°C. CRP and IL-6 were assayed at the Clinical Chemistry department of the VU University Medical Center. High-sensitivity plasma levels of CRP were measured in duplicate by an in-house ELISA based on purified protein and polyclonal anti-CRP antibodies (Dako, Glostrup, Denmark). Intra- and inter-assay coefficients of variation were 5% and 10%, respectively. Plasma IL-6 levels were measured in duplicate by a high sensitivity enzyme-linked immunosorbent assay (PeliKine Compact™ ELISA, Sanquin, Amsterdam, The Netherlands). Intra- and inter-assay coefficients of variation were 8% and 12%, respectively. Plasma TNF-α levels were assayed in duplicate at Good Biomarker Science, Leiden, The Netherlands, using a high-sensitivity solid phase ELISA (Quantikine® HS Human TNF-α Immunoassay, R&D systems Inc, Minneapolis,

MN, United States). Intra- and inter-assay coefficients of variation were 10% and 15%, respectively.

Depression characteristics

Next to CIDI depressive disorder diagnosis, depression characteristics included depressive symptoms severity as measured by the IDS, and depressive symptoms duration, using the Life Chart method[20], in which a detailed account of the presence of depressive symptoms during the past four years was assessed. From this, the percent of time patients reported depressive symptoms was computed. Additionally, age of depression onset was derived from the CIDI interview.

Antidepressant medication

Medication use was assessed based on drug container inspection of all drugs used in the past month and classified according to the World Health Organization Anatomical Therapeutic Chemical classification[21]. Antidepressant medication was only considered when taken on a regular basis (at least 50% of the time) and included selective serotonin reuptake inhibitors (SSRI; N06AB), serotonin-norepinephrine reuptake inhibitors (SNRI; N06AX16, N06AX21), tricyclic antidepressants (TCA; N06AA) and tetracyclic antidepressants (TeCA; N06AX03, N06AX05, N06AX11).

Covariates

Sociodemographic characteristics included age, sex, and years of education. As lifestyle characteristics can be associated with both depression and inflammation, smoking status (never, former, current; assessed by self-report), alcohol intake (<1, 1-14 [women] / 1-21 [men], >14 [women] >21 [men] drinks per week; based on general guidelines that are used in health organizations in the Netherlands[22] and as used in other studies[23]), body mass index (BMI; weight in kilograms divided by height in meters squared) and physical activity (measured with the International Physical Activity Questionnaire[24] in MET-minutes [ratio of energy expenditure during activity compared to rest times the number of minutes performing the activity] per week) were assessed. In addition, several disease related covariates were taken into account including presence of cardiovascular disease (assessed by self-report supported by appropriate medication use [see[25] for detailed

description]), presence of diabetes (fasting plasma glucose level ≥7.0 mmol/l or use of anti-diabetic medication [A10]), and the number of other self-reported chronic diseases for which persons received treatment (including lung disease, osteoarthritis or rheumatic disease, cancer, ulcer, intestinal problem, liver disease, epilepsy and thyroid gland disease). As suggested by Howren et al.[4], to control for possible medication effects, we assessed statin use (C10AA, C10B) and use of systemic anti-inflammatory medication (M01A, M01B, A07EB, A07EC). In women, we additionally assessed use of sex hormones (self-reported use of oral contraceptives or ATC code G03), self-reported menstrual cycle phase (menstrual [0-3 days since start last menstruation], follicular [4-13 days], luteal [14-32 days]), and self-reported postmenopausal status (yes/no).

Statistical analyses

All analyses were conducted using SPSS version 15.0 statistical software. Differences in baseline characteristics across sex were tested for statistical significance using chi-square statistics for dichotomous and categorical variables and independent t-tests and Mann-Whitney U tests for normally and non-normally distributed continuous variables. For subsequent analyses, CRP, IL-6 and TNF-α were ln-transformed to normalize distributions and presented back-transformed. Associations between baseline characteristics and inflammatory markers were tested using independent samples t-tests for dichotomous variables, one-way analyses of variance for categorical variables and Pearson's correlations for continuous variables.

Associations between depressive disorders and inflammatory markers were examined using analyses of (co)variance and (adjusted) means across depression groups (no, remitted, current) were presented. To take the effects of important confounding factors into account, three different models were tested: unadjusted, adjusted for sociodemographics (sex, age, education), and additionally adjusted for lifestyle and disease (smoking status, alcohol intake, BMI, physical activity, cardiovascular disease, diabetes, number of other chronic diseases, statins, anti-inflammatory medication). To investigate possible sex differences, we tested for sex-interactions by including a sex*depressive disorder interaction term. When present, analyses were repeated sex-stratified. For significant associations, Cohen's d was calculated in order to assess effect size. To test whether specific depression characteristics were related to elevated inflammation levels, we performed linear regression analyses for each depression characteristic within the sample of persons

with a current depressive disorder. Sex-interactions were again tested and if present, shown sex-stratified. To investigate possible effects of antidepressant medications, adjusted means of inflammation levels across different medication groups were calculated using analyses of covariance and presented sex-stratified in case sex-interaction was present.

Results

Baseline characteristics of the total sample and for men (N=800) and women (N=1615) separately are shown in Table 1. Women were younger, less often smokers, more often non-drinkers, had a lower BMI, less often cardiovascular disease or diabetes, and less often used statins than men. In addition, women had higher levels of CRP, but lower levels of IL-6 and TNF-α than men. In men, older age, less education, smoking, heavy or non-drinking, higher BMI, lower physical activity, cardiovascular disease, diabetes, higher number of other chronic diseases, statin use and use of anti-inflammatory medication were associated with higher levels of at least one of the inflammatory markers (data not shown). In women, similar associations were found, except for smoking, heavy drinking, and physical activity. The Pearson's correlations between inflammatory markers were modest, likely reflecting only partial biological overlap, and somewhat higher in men (CRP-IL-6: r=.40; CRP-TNF-α: r=.22; IL-6-TNF-α: r=.17) than in women (CRP-IL-6: r=.28; CRP-TNF-α: r=.10; IL-6-TNF-α: r=.09).

Table 2 shows (adjusted) mean inflammation levels across depression groups (controls, remitted, current depressive disorder) based on analyses of (co)variance. In the total sample, current depressive disorders were significantly associated with higher levels of CRP and IL-6 in the unadjusted model. After additional adjustment for sociodemographics, lifestyle and disease factors, these associations disappeared. No associations were found for TNF-α. For current depressive disorders, sex-interactions were found for CRP (p-interaction<.001) and IL-6 (p-interaction=.009), but not TNF-α (p-interaction=.99).

In men, after adjustment for sociodemographics, a current depressive disorder was associated with higher CRP levels (1.33 versus 0.92 mg/l, p<.001, Cohen's d=0.32) and IL-6 levels (0.88 versus 0.72 pg/ml, p=.01, Cohen's d=0.23). Even after full adjustment for lifestyle and disease factors, men with a current depressive disorder had higher CRP levels compared with controls (1.29 versus 1.04 mg/l, p=.02, Cohen's d=0.21), and marginally higher IL-6 levels (0.87 versus 0.76 pg/ml, p=.10,

Cohen's d=0.15). No associations with TNF-α were found in men. Overall, BMI weakened the associations most, followed by number of other chronic diseases. Smoking status, alcohol use and physical activity weakened associations slightly further. Adjustment for cardiovascular disease, diabetes, statins, and anti-inflammatory medication hardly affected associations.

In women, depressive disorders were not associated with inflammatory markers before or after adjustment. Although sex hormone use, menstrual cycle phase and postmenopausal status were strongly associated with inflammation levels, additional adjustment for these factors did not change the results for women. Also, there were no significant postmenopausal status*depression interactions in the associations with inflammation levels (all p>.40) suggesting that associations between depression and inflammation appeared absent in both premenopausal (N=1076) and postmenopausal women (N=539).

To investigate whether specific depression characteristics (severity, duration, age of onset) were associated with higher inflammation levels, linear regression analyses were performed within the subgroup of currently depressed persons (N=1132; Table 3). Women with more severe depressive symptoms had higher levels of TNF-α. No associations were found for duration of depressive symptoms. Men with an older age of depression onset had higher levels of CRP and TNF-α than those with a younger age of depression onset.

Lastly, the association between antidepressant medication use and inflammation levels was examined. To incorporate possible differences in depression severity between persons who were or were not using antidepressants, we selected a control group of medication-free depressed persons with a current diagnosis and an IDS score>25 (N=426). In this medication-free reference group the mean IDS score was comparable to the other medication groups. As effects of TCA and TeCA users were comparable, they were grouped together to increase numbers. Sex-interactions in the association between antidepressant medication group (no medication, SSRI, SNRI, TCA/TeCA) and inflammatory markers were found (CRP: p-interaction=.06, IL-6: p-interaction=.02; TNF-α: p-interaction=.90). Therefore, Figure 1 shows adjusted mean inflammation levels comparing antidepressant users with medication-free persons, for men and women separately. Increased levels of CRP were found for men using SNRI (1.98 versus 1.21 mg/l, p=.02, Cohen's d=0.44) or TCA/TeCA (2.05 versus 1.21 mg/l, p=.02, Cohen's d=0.48) as compared to medication-free depressed men. A trend for higher CRP in TCA/TeCA users was also found for women (2.10 versus 1.53 mg/l, p=.08, Cohen's d=0.28). In men only, IL-6

Table 1. Baseline characteristics

	Total sample	Men	Women	p [a]
Sociodemographics				
Age (years), mean (SD)	41.8 (12.9)	43.7 (12.6)	40.9 (12.9)	<.001
Education (years), mean (SD)	12.2 (3.3)	12.0 (3.3)	12.2 (3.3)	.12
Lifestyle & Disease				
Smoking status				.003
Never, %	27.9	23.5	30.1	
Former, %	32.7	34.3	31.9	
Current, %	39.4	42.3	38.0	
Alcohol intake				<.001
< 1 Drink a week, %	32.9	22.9	37.8	
1-14 (women) / 1-21 (men) drinks a week, %	55.9	66.5	50.7	
> 14 (women) / > 21 (men) drinks a week, %	11.2	10.6	11.5	
Body mass index, mean (SD)	25.7 (5.1)	26.3 (4.6)	25.4 (5.3)	<.001
Physical activity (MET-minutes/week), mean	3673 (3026)	3758 (3324)	3630 (2867)	.36
Cardiovascular disease, %	5.6	9.4	3.8	<.001
Diabetes, %	4.9	7.8	3.5	<.001
Number of other chronic diseases, mean (SD)	0.4 (0.7)	0.4 (0.7)	0.4 (0.7)	.11
Statin use, %	6.6	10.9	4.5	<.001
Anti-inflammatory medication use, %	4.2	4.0	4.3	.75

Depression characteristics				
Depressive disorder				.004
Controls, %	20.5	24.1	18.6	
Remitted depressive disorder, %	32.7	29.8	34.1	
Current depressive disorder, %	46.9	46.1	47.2	
Within currently depressed cases (N=1132)				
Severity (IDS score), mean (SD)	32.5 (12.2)	32.9 (12.6)	32.3 (12.1)	.46
Duration (% of time depressed), mean (SD)	38.5 (30.3)	40.9 (31.6)	37.4 (29.6)	.07
Age of depression onset (years), mean (SD)	27.1 (12.5)	29.7 (13.3)	25.9 (11.8)	<.001
Antidepressant use				.13
No antidepressant, %	57.2	57.5	57.1	
SSRI, %	28.8	25.7	30.3	
SNRI, %	7.2	9.5	6.2	
TCA, %	3.8	3.5	3.9	
TeCA, %	2.9	3.8	2.5	
Inflammatory markers				
C-reactive protein (mg/l), median (IQR)	1.22 (0.54-3.02)	1.09 (0.51-2.67)	1.30 (0.56-3.21)	.001
Interleukin-6 (pg/ml), median (IQR)	0.76 (0.50-1.25)	0.81 (0.53-1.36)	0.72 (0.49-1.19)	.003
Tumor necrosis factor-alpha (pg/ml), median	0.80 (0.60-1.10)	0.80 (0.60-1.10)	0.70 (0.60-1.10)	.001

IDS = inventory of depressive symptoms; IQR = interquartile range; SD = standard deviation; SSRI = selective serotonin reuptake inhibitor; SNRI = serotonin-norepinephrine reuptake inhibitor; TCA = tricyclic antidepressant; TeCA = tetracyclic antidepressant. [a] Based on chi-square test for dichotomous and categorical variables and independent samples t-test for continuous variables testing the difference between men and women; for inflammatory markers the Mann-Whitney U test was used.

Table 2. Adjusted mean inflammation levels across depression groups

	Controls			Remitted depressive disorder			Current depressive disorder		
	Mean	(SE)	p	Mean	(SE)	p	Mean	(SE)	p
	CRP, mg/l [3]								
Total sample		N=494			N=789			N=1132	
Unadjusted	1.09	(1.06)	Ref	1.25	(1.04)	.05	1.40	(1.04)	<.001
Adjusted [1]	1.17	(1.06)	Ref	1.24	(1.04)	.38	1.36	(1.04)	.02
Adjusted [2]	1.27	(1.05)	Ref	1.26	(1.04)	.91	1.30	(1.03)	.66
Men		N=193			N=238			N=369	
Unadjusted	0.83	(1.09)	Ref	1.14	(1.08)	.005	1.36	(1.06)	<.001
Adjusted [1]	0.92	(1.08)	Ref	1.09	(1.08)	.13	1.33	(1.06)	<.001
Adjusted [2]	1.04	(1.08)	Ref	1.03	(1.07)	.96	1.29	(1.06)	.02
Women		N=301			N=551			N=763	
Unadjusted	1.29	(1.07)	Ref	1.30	(1.05)	.94	1.42	(1.05)	.29
Adjusted [1]	1.36	(1.07)	Ref	1.34	(1.05)	.82	1.36	(1.05)	.96
Adjusted [2]	1.45	(1.07)	Ref	1.38	(1.05)	.57	1.30	(1.04)	.16
	IL-6, pg/ml [3]								
Total sample		N=494			N=789			N=1132	
Unadjusted	0.72	(1.04)	Ref	0.75	(1.03)	.49	0.80	(1.03)	.04
Adjusted [1]	0.74	(1.04)	Ref	0.74	(1.03)	.88	0.79	(1.03)	.24
Adjusted [2]	0.78	(1.04)	Ref	0.74	(1.03)	.42	0.77	(1.03)	.91
Men		N=193			N=238			N=369	
Unadjusted	0.68	(1.07)	Ref	0.82	(1.06)	.03	0.89	(1.05)	.001
Adjusted [1]	0.72	(1.07)	Ref	0.80	(1.06)	.20	0.88	(1.05)	.01
Adjusted [2]	0.76	(1.07)	Ref	0.78	(1.06)	.72	0.87	(1.05)	.10
Women		N=301			N=551			N=763	
Unadjusted	0.74	(1.06)	Ref	0.71	(1.04)	.55	0.75	(1.04)	.86
Adjusted [1]	0.77	(1.06)	Ref	0.71	(1.04)	.22	0.75	(1.03)	.63
Adjusted [2]	0.80	(1.06)	Ref	0.72	(1.04)	.13	0.73	(1.03)	.14

	TNF-α, pg/ml [3]								
Total sample		N=494			N=789			N=1132	
Unadjusted	0.82	(1.03)	Ref	0.82	(1.02)	.99	0.86	(1.02)	.23
Adjusted [1]	0.84	(1.03)	Ref	0.82	(1.02)	.60	0.85	(1.02)	.59
Adjusted [2]	0.85	(1.03)	Ref	0.82	(1.02)	.43	0.84	(1.02)	.78
Men		N=193			N=238			N=369	
Unadjusted	0.84	(1.04)	Ref	0.84	(1.04)	.90	0.87	(1.03)	.41
Adjusted [1]	0.85	(1.04)	Ref	0.83	(1.04)	.65	0.87	(1.03)	.70
Adjusted [2]	0.88	(1.04)	Ref	0.83	(1.04)	.33	0.86	(1.03)	.69
Women		N=301			N=551			N=763	
Unadjusted	0.81	(1.04)	Ref	0.81	(1.03)	.97	0.85	(1.02)	.34
Adjusted [1]	0.83	(1.04)	Ref	0.81	(1.03)	.76	0.84	(1.02)	.74
Adjusted [2]	0.84	(1.04)	Ref	0.82	(1.03)	.70	0.83	(1.02)	.90

CRP = C-reactive protein; IL-6 = Interleukin-6; TNF-α = Tumor necrosis factor-alpha.

Based on analyses of (co)variance; [1] adjusted for (sex,) age and education; [2] additionally adjusted for smoking status, alcohol intake, body mass index, physical activity, cardiovascular disease, diabetes, number of other chronic diseases, statins and anti-inflammatory medication. [3] To normalize distributions CRP, IL-6, and TNF-α were ln-transformed; for interpretation purposes presented means were back-transformed.

Table 3. Association[1] of depression characteristics with inflammatory markers in persons with current depressive disorders (N=1132)

	CRP [2]		IL-6 [2]		TNF-α [2]	
Depression characteristic	B	P	β	p	B	P
Severity (IDS score)	-.020	.47	.038	.22	.040	.20
Men					-.048	.36
Women					.085	.02
Duration of depressive symptoms	-.005	.87	-.017	.58	.012	.70
Age of depression onset	.064	.04	-.002	.95	.043	.22
Men	.140	.003			.149	.004
Women	.016	.67			-.025	.55

CRP = C-reactive protein; IL-6 = interleukin-6; TNF-α = tumor necrosis factor-alpha; IDS = inventory of depressive symptoms.

[1] Based on linear regression analyses adjusted for (sex,) age, education, smoking status, alcohol intake, body mass index, physical activity, cardiovascular disease, diabetes, number of other chronic diseases, statins, anti-inflammatory medication; sex-specific associations are shown in case p sex-interaction ≤.05.

[2] CRP, IL-6 and TNF-α were ln-transformed to normalize distributions.

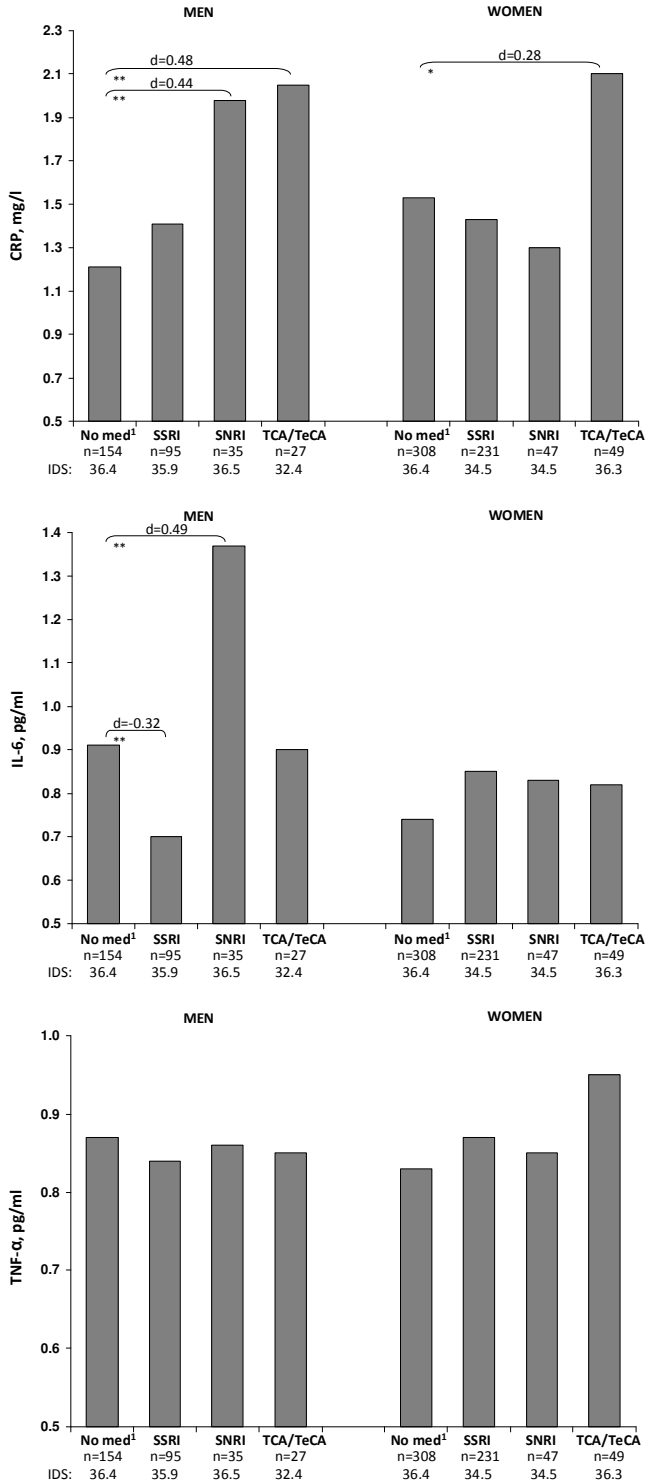
Figure 1. Adjusted mean inflammatory levels across medication groups and sex

based on ANCOVA adjusted for age, education, smoking status, alcohol intake, body mass index, physical activity, cardiovascular disease, diabetes, number of other chronic diseases, statins and anti-inflammatory medication; to normalize distributions CRP, IL-6 and TNF-α were ln-transformed, for interpretation purposes presented means were back-transformed.

IDS = inventory of depressive symptoms score; CRP = C-reactive protein; IL-6 = Interleukin-6; TNF-α = Tumor necrosis factor-alpha; SSRI = selective serotonin reuptake inhibitor; SNRI = serotonin-norepinephrine reuptake inhibitor; TCA = tricyclic antidepressant; TeCA = tetracyclic antidepressant.

[1] Current depressive disorder and IDS ≥ 25 without medication use (= reference); only significant differences from reference are shown: * p < .10; ** p < .05.

Figure 1. Adjusted mean inflammatory levels across medication groups and sex



levels were lower in SSRI users (0.70 versus 0.91 pg/ml, p=.02, in SNRI users (1.37 versus 0.91 pg/ml, p=.01, levels were lower Cohen's d=0.49) compared to medication-free depressed men. No associations were found for TNF-α. For comparison, inflammation levels in persons with cardiovascular disease were 1.84 mg/l for CRP and 1.06 pg/ml for IL-6, suggesting that the putative effects of antidepressant medication are at least of similar magnitude as having cardiovascular disease. Because antidepressant medication effects were found, we checked whether these influenced the findings from Tables 2 & 3, but results remained similar after additional adjustment for antidepressant medication group.

Discussion

The present study examined the association between depressive disorders, depression characteristics, and antidepressant medication with inflammation in a large cohort of depressed persons and controls. After taking a large set of possibly confounding factors into account, it was found that men with current depressive disorders had higher levels of CRP, and marginally higher levels of IL-6, but not of TNF-α. No overall associations were found in women. Increased inflammation was in particular found in depressed men with an older age of depression onset (CRP, TNF-α). Users of SNRI (men only), TCA, and TeCA had increased levels of CRP and IL-6, whereas men using SSRI had significantly lower levels of IL-6.

Our results confirm previous findings of immune dysregulation in depressed persons[3,4]. In men, effect sizes for current depression were small to moderate for both CRP and IL-6. These effect sizes are comparable to those reported by the meta-analysis of Howren[4], in which stronger effects were found for studies using clinical interviews compared with studies using self-report questionnaires. Compared with symptom questionnaires, a depressive disorder diagnosis is less confounded by somatic health problems. Together with the fact that our results were elaborately adjusted for possibly confounding factors, our findings suggest a true relationship between depression and inflammation in men.

No clear associations between depression and inflammation were found in women. This is in line with results from another large and relatively young cohort study in which history of depression was associated with CRP levels in men, but not women[5]. Hormonal changes throughout female life due to phase of menstrual cycle, use of hormonal contraceptives, menopause and use of estrogens greatly impact on inflammation levels[6-8], which could confound a clear association

between depression and inflammation. However, in our study, associations between depression and inflammation appeared absent in both pre- and postmenopausal women and adjusting for several hormonal factors did not change our findings. Another explanation might be that in women, psychosocial factors play a larger role in depression and therefore override the effects of biological factors. For instance, insufficient social support and stressful life events have been found to pose a greater risk for depression among women compared to men[26, 27]. Results of increased inflammation were particularly present in men with late-onset depression. In contrast, characteristics that are more often associated with an early age of onset such as higher severity and longer duration were not consistently associated with increased inflammation. Interesting to note is that women in our study also had an earlier age of depression onset compared to men. A distinct etiology in late-onset versus early-onset depression was also found by Kendler et al.[28]. This study showed that depression with an early age of onset was associated with a family history of depression, while late-onset depression was associated with a family history of vascular disease. Also, subclinical vascular dysregulations, such as atherosclerosis, have been found to relate to late-onset depression[11,12]. These findings are in line with the vascular depression hypothesis which suggests that vascular damage in the brain predisposes to late-onset depression[29]. As immune dysregulation is critically involved in vascular disease[13], this vascular damage could be the result of increased inflammation.

Instead of inflammatory or vascular, the true etiology of depression in this subgroup of men with late-onset depression might have a metabolic nature. Several studies have confirmed an association between CRP, IL-6 and TNF-α with the metabolic syndrome and visceral fat depots release cytokines[30]. The metabolic syndrome and in particular visceral fat have been bidirectionally associated with depression in late life[31, 32], specifically in men. Men possess higher amounts of visceral fat compared to women and are therefore more likely to experience related inflammation and depression. Involvement of metabolic processes is further supported by our finding that the association between depression and inflammation in particular decreased after adjustment for BMI.

Several biological mechanisms could further explain the relationship between depression and inflammation. Depression has been associated with dysregulation of important stress systems of the human body, i.e. the hypothalamus-pituitary-adrenal (HPA)-axis[33] and the autonomic nervous system[34]. Although the HPA-axis in normal situations should temper inflammatory reactions, prolonged hyperactivity of the HPA-axis could result in blunted anti-inflammatory responses to

glucocorticoids resulting in increased inflammation[35, 36]. In addition, both decreased parasympathetic[37] as well as increased sympathetic nervous system activity[38] have been associated with increased inflammation. Furthermore, pro-inflammatory cytokines might inhibit hippocampal neurogenesis[39] which could lead to a reduced hippocampal volume[40] which is also seen in depression[41]. Also, several inflammatory markers have been shown to promote indoleamine-2,3-dioxygenase activation[42], which catalyzes tryptophan, the precursor of serotonin, to kynurenine, thereby indirectly reducing the availability of serotonin[43]. Lastly, specific genes might underlie both increased inflammation and depression, as several inflammation-related genes have been associated with susceptibility to major depression[44].

Our study also shows that inflammation levels differ across persons using different types of antidepressant medication. Highest inflammation levels were found in men using SNRI, TCA or TeCA, while IL-6 levels in men using SSRI were lower compared to medication-free depressed men. Interesting to note is that the first three classes of medication have a combined serotonergic/noradrenergic effect, while SSRI act purely serotonergic. Earlier studies found decreases in inflammatory marker levels after antidepressant treatment, mainly SSRI (see[14] for an overview), while two recent large studies found that use of antidepressants, mainly TCA, was associated with elevated inflammation levels[15]. Possibly, noradrenergic effects are driving increased inflammation mechanisms. Noradrenaline is part of the human stress response and has been suggested to potentiate cytokine production[45]. Use of SNRI, TCA and TeCA has also been observed to disturb functioning of autonomic nervous system[46], blood pressure[47] and the metabolic syndrome[48]. Although it is possible that persons using SNRI, TCA or TeCA are in other ways different from SSRI users and medication-free depressed persons, we constructed our groups in such a way that depression severity levels were very comparable. In addition, we adjusted our analyses for a large set of covariates and have therefore taken possible differences in lifestyle or disease factors into account. On the other hand, evidence suggests that increased inflammatory activity prior to treatment predicts non-response[49, 50]. Possibly, persons with elevated inflammation did not respond to SSRI and were therefore prescribed SNRI, TCA or TeCA.

Associations found in this study were not always consistent across all inflammatory markers. Correlations between inflammatory markers were only modest and were highest between CRP and IL-6. Regulation of the immune system is rather complex and involves many different inflammatory mechanisms. Most consistent findings were found for CRP, which is a very general marker of inflammation. IL-6 and TNF-

α, on the other hand, only tap part of the immune system. This seems to suggest that inflammation is indeed involved in depression, but it is still unclear which part of the immune system is most critically involved. Nonetheless, expected associations with covariates (e.g. age, smoking, alcohol use, BMI, physical activity, somatic disease [medication]) were found for all inflammatory markers.

What do the findings of our study implicate with regard to treatment of depression? Considering the heterogeneity of depression and the fact that current treatment of depression is only effective in about a third to a half of patients[1, 2], this indicates that new treatments are needed for specific subgroups. Our finding of increased inflammation in men with late-onset depression, together with previous findings of high inflammation indicating non-response to antidepressants[49,50], might suggest that this specific subgroup could benefit from alternative treatments, with anti-inflammatory medication being a likely candidate. Preliminary evidence from studies among patients treated with anti-inflammatory agents for other indications suggests that these agents may have beneficial effects on mood[51]. One study found positive effects on mood in medically healthy, major depressed patients[52]. On the other hand we found, like others[14], that SSRI might have a beneficial effect on inflammation, suggesting that SSRI could be effective in depressed patients with immune dysregulation through this anti-inflammatory effect. Furthermore, behavioral interventions, such as exercise, have been shown to normalize immune and metabolic dysregulation[53], as well as to improve depressive symptoms to some degree[54], and might therefore be an indicated treatment for a immune/metabolic depression subgroup. At this moment, these considerations for treatment implications are still very speculative. Follow-up (longitudinal) research should confirm and further delineate an inflammatory (or metabolic) depression subtype, taking into account age, sex, depression characteristics and course. Experimental studies are needed to examine and compare the effects of different types of currently available antidepressants, anti-inflammatory medication and behavioral (exercise) interventions on both immune/metabolic parameters and depression.

Our study has some important strengths such as a large sample size, assessment of multiple inflammatory markers, clinical diagnoses of depression, adequate adjustment for potential confounders, and the ability to examine the role of depression characteristics and antidepressant medication. However, some limitations need to be acknowledged. As our data are cross-sectional, we cannot make any inferences about the direction of the association. Longitudinal studies are needed to investigate whether immune dysregulation is a precursor or the

result of depression (treatment), or whether this relationship is bidirectional. The few available prospective studies have shown contradicting results[9,55,56]. Further, like most other studies, we assessed circulating levels of inflammatory markers, which show a high degree of intra-individual variation which could explain the rather modest overall associations between depression and inflammation in our study.

In conclusion, our study suggests that immune dysregulation plays a role in a subgroup of depressed persons, in particular in men with a late onset depression. Treatment trials should further examine the differential effects of different types of antidepressants on inflammation. Whether a specific treatment strategy (SSRI, anti-inflammatory drugs, exercise) is indicated for a subgroup of late-onset depressed patients with immune dysregulation needs to be further investigated using longitudinal and experimental study designs.

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CHAPTER 6

Differential association of somatic and cognitive symptoms of depression and anxiety with inflammation.

Findings from the Netherlands Study of Depression and Anxiety

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Abstract

Background: Depression and anxiety have been suggested to be associated with a systemic inflammation upregulation. However, results are not always consistent, which may be due to symptom heterogeneity of depression and anxiety disorders as some indications have been found that associations with inflammation are mainly driven by somatic symptoms of depression and anxiety. We therefore set out to evaluate the differential association of somatic and cognitive symptoms of depression and anxiety with inflammation.

Methods: We evaluated baseline data from 2799 participants from the Netherlands Study on Depression and Anxiety (NESDA), an ongoing prospective cohort study. We used the Inventory of Depressive Symptomatology and the Beck Anxiety Inventory to assess depressive symptoms and anxiety symptoms. For both scales somatic and cognitive symptoms scales were calculated. Blood samples were collected to determine high sensitive C-Reactive Protein (hsCRP), interleukin 6 (IL-6) and Tumor Necrosis Factor-α (TNF-α). We used linear regression to analyze the association between depressive and anxiety symptoms and inflammation.

Results: After adjustment for sociodemographic and health indicators, depressive symptoms were associated with higher levels of hsCRP, IL-6 and TNF-α. This association was mainly driven by somatic symptoms and not cognitive symptoms. For anxiety, somatic symptoms were associated with higher levels of CRP, IL-6 and TNF- α, whereas cognitive symptoms were only associated with hsCRP as well (in men only). Lifestyle factors explained most of all significant associations.

Conclusions: Especially through their somatic symptoms, depression and anxiety are associated with inflammation. However, this association was mostly mediated through unhealthy lifestyles among depressed and anxious individuals.

Introduction

Depression and anxiety have been found to be prognostically associated with various somatic conditions, including cardiac disease[1, 2], diabetes[3] and obesity[4]. Low grade inflammation has been proposed as one of the physiological links between both depression and anxiety and adverse somatic outcomes[3, 5]. In the last decade a substantial amount of research has been published on the depression-inflammation relationship, in healthy[6] and cardiac populations[5]. Most of these studies concern cross-sectional research, although some prospective studies have been published[7-10].

Despite the substantial amount of research published on the depression-inflammation link , results are still conflicting[5], with some studies reporting positive associations[11, 12] and others reporting negative findings[13, 14]. Stewart and colleagues[8] found that depressive symptoms predicted an upregulation of interleukin (IL) 6 after a 6-year follow-up in an otherwise healthy sample, but inflammation did not predict depressive symptoms after 6 years. Another study reported that recurrent depressive symptoms were associated with subsequent inflammation, although this association was largely explained by health behaviors[9]. In contrast, Gimeno and colleagues[10] found that after 11 years of follow-up, C-reactive Protein (CRP) and IL-6 were predictive of cognitive symptoms of depression, but not vice versa. It is obvious from the preceding that there is still considerable debate on whether or not depression and inflammation are associated and which factors contribute to this relationship.

A possible explanation for inconsistencies in the depression and inflammation link could be that most studies report only on depression as a whole, whereas it might be more suitable to pay attention to individual depressive symptoms or dimension scores in relation to inflammation[15]. Based on the sickness behavior theory[16], which argues that depressive-like symptoms such as fatigue, sleeping problems, anorexia and motor slowing tend to be more present in the case of upregulated inflammation levels, one could expect that possible associations between depression and inflammation are being missed when taking depression as a whole into account. It could thus be hypothesized that somatic symptoms show a stronger association with inflammation than cognitive symptoms, and this should to be taken into account when investigating the depression-inflammation relationship.

In the case of anxiety, less research is conducted on the associations with inflammation. However, there is some evidence suggesting that anxiety is

associated with inflammation[17-19]. As with depression, anxiety also consists of somatic and cognitive symptoms. One study found that in women somatic symptoms of anxiety were associated with an increased CHD risk, whereas more psychological symptoms of anxiety were not[20].

An additional possible explanation for prior conflicting results of studies examining the link between anxiety/depression and inflammation could be that possible mediating effects of for instance health behaviors such as smoking, physical activity, alcohol consumption and overweight. These factors are not always included in multivariate analyses, even though there is considerable evidence that lifestyle factors are associated with both depressive[4, 21, 22] and anxiety symptoms[23, 24] as well as inflammation[25-27]. Finally, as the majority of studies has a rather small sample size (N< 100), reported effect sizes can be masked. In order to detect true significant associations and to be able to adjust for important confounders or mediators, one needs a sufficient number of participants.

We previously found in a large sample of participants that depression diagnosis was associated with immune dysregulation in men with a late onset depression[28], but we did not distinguish in symptom dimensions of depression. We therefore set out to conduct a study in this same sample in which we will thoroughly investigate the relationship between symptoms profiles of depression and anxiety with inflammation. We hypothesize that 1) mainly the somatic symptoms of depression and anxiety are associated with inflammation, and 2) that the association between (somatic) depressive and anxiety symptoms and inflammation will be partly explained by health behaviors such as BMI, smoking, physical inactivity and alcohol consumption.

Methods

Design and participants

NESDA is an ongoing multi-center cohort study on the course of depressive and anxiety disorders in the adult (18-65 years) population. A total of 2981 participants were recruited from the community (n=564:19%), primary care (n=1610: 54%) and specialized mental health care (n=807: 27%) including controls and persons with a current or past depressive and/or anxiety disorder for the baseline assessment from 2004-2007. Exclusion criteria were a primary clinical diagnosis of a psychiatric disorder like psychotic disorder, obsessive compulsive disorder, bipolar disorder or

severe addiction disorder and not being fluent in Dutch. A detailed description of the NESDA study design and sampling procedures can be found elsewhere[29]. Participants who had missing data on either CRP, IL-6, or TNF-α were excluded from the analyses (n = 63). Additionally, participants who had not returned the questionnaire or had too many missing values on the Inventory of Depressive Symptomatology (IDS) or Beck Anxiety Inventory (BAI) (n = 39) were also excluded. This resulted in a total sample of 2861 participants. Excluded participants were on average more often male (43%), current smokers (50%) and more often used 1-14 glasses of alcohol a week (64%). Their scores on the IDS and Beck Anxiety Inventory BAI were on average slightly higher. Inflammation levels were on average not different.

Depressive and anxiety symptoms

Depressive symptoms - The 30-item IDS self-report version was administered[30]. The IDS assesses the DSM-IV criterion symptom domains for major depressive disorder, and in addition commonly associated symptoms (e.g. anxiety, irritability) and symptoms relevant to melancholic and atypical features. The questionnaire consists of 30 items, each with four answer options (coded 0 through 3). The questionnaire uses a 7-day timeframe for assessing symptom severity. The psychometric properties of the IDS have shown to be acceptable; high correlations were found between the IDS scores on the Hamilton Depression Rating Scale and the Beck Depression Inventory[31].

Principal Component Analysis performed by Wardenaar and colleagues[32] on the IDS revealed three dimensions, a mood cognition dimension, an anxiety arousal dimension and a sleep dimension. However, none of these subscales represent a pure somatic or cognitive symptoms scale as based on the symptoms from the DSM-IV. (somatic: weight loss or gain, insomnia or hypersomnia, psychomotor agitation or retardation, and loss of energy of feeling tired; cognitive: anhedonia, feeling depressed, feelings of worthlessness, concentration problems, and recurrent thoughts of death). The anxiety arousal dimension encompasses somatic symptoms such as psychomotor agitation and psychomotor slowing. However, it also contains somatic complaints, gastrointestinal complaints and panic/phobic symptoms[32], which are not a part of sickness behavior. We therefore created a pure somatic and a pure cognitive symptom scale based on DSM-IV criteria and previous research on somatic and cognitive depression symptoms[33, 34] (Table 1). Both the somatic and the cognitive subscale consisted of 10 items derived from the

Table 1. Overview of the somatic and cognitive symptoms of depression (IDS) and anxiety (BAI) as measured with the items of the Inventory of Depressive Symptoms

Depressive symptoms (IDS)		
Somatic symptoms		Cognitive symptoms
Falling asleep	Merged to sleep variable	Feeling sad
Sleep during the night		Feeling irritable
Waking up to early		The quality of your mood
Sleeping too much		Concentration / decision making
Decreased or increased appetite		View of myself
Decreased or increased weight		Thoughts of death or suicide
Energy level		General interest
Feeling slowed down		Capacity for pleasure or enjoyment (excluding sex)
Feeling restless		Interest in sex
Leaden paralysis / physical		Interpersonal sensitivity

Anxiety symptoms (BAI)	
Somatic symptoms	Cognitive symptoms
Numbness or tingling	Unable to relax
Feeling hot	Fear of worst happening
Wobbliness in legs	Terrified or afraid
Dizzy or light-headed	Nervous
Heart pounding/racing	Fear of losing control
Unsteady	Fear of dying
Feeling of choking	Scared
Hands trembling	
Shaky/unsteady	
Difficulty in breathing	
Indigestion	
Faint/light-headed	
Face flushed	
Hot/cold sweats	

IDS = Inventory of Depressive Symptomatology, BAI = Beck Anxiety Inventory

IDS (Table 1). Because the sleep symptoms were over represented (4 items), we created a variable combining the four sleep items by taking the mean score of all four items. This resulted in Cronbach's α = 0.69 for the somatic symptom dimension and Cronbach's α = 0.89 for the cognitive symptom dimension.

Anxiety symptoms – We used the 21-item BAI[35] to measure symptoms of generalized anxiety and panic symptoms. Respondents are asked to rate how much they have been bothered by each symptom over the past week on a 4-point scale, ranging from 0 (not at all) to 3 (severely, I could barely stand it). The BAI is scored by summing the ratings for all of the 21 symptoms to obtain a total score that can range from 0 to 63. The internal and test-retest reliability and validity of the BAI are well-established[36, 37].

In order to differentiate between somatic and cognitive symptoms of anxiety, previous research has shown that factorial validity analysis revealed that the BAI consists of two subscales accounting for 84% of the variance i.e. a somatic subscale and a subjective – or cognitive - subscale[38]. Factor one consists of 14 items and forms a somatic scale (Cronbach's alpha = .90) (Table 1). Factor two consists of 7 items and forms a cognitive scale (Cronbach's alpha = .88) (Table 1). These two subscales still discriminate adequately between patients with and without an anxiety disorder[38].

Inflammatory markers

As described before[28] markers of inflammation were assessed at the baseline NESDA measurement and include interleukin-6 (IL-6), high sensitive C-Reactive Protein (CRP) and Tumor Necrosis Factor (TNF)-α. IL-6 is a pro-inflammatory cytokine secreted by activated macrophages and CRP is a non-specific acute phase protein synthesized in the liver in response to amongst others stimulation from IL-6. TNF-α is the prototypic ligand of the TNF superfamily and plays a central role in inflammation. After an overnight fast, 50 ml blood was drawn which was immediately transferred to a local laboratory and kept frozen at -80°C. Plasma IL-6 levels were measured in duplicate by a high sensitivity enzyme-linked immunosorbent assay (PeliKine Compact™ ELISA, Sanquin, Amsterdam). The IL-6 assay was standardized against a recombinant human IL-6 standard. The lower detection limit of IL-6 is 0.35 pg/ml and the sensitivity 0.10 pg/ml. Intra- and inter-assay coefficients of variation were 8% and 12%, respectively. High-sensitivity plasma levels of CRP were measured in duplicate by an in-house ELISA based on

purified protein and polyclonal anti-CRP antibodies (Dako, Glostrup, Denmark). The CRP assay was standardized against the CRM 470 reference agent. The lower detection limit of CRP is 0.1 mg/l and the sensitivity is 0.05 mg/l. Intra- and inter-assay coefficients of variation were 5% and 10%, respectively. Plasma TNF-α levels were assayed in duplicate at Good Biomarker Science, Leiden, The Netherlands, using a high-sensitivity solid phase ELISA (Quantikine® HS Human TNF- α Immunoassay, R&D systems Inc, Minneapolis, MN, United States). The TNF-α assay was calibrated against a highly purified E. coli-expressed recombinant human TNF-α. The lower detection limit of TNF-α is 0.10 pg/ml and the sensitivity 0.11 pg/ml. Intra- and inter-assay coefficients of variation were 10% and 15%, respectively. We log transformed IL-6, hsCRP, and TNF-α, because of non-normality which resulted in normal distributed variables.

Covariates

Sociodemographic factors included age, sex, and years of education. In order to ascertain the presence of cardiovascular disease (CVD), self-reports and medication use were used (based on drug container inspection and World Health Organization Anatomic Therapeutic Chemical (ATC) coding; see Vogelzangs and colleagues for a detailed description[39]). Presence of diabetes was based on fasting plasma glucose level≥7.0 mmol/l or use of anti-diabetic medication (ATC code A10).

Participants were asked to bring the containers of the medication used during the month prior to the interview, so the research assistant could copy medicine names. We used the ATC classification[40] to classify frequently used (>50% of all days in past month) medication (Table 2). For antidepressant medication selective serotonin reuptake inhibitors (SSRI; N06AB), serotonin–norepinephrine reuptake inhibitors (SNRI; SNRI; N06AX16 and N06AX21), tricyclic antidepressants (TCA; N06AA), and tetracyclic antidepressants (TeCA; N06AX03, N06AX05 and N06AX11) were classified. Furthermore, we included use of systemic anti-inflammatory medication (M01A, M01B, A07EB, and A07EC) and statin (C10AA, C10B).

Health behaviors were considered as covariates, because they have been linked to both psychopathology and inflammation. Body Mass Index (BMI) was determined as measured weight in kilograms divided by the square of the measured height in meters. Alcohol use was measured with the Alcohol Use Disorders Identification Test[41, 42] and defined as < 1 glass per week, 1-14 glasses per week and > 14 glasses per week, and smoking status was categorized as nonsmoker, former, and current

smoker. Physical activity was assessed using the International Physical Activity Questionnaire[43] and defined as total MET-minutes per week (ratio of energy expenditure during activity compared to rest times the number of minutes performing the activity).

Statistical analyses

Characteristics of the participants compared across men and women using independent t-tests for continuous variables, chi-square statistics and ANOVA's for dichotomous and categorical variables, and Mann-Whitney U for the inflammatory markers.

Linear regression analyses were used to assess the association of depressive and anxiety symptom scores, as well as the association of somatic and cognitive symptoms scores separately with IL-6, CRP and TNF-α. Depressive and anxiety symptoms were considered as predictors and levels of IL-6, CRP, and TNF-α as outcome variables. First a demographics and health model was evaluated, adjusting for age, sex, education level, anti-inflammatory medication and statin use, and presence of CVD and diabetes were taken into account as covariates. Secondly, when significant correlations between inflammatory markers and specific antidepressant medication were present, analyses were further adjusted for those antidepressant medications. We chose to restrict analyses to these medications, because adjusting for all antidepressant medication possibly leads to overadjusting by adjusting for those participants with the most severe depressive and anxiety symptoms. CRP was significantly negatively correlated with SSRI, and positively correlated with TCA, and TeCA use ($p<.05$). TNF-α was positively correlated with TCA ($P<.01$). There were no significant correlations between IL-6 and antidepressant medication use. Because of possible mediating effects of lifestyle factors[44] smoking, alcohol consumption, BMI, and physical activity were added in a final to the previous models.

Since Vogelzangs and colleagues found sex differences in the association between depression and inflammation[28] we tested for the presence of interaction effects for sex and included the following interaction terms for sex (sex*ids, sex*ids somatic, sex*ids cognitive, sex*bai, sex*bai somatic, sex*bai cognitive). When the interaction effect was found significant, regression analyses were repeated stratified for gender.

Results

Baseline characteristics

Table 2 shows the descriptive characteristics of the total study sample (N = 2861) of which 950 were male (33.2%). Men were significantly older than women (p <.01), were less often nonsmoker (p <.01), less often used < 1 glass per week (p < .01), and had higher levels of BMI (p <.01). Furthermore, cardiovascular disease (p <.01) and diabetes (p <.01) were more present in men and men used statins (p <.01) more often than women. Women had higher total BAI scores (p <.01). Men had higher IDS somatic symptoms scores than women (p <.01), whereas women had higher total BAI (p <.01), BAI somatic (p <.01), and BAI cognitive scores (p <.01). Finally, women had higher median levels of hsCRP (p < .01), IL-6 (p <.01) and TNF-α (p <.01) (Table 2).

Symptoms of depression and inflammation

Significant sex interactions were found with IDS total depressive symptoms in predicting CRP (p = .02), which lead us to stratify the concerning analyses for sex. Considering somatic symptoms and cognitive symptoms of depression there were no significant interactions with sex for any of the markers (all p values for sex interactions>.06).

In line with our previous findings[28], demographic and health adjusted regression analyses revealed a positive significant association between total IDS score and CRP (men only: β = .104, p <.01), IL-6 (β = .046, p = .02) and TNF-α (β = .039, p = .04). Somatic symptoms, but not cognitive symptoms, of depression were significantly associated with CRP (β = .078, p <.01), IL-6 (β=.054, p = <.01), and TNF-α (β= .045, p = .02) in the demographics and health model. Additional adjustment for those specific antidepressant use that showed associations with inflammatory markers, only had an effect on the association between total IDS score and TNF-α (adjusted for TCA)(p>.07). Final additional adjustment for lifestyle factors made the remaining associations non-significant for all three markers (p>.12) (Table 3). In the lifestyle adjusted analyses, BMI had the largest reducing effect on the depressive symptoms and inflammation association, followed by an inverse effect of moderate alcohol consumption, and former smoking (CRP) or current smoking (TNF-α).

Symptoms of anxiety and inflammation

Significant interactions were found for sex and total symptoms of anxiety, somatic symptoms of anxiety and cognitive symptoms of anxiety when CRP was analyzed (p<.05), therefore these results are presented stratified for sex.

In men, but not in women, total BAI score was significantly associated with higher levels of CRP in the demographics and health model (β = .106, p =. <01). Overall symptoms of anxiety were not associated with IL-6 (β = .034, p = .07), and TNF-α (β = .035, p = .07). However, in a similar model, somatic symptoms of anxiety were significantly associated with CRP (men only: β = .116, p <.01), IL-6 (β = .050, p = <.01), and TNF-α (β = .038, p = .05) (Table 4). Cognitive symptoms of anxiety and CRP were associated in men only (β = .073, p = .02). Additional adjustments for antidepressant use diminished the association between somatic symptoms and followed by an inverse effect of moderate alcohol intake and former smoking. TNF-α (adjusted for TCA), and cognitive symptoms and CRP (adjusted for SSRI, TCA, and TeCA). Finally, adjusting for lifestyle factors resulted in diminished, non-significant associations for CRP and somatic and total symptoms of anxiety (p>14). From the lifestyle factors, BMI had the largest diminishing effect on all associations,

Discussion

This study shows that higher depressive symptoms were associated with increased inflammatory levels of CRP, IL-6, and TNF-α, but this was mainly driven by the somatic – and not the cognitive – symptoms of depression. This supports the hypothesis that somatic symptoms and cognitive symptoms of depression are differently associated with inflammation. Regarding symptoms of anxiety, total, somatic and cognitive symptoms were all similarly associated with higher CRP (men only). IL-6 and TNF-α levels were only associated with somatic symptoms of anxiety. For all significant associations, health behaviors played an important role, with BMI explaining most of the relationship.

The results from this study support the hypothesis that somatic and cognitive symptoms are differently associated with inflammation suggesting that the association between depression and inflammation is mainly driven by somatic symptoms. This is in concordance with some previous findings. Elovainio and colleagues found that somatic symptoms were more strongly associated with CRP in men than in women, even after full adjustment for covariates[15]. In contrast to

Table 2. Descriptives for the total sample, and for men and women seperately

	Total		Men		Women		
	N = 2861		N = 950		N = 1911		
	N or mean	% or SD	N or mean	% or SD	N or mean	% or SD	*p**
Demographics + health							
Age	41.9	13.0	43.6	12.8	41.1	13.1	<.001
Education (years)	12.2	3.3	12.2	3.3	12.2	3.3	.685
Health							
Diabetes	138	5%	75	8%	63	3%	<.001
Cardiovasculair disease	163	6%	91	10%	72	4%	<.001
Medication use							
Anti-inflammatory drugs	129	5%	38	4%	91	5%	.355
Statins	191	7%	99	10%	92	5%	<.001
Antidepressants	697	24%	224	24%	473	25%	.491
SSRI	481	17%	150	16%	331	17%	.302
SNRI	110	4%	40	4%	70	4%	.473
TCA	77	3%	21	2%	56	3%	.263
TeCA	50	2%	24	3%	26	1%	.025
Lifestyle factors							
Smoking							
No	802	28%	229	24%	573	30%	.003
Former	970	34%	331	35%	639	33%	
Current	1089	38%	390	41%	699	37%	
Alcohol intake							
< 1 glass per week	923	32%	207	22%	716	37%	<.001
1-14 glasses per week	1608	56%	635	67%	973	51%	
> 14 glasses per week	330	12%	108	11%	222	12%	
BMI	25.6	4.9	26.2	4.5	25.3	5.2	<.001
Physical activity (in MET-minutes per week)	3678	3028	3728	3296	3652	2887	.544

Depression and anxiety							
IDS score	21.4	14.1	20.8	14.7	21.8	13.8	.074
IDS somatic score	7.9	5.1	7.5	5.4	8.1	5.0	.004
IDS cognitive score	7.7	6.4	7.6	6.6	7.7	6.3	.725
BAI score	12.0	10.6	11.2	10.6	12.5	10.6	.002
BAI somatic score	7.3	6.9	6.7	6.9	7.5	6.8	.004
BAI cognitive score	4.8	4.5	4.4	4.4	4.9	4.6	.005
Inflammatory markers							
C-Reactive Protein (mg/L) (median, IQR)	1.22	0.54-3.02	1.05	0.49-2.54	1.32	0.56-3.29	.001
Interleukin 6 (pg/L) (median, IQR) (pg/L)	0.75	0.49-1.25	0.79	0.52-1.35	0.73	0.48-1.21	.049
Tumor necrosis factor-alpha (median, IQR)	0.80	0.60-1.10	0.80	0.60-1.10	0.80	0.60-1.10	.476

* Calulated with t-tests, chi-square tests, ANOVA, or Mann-Whitney U.

BMI = body mass index, IDS = Inventory of Depressive Symptomatology, BAI = Beck Anxiety Inventory

Table 3. Regression analyses for depressive symptoms (IDS) and inflammation

	CRP		IL-6		TNF-α	
	ß	p	ß	P	β	p
IDS total score	Men		Total sample		Total sample	
Demographics + health	.104	**<.01**	.046	**.02**	.039	**.04**
Antidepressant medication*	.092	**<.01**	.046	**.02**	.035	.07
Lifestyle	.043	.16	.010	.59	.022	.27
IDS total score	Women					
Demographics + health	.015	.52				
Antidepressant medication*	-.002	.93				
Lifestyle	-.038	.09				
IDS somatic	Total sample		Total sample		Total sample	
Demographics + health	.078	**<.01**	.054	**<.01**	.045	**.02**
Antidepressant medication*	.067	**<.01**	.054	**<.01**	.042	**.03**
Lifestyle	.022	.23	.017	.37	.028	.16
IDS cognitive	Total sample		Total sample		Total sample	
Demographics + health	.021	.27	.028	.13	.035	.07
Antidepressant medication*	.004	.83	.028	.13	.031	.10
Lifestyle	-.028	.12	-.001	.95	.020	.30

Table 3. Demographics + health: age, sex, years of education, anti-inflammatory medication use, statin use, presence of cardiovascular disease and diabetes
Lifestyle: Demographics + health + smoking, alcohol consumption, BMI, and physical activity
IDS = Inventory of Depressive Symptomatology, CRP = high sensitive C-reactive protein, IL-6 = Interleukin 6, TNF-α = Tumor Necrosis factor- alpha
Bold faced: p<.05
*Only adjusted for antidepressant medication use when significant correlation was present with inflammatory marker. CRP analyses were adjusted for selective serotonin reuptake inhibitor (p=.04), tricyclic antidepressants (p<.01), and tetracyclic antidepressants (p<.01). TNF-α analyses were adjusted for tricyclic antidepressants (p<.01). There were no significant correlations between IL-6 and

our findings, Elovainio and colleagues found significant associations between cognitive symptoms and CRP. However, these were unadjusted or for every covariate separately adjusted analyses. We did not present unadjusted or separately adjusted analyses, because of the already known age effects on inflammation[46, 47]. However, contradictory findings have also been reported. Kupper and colleagues found that both somatic and cognitive symptoms were cross-sectionally associated with inflammation in a sample of heart failure patients[48]. Furthermore, cognitive symptoms of depression were associated with subsequent inflammation, whereas change in somatic symptoms over a 12 month period were associated with inflammation[48]. One possible explanation for the differences in the cross-sectional results between these studies could be that our study and Elovainio and colleagues' study consisted of somatic healthy participants, whereas Kupper and colleagues used a sample of heart failure patients, whom probably have higher levels of inflammation to begin with due to their disease status. Furthermore, the heart failure sample is on average older, which possibly affects cognitive functions comparable to cognitive symptoms of depression (i.e. concentration).

As was found in previous research[8, 9, 48], this study shows that lifestyle factors, and mainly BMI, explained a significant part of the association between depressive symptoms and inflammation. Obesity has been found to be associated with depression[4, 49]. A meta-analysis on the prospective association between overweight and depression showed that the relationship is bi-directional: depression predicts obesity and obesity is a risk factor for depression[4]. Obesity has also been found to

Table 4. Regression analyses for anxiety symptoms (BAI) and inflammation

	CRP		IL-6		TNF-α	
	ß	p	ß	P	β	P
BAI total score	Men		Total sample		Total sample	
Demographics + health	.106	<.01	.034	.07	.035	.07
Antidepressant medication*	.094	<.01	.034	.07	.031	.11
Lifestyle	.038	.20	.001	.95	.021	.28
BAI total score	Women					
Demographics + health	.010	.68				
Antidepressant medication*	-.004	.86				
Lifestyle	-.022	.33				
BAI somatic	Men		Total sample		Total sample	
Demographics + health	.116	<.01	.050	<.01	.038	.05
Antidepressant medication*	.105	<.01	.050	<.01	.034	.08
Lifestyle	.045	.13	.015	.43	.023	.25
BAI somatic	Women					
Demographics + health	.026	.27				
Antidepressant medication*	.012	.63				
Lifestyle	-.012	.60				

BAI cognitive	Men		Total sample		Total sample	
Demographics + health	.073	.02	.005	.77	.024	.21
Antidepressant medication*	.059	.06	.005	.77	.021	.27
Lifestyle	.020	.49	-.019	.31	.014	.45
BAI cognitive	Women					
Demographics + health	-.015	.50				
Antidepressant medication*	-.026	.27				
Lifestyle	-.031	.15				

Demographics + health: age, sex, years of education, anti-inflammatory medication use, statin use, presence of cardiovascular disease and diabetes

Lifestyle: Demographics + health + smoking, alcohol consumption, BMI, and physical activity

BAI = Beck Anxiety Inventory, CRP = high sensitive C-reactive protein, IL-6 = Interleukin 6, TNF-α = Tumor Necrosis factor- alpha

Bold faced: p<.05

*Only adjusted for antidepressant medication use when significant correlation was present with inflammatory marker. CRP analyses were adjusted for selective serotonin reuptake inhibitor (p=.04), tricyclic antidepressants (p<.01), and tetracyclic antidepressants (p<.01). TNF-α analyses were adjusted for tricyclic antidepressants (p<.01). There were no significant correlations between IL-6 and antidepressant medication use, and therefore these analyses were not further adjusted.

be associated with higher levels of inflammation[44, 50]. Research suggests that adipose tissue produces IL-6 and TNF-α[44, 50] and this is therefore a plausible link between depressive symptoms and inflammation[50]. Furthermore, Dod and colleagues found that intervening in lifestyle, by changing food intake and enhancing moderate exercise, had significant effects on lowering inflammation levels after 12 weeks[25]. As higher levels of inflammation are found to be associated with adverse health outcomes such as cardiac risk factors[51] and diabetes[3], promoting weight loss in people with depressive symptoms could have beneficial effects on levels of inflammation and possibly future health status.

Regarding anxiety and inflammation, we found a significant association between total anxiety symptoms and IL-6 and CRP (men only) whereas somatic symptoms were associated with CRP (men only), IL-6, and TNF-α. These associations also diminished after lifestyle factors were considered. In line with our findings on total symptoms of anxiety, previous studies also reported on a positive association between anxiety and inflammation[52,53]. Liukkonen and colleagues found that men reporting anxiety symptoms had elevated levels of CRP levels compared to those who did not report symptoms of anxiety. However, their findings were not affected by adjustment for a range of covariates including BMI[52] , whereas our results became non-significant after adjusting for health behaviors (smoking, BMI, physical inactivity, and alcohol use), with BMI having the strongest effect on the association. Furthermore, similar to our findings, they did not find support for this association in women. In contrast, Pitsavos and colleagues did find significant associations for anxiety and inflammation in women[53]. Interestingly, the results published by Liukkonen and colleagues and Pitsavos and colleagues did not diminish after adjustment for health behaviors as our results did. Previous research has shown a positive relationship between BMI and inflammation on one hand[54, 55] and BMI and anxiety on the other hand[56]. One possible explanation could be that our sample partly consists of psychiatric patients, in contrast to the healthy samples used by Pitsavos and colleagues and Liukkonen and colleagues. Around 27% of the participants are recruited in specialized mental health care. It could be that this group of participants has more adverse health behaviors contributing to inflammation and/or anxiety compared to healthy participants. Furthermore, our results suggest that the anxiety-inflammation relationship is mainly driven by somatic symptoms. Somatic symptoms of anxiety consist of hot flushes, respiration, heart pounding, shaking hands and difficulty breathing. This sheds a new light on anxiety and inflammation. In contrast to somatic symptoms of depression, somatic symptoms of *anxiety* are not similar to symptoms of sickness behavior, but may be seen as a reflection of autonomic control, suggesting a role

for the autonomic nervous system (ANS). The ANS is also associated with higher levels of inflammation[57-59] and could possibly be involved in the anxiety-inflammation link. However, studies examining anxiety and the ANS show conflicting results[60-62] as to whether the ANS and anxiety are associated. Possibly, the ANS is only involved in the somatic symptoms of anxiety and affects immune function simultaneously.

This study is conducted on cross-sectional data, implying that no inferences can be made on the direction of the relationship of depression and anxiety with inflammation. There is some evidence that suggests that depressive symptoms are associated with subsequent inflammation[8, 9, 48, 63], but the opposite has been reported as well[10]. Regarding anxiety there is little evidence up to date on the direction of association, though it has been suggested that pro-inflammatory cytokines rise in the presence of anxiety, due to chronic stress[65]. Up to date there is no longitudinal research conducted which could provide insight into the direction of the anxiety-inflammation relationship and a more thorough understanding of mechanism involved in this relationship.
Some strengths can be attributed to our study, such as a large sample size with a wide range on psychopathology thereby increasing the power of our analyses, which made it possible to adequately adjust for potential confounders. In addition, multiple inflammatory markers were assessed. However, some limitations need to be acknowledged. Our data is cross-sectional, which makes it impossible to draw any conclusions on the direction of the relationship between symptoms of depression or anxiety and inflammation. Association of depression and anxiety inflammation with inflammation may be different in prospective research. Furthermore, we assessed circulating levels of inflammatory markers, which show a high degree of intra-individual variation. This could explain why we found the rather modest associations between symptoms of depression and anxiety with inflammatory marker levels in our study.

In conclusion, our results suggest an association between depressive and anxiety with inflammation, in which associations were mainly driven by the somatic symptom components. Nevertheless, adjustment for lifestyle factors diminished all associations to non-significance indicating that it is the poorer lifestyle of depressed and anxious patients that puts them at risk for inflammation.

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PART 3

Depression and immune function in adolescents

CHAPTER 7

Depressive symptoms predict subsequent inflammation in adolescence

Data from the TRacking Adolescents' Individual Lives Survey.

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Abstract

Background: Depression and inflammation have been found to be related both in healthy and diseased adults. As depression and inflammation are also known metabolic and cardiac risk factors, we set out to evaluate the trajectories of depressive symptoms, somatic symptoms of depression, and cognitive symptoms of depression in adolescence over the course of 5 years and their relationship to subsequent levels of inflammation.

Methods: 1166 Dutch adolescents were followed for TRacking Adolescents' Individual Lives Survey (TRAILS) from 2001 until 2008. Three assessments over a period of 5 years took place in which the Youth Self Report (YSR) was administered, and information on demographics and health behaviors was collected. The YSR provided the overall depressive symptoms score, as well as a somatic and a cognitive subscale score. At the third assessment, blood was collected to determine hsCRP. Cluster analysis was used to determine 5-year trajectories of depression. GLM was used to determine the association between the clusters and hsCRP.

Results: Adolescents with steady mild depressive symptoms had higher levels of subsequent hsCRP than those reporting decreasing depressive symptoms after adjustment for demographics. Additional adjustment for health behaviors diminished this relation to non-significance. Consistently high scores on somatic symptoms and cognitive symptoms were associated with higher levels of hsCRP compared to consistently low scores on these dimensions, but this difference became non-significant after adjustment for demographics.

Conclusion: Persistent depressive symptoms were associated with subsequent high levels of hsCRP. Smoking, BMI, and physical activity play an important role in this association, suggesting a mediating role of health behaviors in the depression-inflammation relationship in adolescence.

Introduction

In the past decades a substantial amount of research has evaluated the relation between depression and inflammation both in healthy and diseased adults[1, 2]. Within this body of research, inflammation has been suggested as one of the physiological mechanisms explaining the adverse association between depression and progression of heart disease[3], and to a lesser extent the development of heart disease[4]. Most of the studies to date have been conducted in adult healthy populations [e.g.[5]] and psychiatric [e.g.[6]] or somatic patient populations [e.g.[7, 8]] while only a very limited number of studies considered adolescents.

Still, especially adolescence may be an important period in which this association should be evaluated. Adolescence is a period in life where many of the first onsets of depression or depressive symptoms start to develop[9]. Prevalence estimates of depression range from approximately 0.3% in early adolescence up to 23.2% in late adolescence[9]. It is therefore of interest to evaluate whether the association between depression and inflammation is already present in adolescents. The few studies to date that have investigated the relationship between depression and inflammation in adolescents report inconsistent results[10-12]. Chaiton and colleagues examined the cross-sectional depression-inflammation relationship in adolescents, but failed to find an association[10], while a recent prospective study reported that adolescents who had repeatedly experienced depressive symptoms had higher levels of subsequent C-reactive protein (CRP) than those who did not[11]. This finding suggests that mainly recurrent depressive symptoms are associated with CRP, a finding that is in line with previous reports on depression and inflammation in coronary heart disease patients[7]. Finally, Elovainio and colleagues reported that depression in young Finns was associated with subsequent hsCRP, with an important role for BMI and triglycerides[12], suggesting a mediating effect of overweight which has also been suggested in previous research[13]. An important shortcoming of the prospective studies conducted thus far[11, 12] is that these authors did not look into the trajectories of depressive symptoms over time. Looking into trajectories over time may provide insight in the chronicity of the symptoms and its temporal association with inflammation.

Another important distinction in the association between depression and inflammation may be the differential association of inflammation with cognitive and somatic symptoms of depression, with studies demonstrating the somatic symptoms of depression (e.g., sleeping problems, lack of sleep, eating problems, and psychomotor retardation) to be associated with inflammation and not the

cognitive symptoms in healthy adults[5] and cancer patients[14]. In contrast, a prospective study in patients with heart failure by Kupper and colleagues reported that markers of inflammation were associated with both somatic and cognitive symptom dimensions of depression[8].

To assess whether the course of depressive symptoms in a population based sample of adolescents is associated with subsequent inflammation, we set out to evaluate the trajectories of depressive symptoms, somatic symptoms of depression, and cognitive symptoms of depression in adolescence over the course of 5 years and their relationship to subsequent levels of inflammation. Our first aim was to identify longitudinal development classes for depressive symptoms, somatic symptoms of depression, and cognitive symptoms of depression separately. Secondly, we aimed to investigate whether these classes were differentially associated with subsequent hsCRP.

Methods

Participants

We used data from the Tracking Adolescents' Individual Lives Survey (TRAILS), a large ongoing prospective cohort study of Dutch adolescents. In this study, data from wave 1 (March 2001 to July 2002), wave 2 (September 2003 to December 2004), and wave 3 (September 2005 to August 2008) were used. Sample selection is described in more detail elsewhere[15]. In short, selected municipalities in the north of The Netherlands were asked to provide names and addresses of all inhabitants born between October 1, 1989 and September 30, 1990 or October 1, 1990 and September 30, 1991. This yielded 3483 names. In addition, primary schools within these municipalities were asked to participate in TRAILS. The participation of schools was a prerequisite for eligible children and their parents to be approached for participation in TRAILS. Of all children approached for inclusion in the study (N = 3145), 2230 (76.0%) were enrolled in the study. Parents had to provide a written informed consent. At waves 2 and 3 the adolescents gave additional written informed consent. Of the total baseline sample (N = 2230, mean age = 11.1, SD = .06) 2149 adolescents (96.4 %, mean age = 13.65, SD = .53) took part in wave 2, two to three years after the baseline assessment. At wave 3, 1816 adolescents (81.4%, mean age = 16.27, SD = .73) participated in the study.

For the sample used in this study, adolescents who had too many missings on the Youth Self-Report (YSR) (wave 1 N = 28, wave 2 N = 137, and wave 3 N = 570) and for whom hsCRP values were not available (N = 1017) were excluded from the

analyses. This resulted in a final N of 1166. TRAILS was approved by the Central Committee on Research Involving Human Subjects (CCMO).

Depressive symptoms

Depressive symptoms were measured by the Affective Problems scale of the Youth Self-Report (YSR), a self-report questionnaire assessing emotional and behavioral problems during the past 6 months. The Affective Problems scale contains 13 items, which were scored on a 3-point Likert scale (0 = not true; 1 = somewhat or sometimes true; or 2 = very true). The scale reflects the symptoms of a Major Depressive Episode according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV)[16]. Bosch and colleagues earlier showed in this sample that omission of one sleep item ('I sleep more than most kids') increased the internal consistency of the scale, so we followed this suggestion[17]. The remaining 12 items covered depressed mood, anhedonia, loss of energy, feelings of worthlessness and guilt, suicidal ideation, sleep problems, and eating problems, and had an internal consistency (Cronbach's α) of .73 at wave 1, of .78 at wave 2 and .79 at wave 3.

In order to evaluate the effects somatic and cognitive symptoms of depression, the items were divided into two subscales; a somatic symptoms subscale and a cognitive symptoms subscale (described earlier by Bosch and colleagues[17] and presented in table 1). The internal consistency of the cognitive symptoms scale was .67-.74 for the 3 data waves, while the consistency of the somatic symptoms scale was .58-.67. Total depressive symptoms could be determined for 2202 (wave 1), 2093 (wave 2), and 1660 (wave 3) adolescents respectively. We used corrected-item-mean (CIM) imputation to handle missing data if less than 30% of the items were not answered[18]. From the total sample, 1627 adolescents
completed all 3 YSRs, 478 completed 2, and 118 completed 1 YSR. Missing values are replaced by the item mean, corrected for participants' score on the items compared with the mean score on these items in the total sample[18]. When more items were unanswered, the YSR Affective Problems Scale was considered incomplete and the data was not used in the analyses.

Table 1. Depressive Symptoms used in this study.

Somatic symptoms	Cognitive symptoms
Lack of appetite	Loss of pleasure
Overtired	Crying
Reduced sleep	Self-harm
Trouble sleeping	Suicidal Ideation
Lack of energy	Feelings of worthlessness
	Feelings of guilt
	Sadness

C-reactive protein

At wave 3, 39.5 ml fasting blood was drawn, which was transported to the laboratory within four hours. High sensitive-C reactive Protein (hsCRP) was determined using a immunonephelometric method, BN2, *Cardio*Phase® hsCRP, Siemens with a lower detection limit of 0.175 mg/L. Intra-assay coefficients of variance ranged from 2.1 to 4.4, and inter-assay coefficients of variation coefficients of variance ranged from 1.1 to 4.0. HsCRP was not normally distributed, so we applied a log transformation to achieve normality. In the tables and figures values are presented back transformed for interpretational purposes.

Covariates

We included sex, age, socio-economic status, body mass index (BMI), physical activity, and current smoking as covariates because of their known association with inflammation[19] and depression[19-21].
Socio-economic status was based on baseline data (wave 1) on parental education, income, and occupation according to the International Standard Classification[22].
The lowest 25%, intermediate 50% and highest 25% of the scores were defined as low, middle, or high.
Finally, health behaviors might serve as mediators in the depression-inflammation relationship[7, 11, 19]. Individual indicators of health behavior (i.e. physical activity, BMI and current smoking) were therefore added to the analyses in a second step. Physical activity at wave 3 was calculated by means of metabolic equivalent scores (METs). METs are the ratio of the work metabolic rate to the resting metabolic rate. One MET is defined as 1 kcal/kg/hour and is roughly equivalent to the energy cost of sitting quietly[23]. Smoking was determined at wave 3 by self-report and defined

as no, former, or current smoker. BMI was measured by a research assistant at wave 3 and calculated by dividing the weight (kg) by the square of the height (m^2).

Statistical analyses

Cross-sectional analyses – Linear regression analyses were conducted to examine the cross-sectional association between wave 3 depressive symptoms, somatic symptoms of depression, and cognitive symptoms of depression with hsCRP. After exploring the unadjusted association, the "*Demographics"* model added the variables age, sex, and socio-economic status. The final model, named "*Health behaviors*" additionally included the health behaviors (smoking, BMI and physical activity) .

Course of depression - Latent class ordinal regression modeling in Latent Gold 4.0[24] was used to determine the number of latent classes in the course of depressive symptoms over 5 years (3 data waves) in the TRAILS sample, and to evaluate the course of depressive symptoms, somatic symptoms and cognitive symptoms within these latent classes. Because depressive symptoms, somatic symptoms, and cognitive symptoms were discrete variables, they were handled as ordinal dependent variables. Time was used as a predictor. Eight models were compared with an increasing number of classes (1-8). The Bayesian Information Criterion (BIC) was used to compare the fit of the subsequent models[25]. BIC provides a quantitative index of the extent to which a model maximizes the correspondence between the observed and model predicted responses while minimizing the number of parameters. A BIC difference of > 10 is strong evidence that the models with the lower BIC is better[26, 27].

Association with hsCRP - Trajectory membership was exported to PASW statistics 17. Trajectories were reorganized for all depression scales in such a way that the trajectory with the lowest scores on the depression scales represented the lowest number and the highest score on the scales represented the highest number.
Finally, the General Linear Models procedure in PASW was used to examine whether class membership was differentially associated with hsCRP, in unadjusted and adjusted analyses. In the first model unadjusted associations were tested. The second model evaluates the association adjusted for *demographic* variables; age, sex, and socio-economic status. In the final model *health behaviors* were additionally included. Because health behaviors (smoking, BMI, and physical

activity) are possible mediators in the association between depression and inflammation[7, 19], they were analyzed in a separate model that was added to the demographics model. Mean levels of hsCRP were compared across the clusters using a Bonferroni correction to evaluate differences among classes and their association to hsCRP. P-values were considered significant when $p<.05$.

Results

Sample characteristics

Characteristics of the total sample are summarized in Table 2. The total sample (N = 1166) consisted for 46.5% of boys, with a mean age at wave 3 of 16.2 years. Mean levels of hsCRP at wave 3 were 1.13 (SD = 2.05).

Cross-sectional analyses of depression and hsCRP levels

Cross-sectional linear regression analyses of wave 3 data did not reveal significant associations of depressive symptoms, somatic symptoms of depression, and cognitive symptoms of depression with hsCRP (β=.010, p=.17; β=.02 p=.14; β=.018 p=.39 respectively). Additional adjustments for demographics and health behaviors did not alter the results.

Table 2. Total sample characteristic

N = 1166 (at wave 3)	N(%)*
hsCRP (mg/L) *Mean(SD)*	1.13 (2.05)
Demographics	
Sex (male)	542 (46.5%)
Age (years) *Mean(SD)*	16.22 (0.64)
Socio-economic status	
Low	284 (24.4%)
Middle	534 (45.8%)
High	276 (23.7%)
Health behaviors	
Physical activity (METs) *Mean(SD)*	3460.9 (4577.2)
BMI *Mean(SD)*	21.3 (3.2)
Smoking	
No	513 (44.0%)
Former	318 (27.3%)
Current	328 (28.2%)

*Numbers are presented as N(%) unless otherwise specified.

The course of depression over the 5-year period

Depressive symptoms - Cluster analysis identified the presence of 5 trajectories for YSR depressive symptoms total score over 5 years of follow-up in the best-fitting model, explaining 65.7% of the total variance (Table 3 top panel). The 5 trajectories are visualized in Figure 1a and reflect (cluster 1) "consistently *low depressive symptoms*", (cluster 2) "*decreasing depressive symptoms*", (cluster 3) *"increasing depressive symptoms",* (cluster 4) *"consistently mild depressive symptoms"*, and (cluster 5) *"consistently high depressive symptoms"*. Though the most important difference between the 5 trajectory classes was in the overall level of depression (i.e. the intercept), also the time effect significantly differed across classes (Wald statistic = 92.22, p <.0001).

Somatic symptoms - Four trajectories were identified (Table 3, Figure 1b) in the best fitting model, explaining 62.8% of the total variance and reflecting "*consistently low somatic symptoms of depression*" (cluster 1), *"decreasing somatic symptoms of depression"* (cluster 2)*, "increasing somatic symptoms of depression"* (cluster 3)*, and "consistently high somatic symptoms of depression"* (cluster 4). The largest trajectory contained 30% of the sample and the smallest trajectory contained 19%, with time having a different effect across the classes (Wald = 83.83, p <.001) Figure 1b).

Cognitive symptoms - Three trajectories were identified for YSR cognitive symptoms in the best fitting model. These trajectories explained 50.0% variance and reflected *"low cognitive symptoms of depression"* (cluster 1)*, "mild cognitive symptoms of depression"* (cluster 2), and *"high cognitive symptoms of depression"* (cluster 3; Figure 1c). The effect of time was significantly different across the trajectories (Wald statistic 35.85, p <.001).

Table 3. Identification of the number of latent classes using regression models for Depressive symptoms total score, Somatic symptoms subscore and Cognitive symptoms subscore

Model			Statistics	
Depressive symptoms	LL	Npar	N	BIC
Cluster 1	-14070.7951	24	2223	28326.5488
Cluster 2	-13615.1104	28	2223	27446.0060
Cluster 3	-13517.0762	32	2223	27280.7639
Cluster 4	-1502.8825	36	2223	27283.2030
Cluster 5	***-13485.9691***	***40***	2223	***27280.2027***
Cluster 6	-13478.3165	44	2223	27295.7240
Cluster 7	-13471.0096	48	2223	27311.9365
Cluster 8	-13468.0568	52	2223	27336.8574
Somatic symptoms	LL	Npar	N	BIC
Cluster 1	-11246.1602	12	2222	22584.7943
Cluster 2	-10886.3136	16	2222	21895.9259
Cluster 3	-10824.1430	20	2222	21802.4092
Cluster 4	***-10807.7257***	***24***	2222	***21800.3994***
Cluster 5	-10795.3553	28	2222	21806.4833
Cluster 6	-10789.2724	32	2222	21825.1421
Cluster 7	-10787.3715	36	2222	21852.1648
Cluster 8	-10784.8352	40	2222	21877.9169
Cognitive symptoms	LL	Npar	N	BIC
Cluster 1	-9851.3331	16	2223	19825.9719
Cluster 2	-9493.0712	20	2223	19140.2746
Cluster 3	***-9451.2930***	***24***	2223	***19087.5447***
Cluster 4	-90438.0806	28	2223	19091.9464
Cluster 5	-9430.7559	32	2223	19108.1234
Cluster 6	-9425.4415	36	2223	19128.3212
Cluster 7	-9421.5894	40	2223	19151.4433
Cluster 8	-9418.7375	44	2223	19176.5660

LL= log likelihood

Npar = number of estimated parameters

N = number of subjects in analysis

BIC = Bayesian Information Criterion

The association of the 5-year course of depression with subsequent hsCRP levels
Table 4 shows the results of the General Linear Models ANCOVA, in which trajectory membership of total depression (left), somatic depressive symptoms (middle) and cognitive depressive symptoms (right) was used to predict hsCRP levels at wave 3.

Depression - In the unadjusted model, the 5 trajectories were differentially associated with subsequent levels of hsCRP (p=.007). Pairwise comparisons of the mean hsCRP levels across the trajectories showed that adolescents with "*decreasing depressive symptoms*" had lower levels of subsequent hsCRP compared to adolescents with *"mild depressive symptoms"* (p=.01; Figure 2a). After adjusting for demographics, class membership remained associated with subsequent hsCRP. Additional adjustment for health behaviors however, made the association with inflammation no longer significant across membership classes, with BMI and smoking being significant covariates (p-values <.001) (Table 4, left panel).

Somatic symptoms of depression - Trajectories of somatic symptoms were differentially associated with levels of hsCRP in the unadjusted model (p=.050; Figure 2b). Pairwise comparisons across the 4 trajectory classes showed that hsCRP levels were significantly higher in the high somatic symptoms group compared to the low somatic symptoms group (p=.04). Adjustment for demographics, however, made the association non-significant and the association was even further reduced after additional adjustment for health behaviors (Table 4).

Cognitive symptoms of depression - The 3 classes were differentially associated with subsequent levels of hsCRP (p=.02) in unadjusted analysis, with adolescents who consistently reported '*high cognitive symptoms of depression'* having higher levels of subsequent hsCRP than those who consistently reported '*low cognitive symptoms of depression'* (Figure 2c). After adjusting for demographics, the classes were no longer differentially associated with hsCRP.

Table 4. Results of ANCOVA, comparing levels of hsCRP across class membership for depressive symptoms, somatic symptoms of depression, and cognitive symptoms of depression

	Depressive symptoms				
	F	Df	p	η^2	Significant
Unadjusted	3.54	4.12	**.01**	.007	
Demographics	2.71	4.11	**.03**	.006	1, 2
Health behaviors	1.31	4.11	.26	.002	1, 4, 5
	Somatic Symptoms				
	F	df	p	η^2	Significant
Unadjusted	2.62	3.12	**.05**	.007	
Demographics	2.07	3.11	.10	.004	1, 2
Health behaviors	0.53	3.11	.66	.000	1, 4, 5
	Cognitive symptoms				
	F	df	p	η^2	Significant
Unadjusted	3.87	2.12	**.02**	.012	
Demographics	2.02	2.11	.13	.010	1, 2
Health behaviors	0.20	2.11	.82	.005	1, 4, 5

Demographics: adjusted for age, sex, and socio-economic status.

Health behaviors: adjusted for demographics + smoking, BMI, and physical activity.

1 = age, 2 = sex, 3 = socio-economic status, 4 = smoking, 5 = BMI, and 6 = physical activity

Discussion

This study identified clusters in the course of depressive symptoms, somatic symptoms of depression, and cognitive symptoms of depression over a 5-year period in healthy adolescents and examined whether these course clusters were associated with subsequent systemic hsCRP concentrations. The results demonstrated the presence of 5 trajectories for depressive symptoms that differed in terms of intercept and slope. These trajectories were differentially associated with subsequent levels of hsCRP, with persistent mild to high depression having the highest hsCRP levels. Health behaviors, i.e. smoking and being more overweight explained a large part of this association. Four trajectories for somatic symptoms were identified and 3 trajectories were identified for cognitive symptoms. These trajectories were not associated with subsequent hsCRP in adjusted analyses.

In order to evaluate the relationship between the course of depressive symptoms and hsCRP, cluster analyses for depressive symptoms, somatic symptoms and cognitive symptoms of depression over a 5 year follow-up period were performed. We found 3 (cognitive symptoms) to 5 classes (depressive symptoms) for the

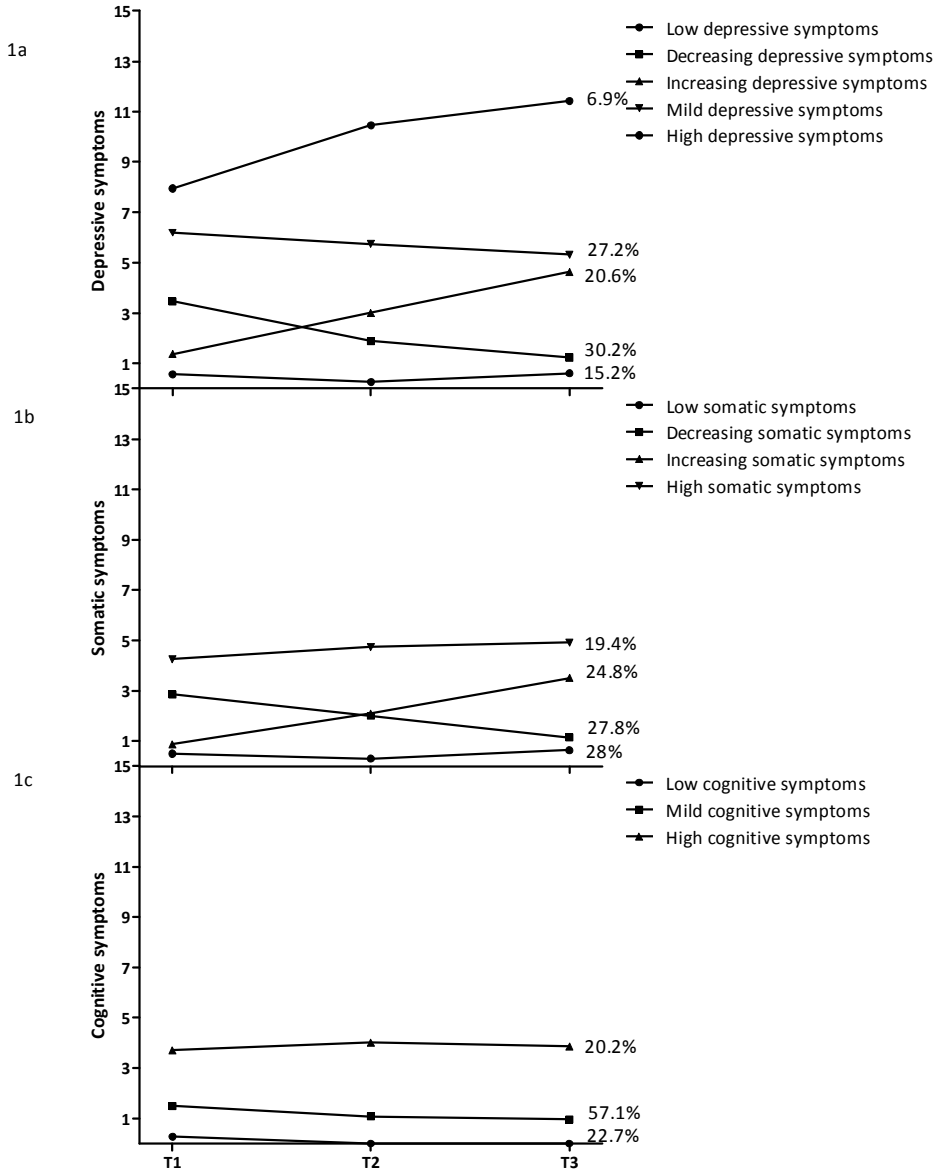
separate depression scales over time, suggesting a substantial degree of heterogeneity in the experience of depression over time. Somatic symptoms of depression seemed to show more variation over time than cognitive symptoms of depression.

Next to the time-stable depression classes, we also identified classes with increasing and decreasing depression (subscale) scores. In contrast, for cognitive symptoms only classes with consistent scores (low, mild, and high) were identified. This suggests that adolescents with high scores on depressive symptoms and somatic symptoms of depression tend to consistently report high scores, whereas adolescents with low scores at baseline can report increasing depressive symptoms or adolescents with mild scores at baseline can report decreasing scores over time.

Our findings support the results of an earlier study on trajectories of depression in adolescents that identified 4 clusters of depression over an 11-year follow-up period, which were comparable to 4 out of the 5 classes identified in our study[28]. They did not, however, identify a mild depressive symptoms class. These differences could be the result of their longer follow-up period, but might also be explained by the different questionnaires used. Wickrama and colleagues used 8 items from the Center for Epidemiological Studies of Depression (CES-D) Scale[28], whereas we used the Affective Problems scale from the Youth Self Report (YSR). One major difference between both questionnaires is that the YSR is especially developed for children and adolescents. The DSM-IV affective subscale of the YSR has strong correlations with the major depressive disorder scale from the Revised Child Anxiety and Depression Scale[29], and thus may be more sensitive in this age group. Despite these differences, Wickrama and colleagues also found that adolescents reporting moderate to high depressive symptoms tend to report moderate to high depressive symptoms over time. Taken together, this suggests that especially those adolescents with high scores on (somatic and affective) depressive symptoms already at the age of 11 are at risk for continued feelings of depression in later adolescent life. It could well be that these consistently mild and high classes of depressive symptoms are a reflection of recurrent or even chronic depressive symptoms, whereas the increasing and decreasing classes could be a reflection of single episodes of depressive symptoms.

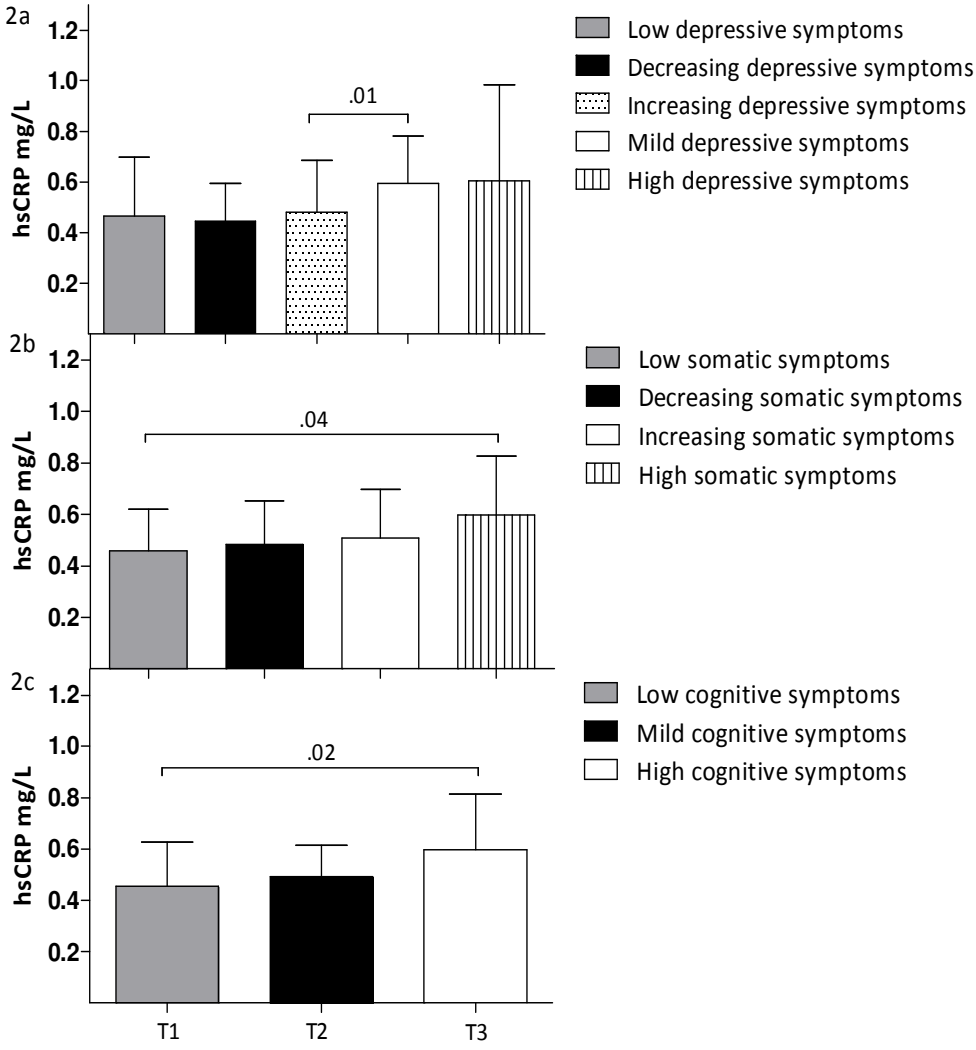
The identified classes were used to examine the association between the course of depressive symptoms, somatic symptoms of depression and cognitive symptoms of depression and subsequent hsCRP levels. The unadjusted results showed that the two subscales and the total depression score were individually associated with subsequent hsCRP, consistently showing adolescents with consistent mild to high

Figure 1. Mean scores for (1a) depressive symptoms, (1b) somatic symptoms of depression, and (1c) cognitive symptoms of depression across classes over time



% reflects percentage of participants in trajectory
T1: wave 1; T2: wave 2; T3: wave 3

Figure 2. Levels of hsCRP (backtransformed) across classes of (2a) depressive symptoms, (2b) somatic symptoms and (2c) cognitive symptoms of depression



hsCRP = high sensitive C-reactive protein. Error bars represent standard error of the mean.
T1: wave 1; T2: wave 2; T3: wave 3

scores having higher levels of hsCRP than those with low or decreasing symptoms. However, both for somatic and cognitive symptoms, differences in hsCRP levels across the classes were no longer significant after adjustment for demographics i.e. age, sex, and education. Taken together, somatic and cognitive symptoms of depression were not differentially associated with inflammation, but total depressive symptoms were, suggesting a synergy of all symptoms of depression when combined using a 5 year perspective.

The results for the depressive symptoms underline previous findings on the prospective association between depression and inflammation, which suggested that associations are most profound when multiple assessments of depressive symptoms were used. For instance, Copeland and colleagues recently published a study on the prospective association between depressive symptoms and inflammation in adolescents and young adults[11]. They found that participants who repeatedly experienced depressive symptoms had higher levels of subsequent CRP compared to those who did not, irrespective of demographic variables and health behaviors[11]. Furthermore, in a large sample of patients with stable coronary heart disease Whooley and colleagues found that depressive symptoms were not associated with inflammation in cross-sectional analysis[30]. However, in the same sample, prospective analyses on the depression-inflammation relationship showed that patients who repeatedly reported depressive symptoms had higher levels of inflammation than those who reported depressive symptoms at one interview only or reported no depressive symptoms[7]. The current results clearly show that especially those adolescents reporting at least mild symptoms of depression at all three assessments had higher subsequent levels of hsCRP. This can also explain why Chaiton and colleagues did not find a cross-sectional association between depressive symptoms and inflammation in adolescents[10]. Taken together, these results demonstrate that inflammation is most strongly associated with recurrent depression or persistent elevated depressive symptoms and that this relationship already exists in adolescence.

We found that the association with hsCRP was explained by adverse health behaviors, especially smoking and BMI. It is well known from previous literature that health behaviors such as smoking[11, 31] and BMI[13, 20, 32] are associated both with depressive symptoms and with hsCRP[19]. The findings of the current study regarding the potentially (partially) mediating role of health behaviors concurs with previous studies in adolescents[12], healthy adults[33], and stable CHD patients[7] in which the strength of the relationship between depressive symptoms and inflammation was affected after including one or more markers of health behavior.

HsCRP is involved in the development of various inflammatory diseases, such as cardiac disease and asthma, but also diabetes. For instance, Toprak and colleagues found that even in young adults, hsCRP was an independent predictor of carotid intima media thickness[34]. It could thus be argued that depressed young adults with higher levels of hsCRP may be more vulnerable for developing cardiac disease in later life. Furthermore, depression and inflammation are both associated with asthma[35], potentially commonly induced by prenatal maternal stress[35]. Research has also shown that adolescents who smoke and have higher levels of hsCRP are at greater risk for abnormal lung function in adulthood[36]. In addition, obesity has been found to be an important environmental risk factor in the development of asthma[37]. Moreover, depression is also associated with a greater risk for diabetes, possibly through inflammation or obesity[38]. Intervening in eating and smoking habits could therefore be a potential therapeutic intervention strategy in adolescents with depression or depressive symptoms in order to affect future risk factors for health outcomes.

The results of this study should be viewed in light of several limitations. Because hsCRP was only determined at wave 3, we were not able to take baseline levels of hsCRP into account in our analyses. Therefore we were not able to evaluate the change in hsCRP levels as well as the potential bidirectional association between depression and hsCRP, although research until now favors the view that repeated exposure to depressive symptoms eventually leads to increased systemic inflammation[7,11]. Furthermore, the internal consistency for the somatic symptoms scale can be considered as insufficient at all three waves and for the cognitive symptoms at the baseline assessment, suggesting that there is still heterogeneity across the subscales and mainly the somatic subscale. The limited number of items in the somatic symptoms scale may serve as a potential explanation for the less than ideal internal consistency of the scale. Several strengths can be attributed to this study as well. First, because of the biennial assessments with 3 assessments over a period of 5 years, we were able to use a prospective design, enabling us to look into the course of depressive symptoms over time. Furthermore, because of the large sample size, we were able to properly adjust for confounding effects of demographics and health behaviors.

In conclusion, this study shows that depressive symptoms have considerable heterogeneity over the course of 5 years in adolescence. These classes could be a reflection of recurrent and single episodes of depression. Somatic symptoms and cognitive symptoms showed a more stable course during the follow-up period. Furthermore, adolescents reporting consistent depressive symptoms over time

have higher subsequent levels of hsCRP. We did not find support for a differential association of somatic symptoms of depression and cognitive symptoms of depression with hsCRP. This suggests that total depressive symptoms are more strongly associated with hsCRP, than subscales of depression are. Finally, the association between depressive symptoms and hsCRP was mainly explained by health behaviors such as smoking and BMI. Interventions focused on improving adverse health behaviors in depressed adolescents might have positive effects on levels of hsCRP and possibly on future health status, and is worth investigating in the future.

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CHAPTER 8

Viral infections and subsequent depression in adolescence

Prospective findings from the Tracking Adolescents' Individual Lives Survey

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Abstract

Background: Research has linked virus infections to mental disorders such as schizophrenia, with a possible role for inflammation. Inflammation has also been associated with depression. However, little is known about depression and virus infections. We therefore set out to evaluate the cross-sectional and prospective association between markers of virus infection and depression in young adolescents.

Methods: We used data from wave 3 and 4 from the TRacking Adolescents' Individual Lives Survey (TRAILS), a Dutch prospective cohort study (N = 1057). Depressive symptoms were assessed using the Youth Self Report (YSR) at wave 3; depression diagnosis was evaluated using CIDI interviews at wave 4. At wave 3, blood was collected to determine the presence of immunoglobulin G (IgG) antibodies in serum to Herpes Simplex Virus (HSV)-1, Cytomegalovirus (CMV), and Epstein Barr Virus (EBV). Antibodies were analyzed as a continuous variable. In addition, a categorical pathogen burden variable was created to reflect the number of infectious markers present (0= no markers to 3 = 3 markers).

Results: HSV-1 was present in 257 (24.3%) adolescents, EBV in 258 (24.4%), and CMV in 261 (24.7%) adolescents. Depression was diagnosed in 127 adolescents (12%). Linear regression showed an inverse association between CMV and depressive symptoms, but only after adjustment for demographics. No associations were found between HSV-1 and EBV with depressive symptoms. Logistic regression analyses did not reveal an association between the presence of HSV-1, EBV, or CMV and depressive symptoms or clinical depression. Furthermore, pathogen burden was not related to depressive symptoms and depression diagnosis.

Conclusions: We only found limited support for an inverse association between CMV for and depressive symptoms. This suggests that depression in adolescence is not affected by the presence of viral infections. However, it could be that the effects of viruses on depression might not come to expression before adulthood.

Introduction

Infectious agents have been linked to mental disorders such as schizophrenia and bipolar disorder, with a possible role for inflammation[1]. Furthermore, it has also been suggested that cognitive functioning in patients with schizophrenia[1] and bipolar disorder[2, 3] is affected by virus infection. For instance, Dickerson and colleagues found that both Herpes Simplex Virus (HSV)-1, and higher levels of hsCRP were associated with impaired cognitive functioning in patients with schizophrenia[1]. Inflammation has also been associated with depression[4-6], with some research suggesting that a higher number of depressive symptoms[7], as well as recurrent depressive symptoms[5, 6] are associated with higher levels of inflammation. Until now, little attention has been paid to a possible relationship between depression and infectious agents. There is some evidence though in support of the association between viruses and depression[8-10]. For instance, Miller and colleagues found that recovering acute coronary syndrome (ACS) patients experiencing depressive symptoms had more latent viruses (Epstein-Barr virus (EBV), Cytomegalovirus (CMV) and HSV-1) than those without depressive symptoms[9]. In line with this, Philips and colleagues found that antibodies to CMV were associated with depression in an elderly sample in such a way that participants with higher CMV antibodies reported higher depressive symptoms[8]. One of the explanations could be that infectious agents such as HSV-1 and EBV[11] may reside in the brain, especially in the limbic system, an area involved in emotion processing. Areas in the limbic system, such as the amygdala, hippocampus, and orbital prefrontal cortex have been associated with depressive disorders, already at a young age[12].

The presence of antibodies to HSV-1 and EBV increases with age[13], but they may already be present in adolescence[13] and often go by asymptomatically in childhood[14]. It could thus be hypothesized that the presence of viruses in the adolescent brain may be associated with psychological disorders, such as depression. We therefore set out to evaluate the cross-sectional and prospective association between markers of virus infection, i.e. the epstein-barr virus, the cytomegalovirus, and the herpes simplex virus 1 and 2, with depressive symptoms and depression diagnosis in young adolescents.

Methods

Study sample

The study is part of the Tracking Adolescents' Individual Lives Survey (TRAILS), which is a large ongoing prospective population study of 2230 Dutch adolescents from the north of the Netherlands. It is a multidisciplinary study on causes and effects of physical and mental health from childhood to adulthood. The design of TRAILS is further described elsewhere[15]. In short, in the five major municipalities in the northern part of the Netherlands all 135 primary schools were asked to participate in this study. Of the children of the schools that decided to participate, children with mental retardation, physical incapability or language problems were excluded. Of all children approached for inclusion in the study (N = 3145), 2230 (76.0%) were enrolled in the study. At wave 1 parents had to provide a written informed consent. At waves 2, 3, and 4 the adolescents gave written informed consent. Of the total baseline sample (N = 2230, mean age = 11.09, SD = .56) 2149 adolescents (96.4 %, mean age = 13.65, SD = .53) took part in wave 2, two to three years after the baseline assessment. At wave 3, 1816 adolescents (81.4%, mean age = 16.27, SD = .73) and at wave 4 1881 adolescents (84.3%, mean age = 19.1, SD = .60) participated in the study. For this study we used data from waves 3 (September 2005 to August 2008) and 4 (October 2008 to September 2010). Immunoglobulin G (IgG) class for HSV-1, HSV-2, CMV, and EBV was determined for 1220 adolescents at wave 3. Data on total depressive symptoms were available for 1660 adolescents at wave 3 and depression diagnosis for 1584 adolescents at wave 4. This resulted in a final sample of 1057 adolescents. TRAILS was approved by the Central Committee on Research Involving Human Subjects (CCMO).

Depressive symptoms

Depressive symptoms were measured at wave 3 using the Youth Self Report (YSR), a self-report questionnaire which assesses the emotional and behavioral problems during the past 6 months. The Affective Problems scale contains 13 items that are scored on a 3-point scale (0=not true, 1=somewhat or sometimes true, 2=very or often true). A previous study using the YSR in the TRAILS sample showed that omission of one sleep item ('I sleep more than most kids') increased the internal consistency of the scale, for which we excluded this item[16]. Scores on the remaining 12 items (depressed mood, anhedonia, loss of energy, feelings of worthlessness and guilt, suicidal ideation, sleep problems, and eating problems) items were

summed up to construct the YSR Affective Problems Scale with an internal consistency (Cronbach's α) of .79 at wave 3. This scale reflects the symptoms of a Major Depressive Episode according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV)[17]. The good reliability and validity of the American version of the YSR were replicated for the Dutch translation[18].

Depression

The Composite International Diagnostic Interview (CIDI) version 3.0 was used at wave 4 to determine depression diagnosis. The CIDI is a comprehensive, fully-structured interview designed for the assessment of mental disorders according to the definitions and criteria of ICD-10 and DSM-IV[19]. The participants were individually interviewed by a trained interviewer. For this study minor and major depressive episodes of depression during the 12 months preceding the interview were included (N = 19 and N = 106 respectively).

Measurement of antibodies

During the third assessment, all participants were asked to donate a blood sample for various biomarker analyses. After consent had been obtained from the parents and the adolescents, 39.5 ml fasting blood was drawn at the schools and delivered to the University Medical Center Groningen within four hours, where serum was extracted and stored at −80 °C until analysis. Infection of the human body by most infectious agents is associated with the development of persistent IgG class antibodies that can be measured in the blood of individuals years after the infection and are therefore suitable for epidemiological studies. IgG serum antibodies to the Herpes virus family (Herpes Simplex Virus Type 1 (HSV-1) Herpes Simplex Virus 2 (HSV-2) Epstein Barr Virus (EBV), Cytomegalovirus (CMV), were measured using solid-enzyme immunoassay methods, as previously described[20]. For each assay, a result was defined as positive or negative based on comparison with the reactivity of specific antibody standards saved along with the blood samples. The standards that tested antibodies to the herpes viruses consisted of samples with defined levels of reactivity of the specific herpes antigens[21]. All serological tests were carried out by standard procedure at the Stanley Laboratory of Developmental Neurovirology, Baltimore, Maryland, USA. A categorical variable reflecting pathogen burden was calculated as the sum of the number of virus

markers present at wave 3 defined as follows: no virus = 0; 1 virus = 1; 2 viruses = 2; 3 viruses = 3.

Covariates

Age, sex and socio-economic status were included in the analyses as these demographic variables are associated with depressive symptoms[22]. Socio-economic status was based on baseline data (wave 1) on the education, income, and occupation from either the adolescents' father or mother using the International Standard Classification[23], and the lowest 25%, intermediate 50% and highest 25% of the scores were defined as low, middle, or high.

As inflammation has been found to be associated with depression[4], but also with viral infections, the possible mediating effects of high sensitive C-reactive protein (hsCRP) was explored as well by adding hsCRP to the analyses in an additional step. hsCRP was determined using a immunonephelometric method, BN2, *Cardio*Phase® hsCRP, Siemens with a lower detection limit of .175 mg/L. Intra-assay coefficients of variance ranged from 2.1 to 4.4, and inter-assay coefficients of variation coefficients of variance ranged from 1.1 to 4.0. HsCRP was not normally distributed, so we applied a log transformation to achieve normality.

Health behaviors, i.e. smoking[24], physical activity[25], and body mass index (BMI)[26], that are associated with depression were included in a subsequent step to explore their possible mediating effects. Smoking, physical activity, and BMI, measured at both wave 3 and wave 4. Smoking was defined as no, former, or current smoker[27]. Physical activity was calculated by means of metabolic equivalent scores (METs). METs are the ratio of the work metabolic rate to the resting metabolic rate. One MET is defined as 1 kcal/kg/hour and is roughly equivalent to the energy cost of sitting quietly. A MET also is defined as oxygen uptake in ml/kg/min with one MET equal to the oxygen cost of sitting quietly, equivalent to 3.5 ml/kg/min[27]. BMI was calculated by dividing the weight (kg) by the square of the height (m^2).

Statistical analysis

T-test, ANOVA, and χ^2 were used to assess differences in proportions and means of covariates between adolescents with and without depression diagnosis. HSV-1 was present in 257 (24.3%) adolescents, EBV in 258 (24.4%), and CMV in 261 (24.7%)

adolescents. Because only 12 adolescents were found seropositive for HSV-2 (< 2%), we did not include this marker in further analyses.

We used linear regression analyses to determine the cross-sectional association between HSV-1, EBV, and CMV and depressive symptoms at wave 3 in 3 separate unadjusted analyses. In addition, the association between pathogen burden and depressive symptoms was analyzed. Prospective logistic regression analyses were conducted to explore the association between the individual virus markers and depression diagnosis at wave 4 and to examine the association between pathogen burden and depression diagnosis.

All analyses were adjusted for potential confounders. In the first step, demographic variables (age, sexe, and SES) (demographics) were added. Additionally, hsCRP was included to demographics model to adjust for possible mediating effects (hsCRP). In the final step, physical activity, smoking, and BMI were added to the hsCRP model (health behaviors). In the prospective analyses only, depressive symptoms determined at wave 3 were added to the health behaviors model. All analyses were conducted in PASW statistics 17.

Results

Sample characteristics

Table 1 shows the sample characteristics at wave 4 according to the presence of a depression diagnosis in the past 12 months. Of the 1057 adolescents 127 were diagnosed with a major or minor depressive disorder in the past 12 months. Compared to adolescents without a depression, those with a depression were on average older (p=.02), more often female (p<.001), more often had a low socio-economic background and less often had a high socio-economic background (p=.03). Finally, depressed adolescents were more often current smoker and less often former smoker (p=.03).

Cross-sectional analysis of virus infection and depressive symptoms

Linear regression analyses revealed evidence for a cross-sectional association between EBV (p=.03), but not HSV-1 (p=.26) and CMV (p=.17) and depressive symptoms in adolescents (Table 2). Adjusting for demographics, hsCRP, and health behaviors made the association between EBV and depressive symptoms non-significant (p≥.13), but hardly changed the magnitude of the association.

Table 1. Sample characteristics (N = 1057)

	Depressed (N = 127)		Not depressed (N=		
	N or M	% or SD	N or M	% or SD	p-value
Age	19.1	0.6	19.0	.5	<.01
Sex (male)	34	26.8	448	48.2	<.01
Socio-economic					
Low	26	20.5	149	16.0	.131
Middle	67	52.8	453	48.7	
High	34	26.8	328	35.3	
BMI	23.4	4.6	22.9	3.7	.21
Smoking					
No	41	32.3	443	47.6	<.01
Former	35	27.6	267	28.7	
Current	51	40.2	220	23.7	
Physical activity	3180.7	3345.0	3548.9	4956.1	.42
hsCRP mg/L median	.50	.20-1.6	.40	.20-1.00	.04
HSV1 median (IQR)	.16	.11-.96	.16	.11-.96	.70
HSV 2 median (IQR)	.07	.05-.10	.07	.06-.11	.16
EBV median (IQR)	.78	.14-1.18	.69	.12-1.08	.43
CMV median (IQR)	.32	.22-.80	.32	.23-.98	.69
Pathogen burden					
None	53	41.7	433	46.6	.23
One	54	42.5	345	37.1	
Two	19	15.0	120	12.9	
Three	1	0.8	32	3.4	

Virus and hsCRP are determined at wave 3, socio-economic status is determined at wave 1. All other variables wave at 4. BMI = Body Mass Index, hsCRP = high sensitive C-Reactive protein, HSV 1 = Herpes Simplex Virus 1, HSV 2 = Herpes Simplex Virus 2, EBV = Epstein Barr Virus , CMV = Cytomegalovirus.

The association between CMV and depressive symptoms showed a significant inverse association between CMV and depressive symptoms (p = .04) after adjustment for demographics, with sex being the most important covariates (Table 2). However, the magnitude of the association was hardly affected, suggesting that the significant association is a result from sex. The results remained essentially unchanged for HSV-1.

When the presence of a virus infection was analyzed as a pathogen burden, no significant results were found for number of viruses predicting depressive symptoms (p=.44).

Table 2. Virus infection as a predictor for depressive symptoms (N = 1057)

	Depressive symptoms at wave 3		
	β	95%CI	*p*-value
HSV 1			
Unadjusted	.06	-.09-.35	.26
Demographics*	.03	-.11-.31	.36
hsCRP	.03	-.12-.30	.40
Health behaviors**	.02	-.15-.27	.60
EBV			
Unadjusted	.07	-.04-.82	**.03**
Demographics*	.05	-.09-.66	.13
hsCRP	.04	-.10-.66	.14
Health behaviors**	.03	-.18-.57	.30
CMV			
Unadjusted	-.04	-.38-.07	.17
Demographics*	-.06	-.46--.02	**.04**
hsCRP	-.06	-.46--.02	**.04**
Health behaviors**	-.06	-.47--.01	**.04**

*Adjusted for wave 3 age, sex, and socio-economic status.

**Adjusted for demographics and wave 3 BMI, smoking, and physical activity

Bold faced p<.05

Prospective analysis of virus infection and depression diagnosis

Table 3 shows the results for the logistic regression analyses with depression diagnosis as outcome measure. None of the viruses were associated with a subsequent depression diagnosis (p>.24). Essentially the same results were found when using pathogen burden as a predictor for subsequent depression diagnosis (p=.84).

Discussion

The results from this study show an inverse association between CMV and depressive symptoms. We did not find support for an association of HSV-1 and EBV with the presence of depressive symptoms or any of the virus markers with subsequent depression diagnosis in adolescents. To our surprise CMV was associated inversely with depressive symptoms, but only after adjustment for

Table 3. Virus infection as a predictor for depression diagnosis (N = 1057)

	Depression		
	OR	95%CI	*p*-value
HSV 1			
Unadjusted	.97	.79-1.19	.80
Demographics*	.95	.77-1.17	.63
hsCRP	.94	.76-1.15	.54
Health behaviors**	.92	.74-1.13	.43
Depressive symptoms	.90	.72-1.12	.33
EBV			
Unadjusted	1.17	.83-1.16	.37
Demographics*	1.06	.75-1.51	.74
hsCRP	1.05	.74-1.50	.79
Health behaviors**	1.03	.72-1.47	.88
Depressive symptoms	.98	.67-1.44	.92
CMV			
Unadjusted	.96	.78-1.18	.71
Demographics*	.90	.73-1.12	.35
hsCRP	.91	.73-1.12	.37
Health behaviors**	.88	.71-1.09	.24
Depressive symptoms	.94	.75-1.18	.59

*Adjusted for wave 4 age, sex, and socio-economic status.

**Adjusted for demographics and wave 4 BMI, smoking, and physical activity

demographics. Sex was found to be the most important confounder and probably explains the inverse association between CMV and depressive symptoms. It should be kept in mind that the magnitude of the association was reasonably small and therefore probably not clinically relevant.

To our knowledge this is the first study to report on viral infections and depression in a young, adolescent population of healthy individuals. Previous reports have been published on presence of viruses and depression in adult samples, and reveal results opposite to ours[8-10]. For instance, Miller and colleagues found in sample of patients with ACS that the presence of viruses was positively associated with depression. Moreover, especially those patients presenting with three viruses had more severe depressive symptoms[9]. In our sample, pathogen burden was still relatively low, with only a small number of participants presenting with three viruses, which may be an explanation for the discrepancy in results. Further support for the depression – viral infection link comes from a study in elderly participants. Philips and colleagues found that the seropositivity for CMV alone was

not associated with depressive symptoms, but that participants with higher antibody titers to CMV reported more depressive symptoms[8], suggesting that the depressive symptoms are a reflection of recent or reactivated infection[8]. Increases in antibody titers to CMV, but also EBV have been associated with aging and could reflect increased frequency of virus reactivation in older people[28]. Psychological stress is one of the mechanisms that can reactivate latent viruses[29-31] and it is plausible that the young sample used for this study, experienced relatively little psychological stressors that can contribute to virus reactivation.

The results of our study suggest that virus infection is not associated with subsequent depression diagnosis, i.e. depression emerges independently of virus infection. However, based on results from other studies, it could be hypothesized that depression in the presence of latent viral infections can reactivate the viruses[29-32]. This could possibly lead to microglial activation, a process involved in neuroinflammation[33, 34] which has been implicated to play a role in the development and progression of depression[35]. This neuroinflammatory induced depression could then possibly reactivate the virus again, suggesting a vicious circle. This vicious circle could serve as a possible physiological explanation for recurrent or persistent depression and warrants further research.

The longitudinal nature of this study provided us with the opportunity to take a closer look into the prospective association between viral infections and depression in adolescents. However, some limitations need to be addressed as well. First, antibody status does not provide information regarding the specific time in life the viral infection took place, and thus the effects of longer or shorter duration of viral infections cannot be taken into account. Furthermore, because we only have data on antibodies from the wave 3 assessment available, we are not able to look into the possible long term effects of depression on virus reactivation. Because of the relatively young age of this sample, the results should be interpreted carefully, as the results are probably not generalizable to adults.

In conclusion, we found little support for a cross-sectional association between CMV and depressive symptoms, but no prospective associations between virus infection and depressive symptoms or subsequent depression. These findings can be attributed to the relatively young age of the sample used for this study. This does not rule out an association between viral infections and depression. Further research is needed to investigate a possible vicious circle between depression and virus reactivation.

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Chapter 9

General Discussion

General discussion

The main objective of this thesis was to investigate the relationship between depression and inflammation over the lifespan. More specific, this thesis aimed to evaluate the association between recurrent and single episodes of depression, and to examine differences between somatic and cognitive symptoms of depression in their relationship with inflammation in various study samples. Within these research objectives, the effects of health behaviors on the depression-inflammation link were investigated. Finally, another possible pathway between depression and immune function was investigated; the prospective association between viral infection and depression.

In order to look into depression and inflammation in light of a life perspective, data from 3 prospective cohort studies were used that included samples increasing in age. The results presented in this thesis are summarized in table 1.

The youngest sample came from the TRacking Adolescents' Lives Survey (TRAILS) and was used to evaluate trajectories of depressive symptoms, somatic symptoms and cognitive symptoms of depression and their association with subsequent high sensitive C-reactive protein (hsCRP) in 1166 adolescents (chapter 7). Data was available from 3 time points assessed biennially over a 5-year follow up period. In chapter 8, the presence of herpes simplex 1 (HSV-1), epstein barr virus (EBV), and cytomegalovirus (CMV) was investigated in association with depressive symptoms and depression diagnosis in 1057 adolescents.

Data from the Netherlands Study of Depression and Anxiety (NESDA) was used to examine depression and inflammation in a middle-aged community sample. In chapter 5 the cross-sectional association between depression diagnosis, as measured with the Composite Interview Diagnostic Instrument (CIDI)[1], with hsCRP, interleukin 6 (IL-6), and tumor necrosis factor α (TNF-α) was investigated in 2415 participants. The differential associations between somatic and cognitive symptoms of depression and anxiety with hsCRP, IL-6, and TNF-α were examined in 2681 participants (chapter 6).

Table 1. Summary of the associations between depression, depressive symptoms, and anxiety symptoms with inflammation presented in this thesis reflected by Cohen's d	Sample		
	CHD patients	Community sample	Adolescents
Diagnosis versus symptoms			
Diagnosis		++++(hsCRP)	- (HSV-1)
Symptoms	+[a] / +++[b] (hsCRP)[c]	+ (hsCRP, IL-6, TNF-α)	- (hsCRP)
Recurrent versus single episode	++ (hsCRP)		+++ (hsCRP)
Somatic versus cognitive			
Somatic symptoms of depression		++ (hsCRP)	- (hsCRP)
Cognitive symptoms of depression		- (hsCRP, IL-6, TNF-α)	- (hsCRP)
Anxiety symptoms		+ (hsCRP)	
Somatic symptoms of anxiety		++ (hsCRP, IL-6)	
Cognitive symptoms of anxiety		- (hsCRP, IL-6, TNF-α)	
Health behaviors	‡	‡	‡

CHD = Coronary Heart Disease (a = chapter 2, b = chapter 3 & 4), Community sample (Chapter 5, 6), Adolescents (Chapter 7, 8)

+ indicates an association before adjustment for health behaviors and reflects Cohen's d: +: d <.10; ++ d = .10 -.20; +++ d = .20 - .30; ++++ d >.30

-indicates no association, ‡ = significant mediating effect of health behaviors on the association between depression and inflammation, c = unadjusted association.

MDD = major depressive disorder; IDS = Inventory of Depressive Symptomatology; BAI = Beck Anxiety Inventory

The heart and soul study is a long term prospective cohort study, from which data of 667 patients with stable coronary heart disease (CHD) were used. Data from the baseline assessments and 5 consecutive annual assessments were available to evaluate the prospective bi-directional association of depression with high sensitive hsCRP, IL-6, and fibrinogen (chapter 3), and with total white blood cell (WBC) count (chapter 4). Furthermore, the presence of single episodes of significant depressive symptoms was compared to recurrent significant depressive symptoms in their bidirectional association with inflammation.

Depression and inflammation

Various studies have reported on the association between depression and inflammation both in healthy samples and in patients with CHD. Results reported range from negative associations to positive associations. A 2009 meta-analysis conducted by Howren and colleagues provided more insight and reported an overall positive association between depression and hsCRP (Cohen's d = .15) and IL-6 (Cohen's d = .25) in healthy and clinical samples with a rather modest effect size[2].

Following this meta-analysis, we conducted a meta-analysis specifically aiming at patients with established CHD using a more strict definition of CHD (chapter 2). We only included patients with established CHD and not subclinical disease such as atherosclerosis, and end stage heart disease, such as heart failure. Overall, we found a small to moderate and significant effect size (Cohen's d = .30) for depressive symptoms and CRP, but not for IL-6. This is in contrast with the findings from Howren and colleagues who reported a smaller effect size for CRP and a significant effect size for IL-6[2]. This could be due to our more strict inclusion criteria, which excluded studies with possible or subclinical CHD, but also excluded more advanced stages of heart disease. One major limitation of our meta-analysis is that we were not able to properly adjust for potentially mediating variables[3], such as health behaviors. Furthermore, only cross-sectional studies were included which makes it impossible to draw conclusions on the direction of the association between depressive symptoms and CRP.

Diagnosis versus symptoms

The meta-analysis from Howren and colleagues showed that depression diagnosis was more strongly associated with inflammation than depressive symptoms were[2].

On average the effect size for depression diagnosis could be considered moderate, whereas the effect size for depressive symptoms was small. In our meta-analysis, we found, compared to Howren and colleagues, a larger effect size for CRP and no significant effect size for IL-6 in relation to depressive symptoms in patients with CHD. This suggests that results can differ according to the study sample used in terms of age and disease status, but also type of depression assessment (self-report versus clinical interview).

In the oldest sample presented in this thesis, patients with CHD, we found that depressive symptoms averaged over a 6 year period were associated with subsequent inflammation (hsCRP, IL-6; chapter 3, and WBC count; chapter 4) (table 1). Depression was also associated with higher levels of inflammation in middle-aged men (chapter 5). More specifically, current depression diagnosis was associated with higher levels of hsCRP and marginally higher levels of IL-6, but not TNF-α in men only. Especially men with a late-onset of depression had higher levels of inflammation. In a subgroup of clinically depressed participants, hsCRP and IL-6 levels were higher when participants used tricyclic (TCA) and tetracyclic (TeCA) antidepressants, and in men using serotonin noradrenergic reuptake inhibitors (SNRI). This is in line with previous studies that reported on antidepressant medication use and inflammation which showed that TCA use was associated with higher levels of inflammation[4], whereas SSRI use was associated with a decrease in inflammation[5]. This may be a reflection of noradrenergic effects on inflammation. Comparable to a diagnosis of depression, depressive symptoms were also found to be associated with increased levels of IL-6, hsCRP, and TNF-α in the same study sample (chapter 6), even though the effect sizes were smaller than for clinical depression (Table 1) and mediated by health behaviors. Finally, we did not find support for a cross-sectional association between depressive symptoms and inflammation in adolescents.

Depression and inflammation were associated in both middle-aged participants and patients with CHD. However, the effect sizes were strongest in CHD patients and when a depression diagnosis was used (table 1). This suggest that there may be a dose-response relationship in the association between depression and inflammation, with the most severe depression being most strongly associated with inflammation and with the highest effect sizes being present in patients with the most disease burden (table 1). Earlier research has indeed shown that depression diagnosis is moderately associated with inflammation[6-8]. In contrast, a recently published study reported no association between clinical depression and a broad range of cytokines, but this study consisted of a relatively small sample (depressed

N = 68)[9]. Furthermore, our results showed that especially late-onset depression was associated with inflammation. Late-onset depression has been associated with a family history of vascular disease[10] and atherosclerosis, a subclinical risk factor for cardiac disease[11, 12]. This suggests that the association between late-onset depression and higher levels of inflammation are possibly a reflection of subclinical disease, which becomes more prevalent with aging. Moreover, in comparison to the meta-analysis from Howren and colleagues[2], we found stronger effect sizes for the studies presented in this thesis on patients with CHD (chapters 2, 3, and 4). This is possibly due to the prospective data we used which enabled us to disentangle the relationship between recurrent depressive symptoms and inflammation, compared to a single episode of depressive symptoms. Another possible explanation comes from the vascular depression hypothesis, which states that depression can be the result of vascular damage in the brain[13], e.g. white matter changes[14]. As higher levels of inflammation are involved in vascular diseases[15], this damage could be the result of inflammation.

Single episode versus recurrent depression and inflammation

Research has suggested that mainly recurrent depression is associated with adverse health outcomes. For instance, greater progression of coronary artery calcification[16], hypertension[17], but also new cardiac events in post myocardial patients[18] are found to be more prevalent in those who suffer from depression more frequently. It could be hypothesized that this also accounts for inflammation, as inflammation is associated with both depression and (risk factors for) CHD.

This hypothesis has been evaluated in this thesis in a sample of 667 patients with stable CHD and in a sample of 1166 adolescents. The results showed that recurrent significant depressive symptoms were associated with higher levels of subsequent hsCRP (chapters 3 and 7), IL-6 (chapter 3), and WBC count (chapter 4) (table 1). Furthermore, the results showed that the associations with hsCRP and IL-6 were mediated by health behaviors, whereas the association with WBC count was not. A possible explanation for this difference could be that recurrent significant depressive symptoms are a reflection of chronic stress, which may stimulate hematopoietic stem cells in the bone marrow to produce WBCs[19]. In this cascade, the role of health behaviors might be less important than in the more downstream depression-cytokine relationship. One major limitation in the WBC study was that we only looked into total WBC count and that we were not able to differentiate between the individual WBC's, such as monocytes and neutrophils. This is

important, as there is evidence that especially monocytes are associated with atherogenesis[20], history of cardiac disease[21], and psychological complaints[22].

We did not find support for a prospective association between inflammation and subsequent significant depressive symptoms. This is in concurrence with findings from other studies who also reported that depression predicted higher inflammation, but not vice versa[23, 24]. However, one study reported that inflammation preceded depressive symptoms[25], though this study only looked into 4 items of the General Health Questionnaire which does not cover all the symptoms of depression. In accordance with the results we reported on patients with CHD, we found that adolescents with persisting depressive symptoms over a 5-year follow-up also had higher levels of subsequent hsCRP, suggesting that the pro-inflammatory effects of recurrent depressive symptoms are not restricted to older samples only (chapter 7). Also, the association between recurrent depressive symptoms and hsCRP in adolescents was mediated by health behaviors.

The results on recurrent versus single episodes of depression presented in this thesis confirm previous findings in various populations. Earlier retrospective studies[26, 27] and one prospective study[24] conducted in various age samples reported that participants with recurrent depressive symptoms had higher levels of inflammation than those who reported a single episode of depression[24, 26, 27]. Integrating these findings with the results from this thesis provides substantial evidence that the association between depression and inflammation is especially apparent in those who repeatedly suffer from depressive symptoms and that this association is already present from early age on. However, we did not find support for an association between inflammation and duration of depressive symptoms in a middle-aged sample (chapter 5). One major difference between this study and the studies conducted in adolescents (chapter 7) and CHD patients (chapter 3 and 4) is that these were prospective studies, whereas duration of depressive symptoms in the middle aged sample was determined retrospectively which may be affected by recall bias.

A possible explanation for the above described results could be that the recurrent depressive symptoms reflect a more chronic state of depression or chronic stress. In reaction to stress the hypothalamus is activated to produce corticotrophin-releasing hormone (CRH) which stimulates the pituitary gland to produce adrenocorticotropic hormones (ACTH). Under the influence of ACTH, the adrenal cortex secretes cortisol, collectively termed as the hypothalamus-pituitary-adrenal (HPA) axis response to stress[28, 29]. Stress can be divided into acute stress and

chronic stress, with acute stress lasting for a period of minutes or hours and chronic stress being more persistent and experienced for several hours per day, during weeks or months[29]. Acute and chronic forms of stress have different effects on the immune system. In the case of acute stress, cortisol is immunosuppressive, i.e. enhancing the production of anti-inflammatory markers and inhibiting the secretion of pro-inflammatory markers[30]. However, it has been found that under the influence of chronic stress the immune system is less responsive to the anti-inflammatory actions of cortisol. This glucocorticoid (GC) resistance of T cells results in an increased production of cytokines and may result in an upregulation of systemic inflammation[28, 31-33]. Another possible pathway linking stress to inflammation is the autonomic nervous system (ANS) (for an overview see[5, 32, 34-37]). Under the influence of stress the sympathetic nervous system (SNS) can be activated which results in increased levels of catecholamines. Catecholamines are found to be associated with higher levels of inflammation. Moreover, there is some evidence that reduced activity of the parasympathetic nervous system is associated with reduced acetylcholine sensitivity, which is hypothesized to be associated with dysregulated inflammatory control and thus possibly higher levels of inflammation[34]. Furthermore, the immune responses to stress can be defined in terms of their end effects as 1) immunoprotective, which is characterized by a rapid and robust immune response, 2) immunoregulative, which concerns inhibiting of immune cells, and 3) immunopathological, which involves chronic inflammation[29]. It has been suggested that these latter immune responses depend on resilience, the capacity of psychological and interacting physiological systems to recover from challenging conditions[29] and thus determine whether the response has a negative or positive influence. It could also be suggested that in this stress-immune model health behaviors play a mediating role by either stimulating persisting experience of stress or directly upregulate systemic inflammation. Stress and more specific, HPA-axis alterations have been found to be associated with obesity[38, 39] and smoking behaviors[40]. Taking all this together results in a stress-behavior-immune model as depicted in figure 1. Under the influence of chronic stress cortisol is secreted by the HPA-axis. This prolonged stress reaction dysregulates the pro-inflammatory processes through GC resistance.

Furthermore in response to stress, the ANS upregulates inflammation, either in synergy with the HPA-axis or on its self. In this final pathway, the negative effect of GC on health behaviors could have an additional upregulating effect on inflammation. This stress-behavior-immune model is worth to be considered in future research.

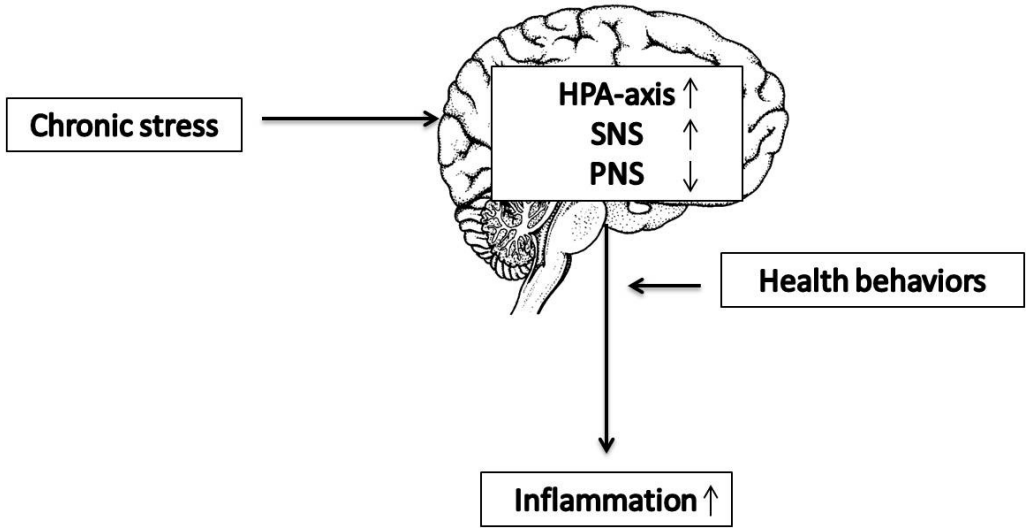

Figure 1: Graphical representation of stress-behavior-immune model. Chronic stress activates the HPA-axis and the sympathetic nervous system (SNS), whereas it reduces the activity of the parasympathetic nervous system (PNS), resulting in immunopathological responses through adverse health behaviors.

Somatic versus cognitive symptoms of depression and inflammation

Research suggests that mainly the somatic symptoms of depression (e.g. sleeping difficulties, psychomotor retardation or activation, changes in eating behavior, and fatigue), and not the cognitive symptoms of depression (e.g. difficulties concentrating, loss of interest, and depressed mood) are associated with worse prognosis in CHD patients[41-43]. Based on the sickness behavior theory, this idea has been adopted and applied to depression-inflammation research. The sickness behavior theory states that motivational behaviors, such as less moving and eating, and more sleeping, emerge in the presence of higher levels of inflammation as a reflection of sickness[44]. These motivational behaviors show a resemblance with the somatic symptoms of depression. It could thus be argued that the worse health outcomes associated with somatic symptoms of depression are associated with inflammatory processes that may reflect the presence of cardiac disease. With this idea in mind we investigated whether somatic symptoms and cognitive symptoms are differentially associated with inflammation in a middle-aged sample and an adolescent sample.

In line with this idea, we found that somatic symptoms of depression, but not cognitive symptoms of depression were significantly associated with hsCRP, IL-6 and TNF-α in middle-aged participants (chapter 4), but health behaviors played an important mediating role in this relationship. This is in line with findings from previous studies Elovainio and colleagues who found that somatic symptoms were more strongly associated with inflammation in men than in women[45]. However, these results were not mediated by the effects of health behaviors[45]. Contrasting results have also been published though. Kupper and colleagues found that both somatic and cognitive symptoms were cross-sectionally and prospectively associated with inflammation[46]. One possible explanation for this difference could be that the study from Kupper and colleagues consisted of an older sample of heart failure patients. Levels of inflammation have been found to increase with age and patients with heart failure patients are exposed to inflammation for a longer period of time, due to their condition. Prolonged exposure to inflammation can result in a priming effect, in which the pro-inflammatory response to a pathogen or invader is exaggerated[47] and might therefore result in greater depressive symptoms. Furthermore, depressive symptoms are possibly associated with vascular damage, as suggested by the vascular depression hypothesis[13].

In contrast to the findings in the middle-aged sample, we did not find support for a differential association between somatic and cognitive symptoms of depression and inflammation cross-sectionally and over a 5-year follow up period in adolescents. This suggests that somatic symptoms of depression are experienced in the *presence* of systemic inflammation, but not until adulthood. This may represent another possible pathway through which depression and inflammation interact next to the stress-behavior-immune model. Based on the sickness behavior theory, which suggests that somatic symptoms of depression emerge in the presence of upregulated inflammation[44], it could be argued that somatic symptoms are preceded by inflammation. According to the sickness behavior theory, these somatic symptoms can be viewed as a motivational state. This is supported by studies in cancer patients. Patients with cancer treated with IFN-α therapy were found to develop the somatic symptoms of depression. Only a subgroup of patients developed cognitive symptoms of depression, but these could effectively be treated with an antidepressant (Paroxetine)[48, 49]. This suggests that the somatic symptoms are a direct response to the inflammatory process present in the body. This is also supported by a study from Kupper and colleagues who found that *change* in somatic symptoms over a 12-month period was associated with inflammation at 12 months follow-up, and not baseline levels of somatic symptoms of depression. This suggests that those who had higher levels of somatic symptoms

at follow-up than they had at baseline, were also having higher levels of inflammation[46]. In fact, this could be the effect of higher levels of inflammation at follow-up. Taken together, somatic symptoms of depression are possibly a direct response to inflammation, but not until adulthood. This association might be a reflection of (subclinical) disease, but data on this is currently lacking.

Anxiety symptoms and inflammation

As with depressive symptoms, anxiety symptoms are associated with higher risk for CHD in healthy[50] and cardiac patients[51]. Some research has even suggested that mainly the somatic symptoms of anxiety are associated with CHD risk[52] and inflammation could possibly play a role in this relationship.

Chapter 6 provides preliminary evidence for an association between symptoms of anxiety and inflammation in middle-aged men (table 1). This is in line with previous findings[53-55], though our results suggest that the anxiety-inflammation relationship is mainly driven by somatic symptoms. This sheds a new perspective on the association between anxiety and inflammation. Somatic symptoms of anxiety consist of hot flushes, respiration, heart pounding, shaking hands and difficulty breathing. In contrast to somatic symptoms of depression, somatic symptoms of anxiety are not similar to symptoms of sickness behavior, but merely a reflection of increased sympathetic autonomic control, suggesting a role for the sympathetic nervous system (SNS). The SNS has been associated with higher levels of inflammation[5, 35, 3]6 and could thus be involved in the anxiety-inflammation link. However, studies examining anxiety and the ANS show conflicting results[56-58] as to whether the ANS and anxiety are associated. Possibly, the ANS is only involved in the somatic symptoms of anxiety and affects immune function simultaneously. As with depression, health behaviors played an important mediating role in the association between anxiety and inflammation and may serve as a therapeutic target for interventions. Based on the results presented in this thesis it could be hypothesized that inflammation plays a role in the link between anxiety and CHD. Further research on this subject in which inflammation, ANS function and symptoms of anxiety are included is therefore warranted.

Viral infection and depression

There is some evidence suggesting that the presence of viruses is associated with depression in adulthood, but also CHD. It may be another physiological explanation of the relationship between depression and CHD.

In this thesis the association between viral infection and both depressive symptoms and clinical depression was evaluated in adolescents. We found little cross-sectional evidence for an inverse association between CMV and depressive symptoms. For the other virus markers we found no association with the development of depressive symptoms and subsequent depression (chapter 8). However, the possibility of an association between viral infections and depression cannot be ruled out based on these results only. First, previous research has shown positive associations in adult populations[59-61] and secondly, a clear association might not be present until (frequent) reactivation of the virus[59]. Reactivation can be determined by antibody titers and is found to be more prevalent with aging[62]. One of the mechanisms behind reactivation of latent viruses is psychological stress[63-65], resulting in the activation of microglia, which is involved in neuroinflammation[66,67]. Neuroinflammation in the limbic area has been implicated to play a role in the development and progression of depression[68], which might reactivate a dormant virus. This whole process might be a reflection of a vicious circle and could serve as another possible physiological explanation for recurrent or persistent depression and is worth to be investigated in future research.

Clinical implications of adverse health behaviors

Throughout this thesis, health behaviors were found to play an important mediating role in the association between depressive symptoms and inflammation. In all chapters, health behaviors were considered as mediators of the relationship between depression and inflammation, and were therefore added to the analyses as a separate group of variables. In support of previous research[23, 27], this thesis showed that markers of (un)healthy behaviors play an important role in the association between depressive symptoms and inflammation in all samples (Chapters 3, 6, 7), but not in the presence of depression diagnosis (Chapter 5). Especially BMI and (former) smoking were found to be important health behaviors in this relationship (chapter 6 and 7). This is consistent with other findings[23, 69, 70]. It is well known from previous literature that smoking[24, 71] and BMI[72-74] are associated with both depressive symptoms and inflammation[3]. Obesity has been found to be associated with both depression[72] and inflammation[75]. A meta-analysis on the

prospective association between overweight and depression showed that the relationship is bi-directional: depression predicted obesity and obesity was a risk factor for depression[72]. An alternative explanation for this bidirectional association is that there is a third, common denominator, i.e. a genetic predisposition to both higher weight and depression. Obesity has also been found to be associated with higher levels of inflammation[3, 74] and this may be attributed to the pro-inflammatory characteristics of adipose tissue that is associated with IL-6 and TNF-α production[3, 74].

Higher levels of inflammation are involved in the development of various inflammatory diseases, such as atherosclerosis[20, 76, 77], asthma[78], and it has been suggested that depression may be associated with diabetes through inflammation[79]. These effects are already present in adolescence as hsCRP levels were found to be independent predictors of carotid intima media thickness in adolescents[80]. Taken together, it could be argued that depressive symptoms contribute to the development and progression of somatic diseases through inflammation with a possible role for adverse health behaviors. Promoting a healthy lifestyle could thus be an important therapeutic intervention strategy in lowering levels of inflammation and may finally have a positive effect on health outcomes. This is supported by Dod and colleagues who found that intervening on lifestyle by changing participants dietary intake and enhancing moderate exercise, had positive effects on lowering inflammation levels after 12 weeks[81]. Improving a healthy lifestyle is an important key priority of the American Heart Association in both adolescents[82] and adults[83, 84] and it has been shown that maintaining a healthy lifestyle during adolescence is associated with a better cardiovascular risk profile in later life[85]. Promoting a healthy lifestyle should therefore be part of standard treatment of depression or depressive symptoms, already in adolescence and adults without overt CHD in order to lower the possible adverse effects of inflammation on health.

Methodological considerations

Many methodological strengths come with the use of large observational studies to examine the association between depression and inflammation, but there are also important limitations that need to be addressed and taken into account when interpreting the results.

One of the major strengths that accounts for all the observational studies conducted in this thesis is the large sample sizes (N > 667). Large samples sizes are especially important in order to detect true relationships, reflected in statistical power. With this we were able to provide more insight on the associations between depression and inflammation and relevant characteristics of this association.

In this thesis health behaviors were considered as possible mediators in the association between depression and inflammation. In order to consider health behaviors as a mediator, the variables must confer to three criteria (also see figure 2)[86]:

1. Depression must be significantly associated with inflammation.
2. The association between depression and health behaviors must be significant.
3. Health behaviors must significantly predict inflammation in an equation which also includes depression

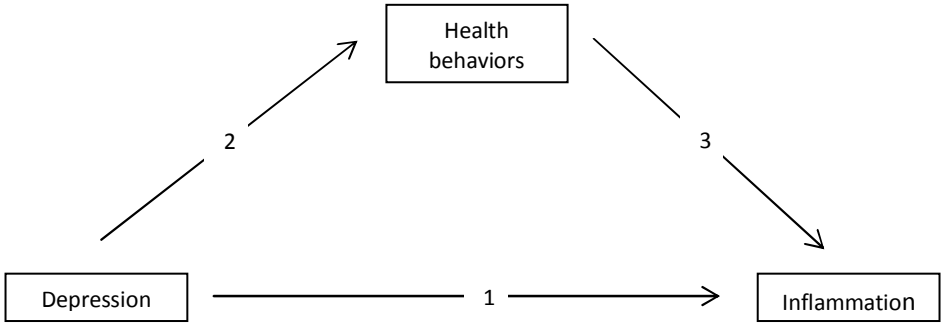

Figure 2: Graphical representation of mediation model including depression as a predictor of inflammation, with health behaviors as a mediator.

Theoretically, health behaviors can be considered as mediators in the depression and inflammation relationship, as been proven by our and other studies; 1) depression was associated with inflammation in every study (Chapters 2-7), 2) previous research has shown that depression is associated with health behaviors such as BMI[72], physical activity[87], and smoking[88], and 3) some of our results have shown that BMI (chapter 5,6,7) and smoking (chapter 6 and 7) are significantly

associated with inflammation with depression in the equation. Statistically however, in the analyses conducted in this thesis (regression analyses and general linear models), it is not possible to determine if health behaviors were actually acting as mediators or as confounders. These analyses are restricted to adding covariates and do not distinguish between confounding or mediating effects of these covariates. More advanced methods, such as structural equation modeling, can be used to determine estimations for paths 1, 2, and 3 (figure 2). Preferably a prospective design to evaluate change over time in the outcome variable and multiple mediators[89] are needed to elucidate the mediating effects of health behaviors in the depression-inflammation relationship.

Finally, due to the observational nature of the studies used in this thesis it is not possible to draw any conclusions regarding causality. Causality can only be determined with a randomized control trial design, in which participants are randomly assigned to either an "exposed" group in which they are exposed to a manipulated condition, or a "control" group. It cannot be ethically justified to "expose" participants to depression and is thus not feasible in this research area. Longitudinal data is therefore the next best thing. In this thesis we were able to use longitudinal data in 4 studies and this presented us with the opportunity to determine that (recurrent) depression precedes inflammation. However, we were not able to rule out effects of, for instance high levels of inflammation preceding the baseline assessments. Therefore long term observational cohort studies consisting of frequent and careful assessments of depression and inflammation plus confounding and mediating variables, are needed to further elucidate the association between depression and inflammation.

Future directions

Future research should consist of a more thorough follow-up, in which depression, depressive symptoms and inflammatory markers should be assessed together, with attention for hsCRP, IL-6, TNF-α, and individual WBC's. As anxiety is another important predictor of adverse cardiac outcomes, and there is preliminary evidence that anxiety is associated with inflammation, this pathway should be considered in new research initiatives.

As health behaviors were found to play an important role in the relationship of depression and anxiety with inflammation, it is worth to further investigate the effects of intervening in health behaviors in patients with depression or anxiety.

This should be examined, preferably with a randomized control design, by promoting a healthy lifestyle aiming at smoking cessation and lowering dietary intake in both patients with cardiac disease and cardiac disease free people. Finally, the association between viral infections and depression needs to be further elucidated in adolescents and adults to provide more insight in the possible vicious circle regarding depression and viruses.

Overall conclusion

This thesis extends previous studies on the association between depression and inflammation. We found that this relationship is already present in adolescence, but becomes stronger with increasing age. Especially a depression disorder is most strongly associated with inflammation. Particularly recurrent depressive symptoms precede inflammation, but inflammation does not precede recurrent depressive symptoms. Moreover, somatic symptoms, but not cognitive symptoms, seem to be associated with inflammation, possibly as a reflection of sickness behavior. Furthermore, we found preliminary evidence for an association between symptoms of anxiety and inflammation.

Adverse health behaviors, especially BMI and smoking explain the associations found for depression and anxiety with inflammation. These health behaviors can serve as a possible therapeutic target to reduce levels of inflammation which may have positive effects on health outcomes. Finally, we did not find an association between viral infections and depression probably because of the young age of the sample. More research is needed to further elucidate the virus-depression relationship.

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Summary

General introduction

Depression is found to be associated with subsequent cardiovascular risk in initially healthy people. Also, people who already have established coronary heart disease (CHD), have a higher risk of developing a depression or feelings of depression. Around 20% of all CHD patients suffer from depression in the months following a myocardial infarction (MI). Another 20% report depressive symptoms that do not fulfill the criteria for a depression diagnosis. Together, these rates are around three times as high as in the general population. Furthermore, these depressive episodes of depressive symptoms have a negative influence on cardiac morbidity and mortality after a MI. The past decennia a range of potential (physiological) mechanisms have been proposed to explain the negative influence of depression on CHD and one of these is inflammation. Inflammation is a collective term that describes the body's response to infection or injury and is involved in destroying the invader and initializing tissue repair in the affected area. Inflammation can be determined with a range of inflammatory markers measured in the blood. Inflammation is also involved in the development and progression of atherosclerosis, which is a risk factor for CHD. There is some evidence suggesting that depression and inflammation are associated in both healthy people and patients with cardiac disease. However, little is known about the direction of the relationship, does depression cause inflammation or does inflammation cause depression?

The general aim of this thesis was to thoroughly investigate the association of depressive symptoms and depression with inflammation across the lifespan, especially focusing on the nature of the depression (clinical vs. subclinical depression, type of symptoms (somatic versus cognitive), and recurrence) and the directionality of the relation between depression and inflammation. Furthermore, in this thesis the influence of adverse health behaviors on the depression-inflammation relationship will be examined. Finally, other possible pathways will be investigated; the association between anxiety and inflammation and the relationship between viral infections and depression. The inflammatory biomarkers included in this thesis were C-reactive protein (CRP), interleukin (IL) 6, tumor necrosis factor (TNF) α, fibrinogen, and white blood cell count.

Depression and inflammation

Systematic reviews of the literature and meta-analyses have shown that depression is associated with CRP and IL-6 in various populations, including CHD patients and psychiatric patients. These studies showed that the strength of the association is stronger as the depression becomes more severe (symptoms versus diagnosis). Following an earlier meta-analysis, we conducted a meta-analysis in chapter 2, for which we used a more strict definition of CHD than previous meta-analyses did. Our results showed a small to medium effect size for the association between depressive symptoms and CRP, but no significant effect for the association with IL-6. The association between depression diagnosis and inflammation was further examined in chapter 5 in 2415 middle-aged participants with and without a current depression diagnosis. The results showed that men with a current depression, but not women, had higher levels of inflammation (CRP and IL-6) than men without a current depression. More specifically, especially men with an older age of onset of depression had the highest level of inflammation (CRP and TNF-α). Furthermore, when we compared the use of specific antidepressant medication, the results showed that levels of CRP and IL-6 were higher in men using serotonin-norepinphrine reuptake inhibitors, and in men and women using tri- and tetracyclic antidepressants (CRP). However, in men, CRP decreased when using selective serotonin reuptake inhibitors. These findings clearly show a relationship between depression and inflammation and suggest that the association is especially present when the age of onset of depression is higher. Comparable to chapter 5, we found in chapter 6 that depressive symptoms were associated with higher levels of inflammation. However, adverse health behaviors (smoking, BMI, physical inactivity, and alcohol use) played an important role in this association. Taken together, these results show that the relationship between depression and inflammation was strongest in participants with a depression diagnosis, which suggests a dose-response relationship; the most severe depression is most strongly associated with higher levels of inflammation. Furthermore, our results showed that especially late-onset depression was associated with inflammation. Previous research reported that late-onset depression is associated with a family history of vascular disease and atherosclerosis. This suggests that the association between late-onset depression and higher levels of inflammation are possibly a reflection of subclinical disease, which becomes more prevalent with aging.

In chapter 3 and 4 we investigated the direction of the association between depressive symptoms and inflammation in an older sample of CHD patients. We found that depressive symptoms averaged over a six year period were associated

with subsequent inflammation (CRP, IL-6; chapter 3, and WBC count; chapter 4), but not vice versa. This suggests that depressive symptoms predict levels inflammations, but that inflammation does not predict future depressive symptoms. The results presented in chapter 7 underline these findings. We examined the association between depressive symptoms and CRP in 1166 adolescents over a seven year period and found that depressive symptoms precede higher levels of inflammation. The associations found in chapter 3 and chapter 7 were explained by the presence of adverse health behaviors, e.g. physical inactivity, smoking, and overweight, suggesting an important role for lifestyle factors. Taken all these studies together, we showed that depression and depressive symptoms in combination with adverse health behaviors predict higher levels of inflammation.

Single episode versus recurrent depression and inflammation

Research has suggested that mainly recurrent episodes of depression are associated with the adverse effects on health outcomes. It has been shown that greater progression of coronary artery calcification, hypertension, but also new cardiac events in post MI patients are more prevalent in those who suffer from depression more often. Possibly this also accounts for inflammation. In chapter 3 and chapter 4 we assessed whether CHD patients who repeatedly reported significant depressive symptoms had higher levels of inflammation than those who did not. In a sample of 667 stable CHD patients we found that those who reported significant depressive symptoms at two or more yearly assessments, compared to those who reported no significant depressive symptoms or at one assessment only had higher levels of IL-6, CRP (chapter 3) and white blood cell count (WBC) (chapter 4). Furthermore, the results showed that the relationship with IL-6 and CRP was mainly explained by adverse health behaviors, such as physical inactivity, overweight, and smoking. This was not the case for WBC count. In chapter 7, we evaluated the course of symptoms of depression in 1166 healthy adolescents over a period of seven years. The results showed that adolescents who persistently reported mild to high depressive symptoms had higher levels of CRP at follow-up. Comparable to chapter 3, we found that health behaviors played an important role in the association. Taken together, these results suggest that a cluster consisting of repeated experience of depressive symptoms, poor health behaviors and higher levels of inflammation could be a plausible explanation why people with depressive feelings are at greater risk for cardiac disease, new cardiac events or cardiac death than those who don't experience depressive feelings.

Somatic versus cognitive symptoms of depression

Research suggests that mainly the somatic symptoms of depression (e.g. sleeping difficulties, psychomotor retardation or activation, changes in eating behavior, and fatigue), and not the cognitive symptoms of depression (e.g. difficulties concentration, loss of interest, and depressed mood) are associated with a worse prognosis in patients with CHD. These somatic symptoms are comparable to the symptoms seen in sickness behavior. These symptoms emerge in the presence of upregulated levels of inflammation as a reflection of sickness. It could be hypothesized that the inflammatory processes present in cardiac disease are reflected in somatic symptoms of depression. In chapter 6 we cross-sectionally tested whether somatic and cognitive symptoms of depression were differentially associated with inflammation in a sample of 2861 participants with and without depression. The results showed that the somatic symptoms were associated with inflammation whereas the cognitive symptoms were not. However, the association was mainly explained by the effects of health behaviors, which suggests that the poor lifestyle of depressed patients puts them at risk for higher levels of inflammation. Chapter 7 discussed if somatic and cognitive symptoms of depression preceded higher levels of inflammation (CRP) in 1166 adolescents. In contrast to chapter 6, we did not find support for a differential association between somatic and cognitive symptoms with inflammation, both cross-sectionally and prospectively. This suggests that the association between somatic symptoms of depression and inflammation is only present in adults, possibly as a reflection of sickness behavior.

Anxiety symptoms and inflammation

It has been shown that next to depression, experiencing feelings of anxiety also has a negative influence on the development and progression of CHD, with some research suggesting that mainly the somatic components of anxiety (trembling, sweating, heart pounding) play an important role. In chapter 6 we examined if symptoms of anxiety were associated with inflammation, and if somatic and cognitive symptoms of anxiety were differentially associated with inflammation. Data from 2861 participants with and without a depression were analyzed. The results showed that especially men reporting symptoms of anxiety had higher levels of CRP and IL-6. Furthermore, the somatic symptoms of anxiety explained most of this association. Comparable to the findings on depressive symptoms adverse health behaviors played an important role. This suggests that men with

feelings of anxiety, together with an unhealthy lifestyle have a higher risk for upregulated levels of inflammation. This unhealthy lifestyle could serve as a possible therapeutic target in order to lower the risk for future CHD but more research is needed to further elucidate this.

Viral infections and depression

Earlier research has linked viral infections to mental disorders such as schizophrenia, with a possible role for inflammation. However, there is little known about an association between viral infections and depression. In chapter 8 we therefore set out to evaluate the cross-sectional and prospective association between markers of viral infection (epstein barr virus, cytomegalovirus and herpes simplex virus 1) and depression in 1057 adolescents. The results showed that viral infections were not associated with current depressive symptoms or future depression status in this relatively young sample. However, this does not rule out the possible effects of virus infections on depression status. As positive associations have been reported in adult populations it could be argued that associations are not visible until adulthood. More research is needed to further elucidate the association between viral infections and depression.

Concluding remarks

The overarching aim of this thesis was to provide more insight in the association between depression and inflammation using a life perspective. Overall it can be concluded depression and depressive symptoms are associated with inflammation. This relationship was found to be already present in adolescents and the strength of the depression-inflammation association increases with age. Moreover, this thesis showed that recurrent depressive symptoms predict subsequent inflammation, but inflammation does not predict (recurrent) depressive symptoms. Furthermore, this thesis showed that somatic symptoms of depression are cross-sectionally associated with inflammation, but cognitive symptoms are not. This is possibly a reflection of sickness behavior. Next to depression, we also found preliminary evidence for an association between anxiety and inflammation. Another important and consistent finding was the role of health behaviors in the association between both depression and anxiety with inflammation. The presence of adverse health behaviors, such as smoking, physical inactivity, and overweight

explain most of the associations found. In conclusion, this thesis showed that a cluster of (recurrent) depression or anxiety, and adverse health behaviors predict higher levels of inflammation. This possibly puts this specific group of depressed or anxious people at risk for future cardiac disease. These health behaviors could therefore serve as a possible therapeutic target to reduce levels of inflammation which may result in lowering the risk for future cardiac disease

Samenvatting

Algemene inleiding

Gezonde mensen met depressieve klachten hebben een verhoogd risico op het ontwikkelen van coronaire hartziekten (CHZ). Daarnaast is gebleken dat CHZ patiënten, en met name patiënten die recent een hartinfarct hebben gehad, vaker last hebben van een depressie of depressieve symptomen. Ongeveer 20% van alle CHZ patiënten ontwikkelt een depressie in de maanden nadat zijn een hartinfarct hebben gehad. Nog eens 20% rapporteert depressieve gevoelens te ervaren die niet voldoen aan de criteria voor een diagnose depressie. Daarmee komen depressie en depressieve gevoelens ongeveer drie keer zo vaak voor bij CHZ patiënten dan bij de algehele bevolking. Daarnaast heeft een depressie bij CHZ patiënten negatieve gevolgen voor de hartprognose; depressieve hartpatiënten hebben een verhoogd risico op nieuwe hartproblemen en vervroegd overlijden. De afgelopen decennia zijn er verschillende potentiële (fysiologische) mechanismen onderzocht die de negatieve invloed van depressie op hartproblemen zouden kunnen verklaren. Een van deze mechanismen is inflammatie. Inflammatie is een verzamelnaam voor de afweerreactie van het lichaam tegen vreemde organismes (zoals virussen en bacteriën) en treedt op bij verwonding en ziekte. Inflammatie is betrokken bij het onschadelijk maken van deze vreemde organismes alsmede het repareren van schade aan het weefsel. De mate van inflammatie kan bepaald worden door de aanwezigheid van verschillende markers in het bloed te meten. Inflammatie is ook betrokken bij de ontwikkeling en het verloop van atherosclerose (ook wel aderverkalking), een belangrijke risicofactor voor het ontstaan van CHZ. Eerder onderzoek heeft eveneens een verband aangetoond tussen depressie en inflammatie. Echter, er zijn ook negatieve verbanden gevonden en er is nog maar weinig bekend over de kenmerken van de relatie tussen depressie en inflammatie. Zo is het onduidelijk of depressie een voorspeller is van verhoogde inflammatie of dat juist inflammatie voorafgaat aan depressieve klachten. Daarnaast is het niet bekend of het in de relatie tussen depressie en inflammatie gaat om specifieke symptomen van depressie of de duur van de depressieve klachten.

Het doel van deze thesis was om een beter inzicht te krijgen in de relatie tussen depressie en inflammatie over de gehele levensloop. Meer specifiek hebben we onderzocht welke richting de relatie tussen depressie en inflammatie uitgaat en of het herhaaldelijk ervaren van depressieve klachten (recidiverende depressie) anders geassocieerd is met inflammatie dan het eenmalig ervaren van depressieve klachten. Tevens hebben we onderzocht of de meer somatische symptomen van depressie (o.a. eetproblemen en slaapproblemen) verschillend samenhangen met inflammatie in vergelijking met de meer cognitieve symptomen (o.a. gevoelens van

somberheid en concentratieproblemen). Binnen deze studies hebben we ook naar de effecten van gezondheidsgedrag gekeken (roken, overgewicht en fysieke inactiviteit), omdat eerder onderzoek heeft aangetoond dat gezondheidsgedrag sterk samenhangt met zowel depressie als inflammatie. Als laatste hebben we andere mogelijke relaties tussen psychische klachten en immuunactivatie onderzocht , die tussen depressie en virusinfectie en tussen angst en inflammatie. Om inflammatie te bepalen voor dit proefschrift zijn c-reactief proteine (CRP) interleukine (IL) 6, tumor necrosis factor (TNF) α, fibrinogeen en witte bloedcellen (WBC) gemeten.

Depressie en inflammatie

Uit literatuuronderzoek is gebleken dat depressie en CRP en IL-6 geassocieerd zijn in diverse populaties, waaronder psychiatrisch patiënten en hartpatiënten. Tevens is aangetoond dat het verband sterker is naarmate de depressieve klachten ernstiger zijn (diagnose versus symptoom). In navolging van eerdere literatuurstudies en meta-analyses, hebben we in hoofdstuk 2 een meta-analyse uitgevoerd waarin de relatie tussen depressie en inflammatie onderzocht werd in CHZ patiënten. Hiervoor hebben we studies geïncludeerd die een stricte definitie van CHZ hanteerden. Uit de resultaten kwam een klein tot medium effect naar voren voor de associatie tussen depressieve symptomen en CRP, maar vonden we geen significant effect voor IL-6. De relatie tussen depressie diagnose en inflammatie hebben we verder onderzocht in hoofdstuk 5 in 2415 personen van middelbare leeftijd met en zonder diagnose depressie. Uit deze studie bleek dat vooral mannen met een huidige depressie hogere CRP en IL-6 waardes hadden dan mannen zonder depressie. Meer specifiek bleek dat de mannen waarbij de depressie pas op latere leeftijd voor het eerst tot uiting kwam (late onset) een sterker verband liet zien met inflammatie. Eerder onderzoek heeft aangetoond dat deze “late onset” depressie geassocieerd is met een familiegeschiedenis waarin van CHZ en atherosclerose vaker voorkomen. Dit suggereert dat de relatie tussen de late onset depressie en inflammatie die we gevonden hebben mogelijk een reflectie is van subklinische ziektes, die meer prevalent worden naarmate men ouder wordt. Tevens hebben we in een subgroep van depressieve personen hebben aangetoond dat diegene die tricyclische en tetracyclische antidepressiva gebruikten en mannen die serotonine-noradrenaline-heropname-remmers gebruikten hogere inflammatie hadden. Daarentegen bleken personen die serotine-heropname-remmers gebruikten lagere inflammatie te hebben.

Vergelijkbaar met de resultaten uit hoofdstuk 5, lieten de resultaten uit hoofdstuk 6 zien dat ook depressieve klachten geassocieerd zijn met hogere inflammatie, maar het verband was minder sterk. Daarnaast bleek een ongezonde leefstijl (bepaald met roken, overgewicht, fysieke inactiviteit en overmatig alcoholgebruik) een belangrijke rol te spelen in deze relatie.

In hoofdstuk 3 en 4 hebben we binnen de oudste groep deelnemers, CHZ patiënten, onderzocht in welke richting de relatie tussen depressie en inflammatie uitgaat. Uit de resultaten kwam duidelijk naar voren dat CHZ patiënten die gemiddeld meer depressieve klachten rapporteerden hogere inflammatie hadden na zes jaar (gemeten met CRP, IL-6, fibrinogeen en WBC). Daarentegen bleken hogere inflammatiewaardes niet voorspellend te zijn voor het later ervaren van depressieve klachten. Dit suggereert dat depressie een voorspeller is voor inflammatie en dat inflammatie geen voorspeller is voor het ervaren van depressieve gevoelens. Deze bevindingen werden onderschreven door de resultaten uit hoofdstuk 7. Hier hebben we 1166 adolescenten gedurende 7 jaar gevolgd en vonden we dat het ervaren van depressieve symptomen voorafgaat aan hogere CRP waardes. Daarnaast bleek dat de associaties die we in hoofdstuk 3 en 7 vonden verklaard werden door een ongezonde leefstijl, zoals roken, overgewicht en fysieke inactiviteit.

Samengevat laten deze studies zien dat depressie en depressieve symptomen geassocieerd zijn met inflammatie. De relatie bleek het sterkst te zijn bij diegene die daadwerkelijk een depressie diagnose hadden. Dit suggereert dat er mogelijk sprake is van een dosis respons relatie; de meest ernstige depressie is geassocieerd met de hoogste inflammatie. Ook kwam naar voren dat vooral de depressie die zich pas op latere leeftijd voor het eerst manifesteert geassocieerd is met inflammatie. Daarnaast bleek uit de resultaten dat het ervaren van depressieve gevoelens hogere inflammatie voorspelt en niet vice versa. In vrijwel alle studies kwam naar voren dat gezondheidsgedrag een belangrijke rol speelt in de relatie tussen depressie en inflammatie, waaruit geconcludeerd kan worden dat een combinatie van depressieve gevoelens in combinatie met een ongezonde leefstijl voorspellend is voor hogere inflammatie.

Eenmalige versus recidiverende depressie

Eerder onderzoek heeft aangetoond dat met name recidiverende depressieve klachten geassocieerd zijn met een slechte gezondheid. Zo is gebleken dat de

prevalentie van atherosclerose, een hoge bloeddruk en nieuwe hartproblemen bij CHZ patiënten hoger is bij mensen die vaker depressief zijn. In hoofdstuk 3 en 4 hebben we bij 667 CHZ patiënten onderzocht of inflammatie ook hoger was bij diegene die vaker depressieve gevoelens rapporteerden in vergelijking met diegene die eenmalig of geen depressieve gevoelens rapporteerden. De resultaten lieten zien dat patiënten die tijdens de studie vaker depressieve klachten rapporteerden hogere CRP en IL-6 waardes hadden (hoofdstuk 3) en meer WBC (hoofstuk 4). In hoofdstuk 7 hebben we in 1166 adolescenten het beloop van depressieve symptomen in relatie tot CRP onderzocht. Hieruit kwam naar voren dat het ervaren van milde tot ernstige depressieve klachten gedurende een periode van 7 jaar al in de adolescentie voorafgaat aan hogere CRP waardes. Zowel uit de resultaten bij CHZ patiënten, als bij de adolescenten bleek dat gezondheidsgedrag een grote rol speelt in de relatie tussen depressieve klachten en CRP en IL-6. Samengevat suggereren deze resultaten dat een cluster bestaande uit recidiverende depressieve klachten, een ongezonde leefstijl en hogere inflammatie een plausibele verklaring zou kunnen zijn waarom mensen met depressieve klachten een verhoogd risico hebben op het ontstaan van CHZ danwel een slechtere prognose van bestaande CHZ hebben dan mensen zonder depressieve klachten.

Somatische versus cognitieve symptomen van depressie

Onderzoek heeft aangetoond dat vooral de somatische symptomen van depressie (o.a. slaapproblemen, eetproblemen, vermoeidheid) en niet de cognitieve symptomen van depressie (concentratieproblemen, sombere stemming, gevoelens van lusteloosheid) geassocieerd zijn met een verslechterde prognose van hartpatiënten. Deze somatische symptomen zijn vergelijkbaar met de symptomen die worden beschreven bij ziektegedrag als reactie op inflammatie (ook wel de sickness behavior theory).

In hoofdstuk 6 is specifiek gekeken naar het verschil tussen somatische en cognitieve symptomen van depressie in relatie tot inflammatie. Daarvoor zijn de data van 2861 volwassen deelnemers gebruikt. Uit de resultaten bleek dat deelnemers met meer somatische symptomen hogere inflammatie hadden. Dit werd niet voor cognitieve symptomen gevonden. Echter, wanneer er gecorrigeerd werd voor de effecten van gezondheidsgedrag, waren de resultaten niet langer significant. Deze resultaten suggereren dat somatische symptomen van depressie in combinatie met een ongezonde leefstijl zorgt voor hogere inflammatie. In tegenstelling tot de bevindingen uit hoofdstuk 6, werden er in hoofdstuk 7 geen

verschillen gevonden tussen de somatische en cognitieve symptomen van depressie in relatie tot inflammatie bij adolescenten en bleek juist dat somatische en cognitieve symptomen van depressie samen voorafgaan aan meer inflammatie. Samengevat suggereren deze resultaten dat somatische symptomen van depressie alleen geassocieerd zijn met inflammatie in volwassenen, mogelijk als een reflectie van ziektegedrag.

Angst en inflammatie

Uit eerder onderzoek is gebleken dat naast depressie ook het ervaren van angst een negatieve invloed heeft op zowel het ontwikkelen van CHZ als het beloop hiervan. Er zijn zelfs aanwijzingen waaruit blijkt dat in het bijzonder de somatische component van angstklachten (o.a. beven, zweten en verhoogde hartslag) een belangrijke rol spelen in deze relatie. In hoofdstuk 6 hebben we onderzocht of symptomen van angst en inflammatie samenhangen en of er een verschil aanwezig is tussen de somatische en cognitieve symptomen. Hiervoor zijn de gegevens van 2861 proefpersonen met en zonder diagnose depressie geanalyseerd. De resultaten lieten zien dat vooral mannen met angstklachten hogere IL-6 en CRP waardes hadden. Verder kwam er naar voren dat vooral de somatische symptomen van angst deze relatie verklaarden. Net als bij de relatie tussen depressieve symptomen en inflammatie bleek echter dat gezondheidsgedrag een belangrijke rol speelt in deze relatie. Dit betekent dat mensen met angstklachten en een ongezonde leefstijl een groter risico hebben op hogere inflammatie. Mogelijk kan ingrijpen in deze ongezonde leefstijl dienen als onderdeel van de therapie van patiënten met angst om zodoende het risico op CHZ te verlagen. Toekomstig onderzoek zal hier meer inzicht in kunnen geven.

Virusinfecties en depressie

Eerder onderzoek heeft aangetoond dat virusinfecties geassocieerd zijn met psychische stoornissen zoals schizofrenie, waarbij inflammatie mogelijk een rol speelt. Echter, er is nog weinig bekend over een relatie tussen virusinfecties en depressie. In hoofdstuk 8 hebben we onderzocht of de aanwezigheid van virussen (epstein barr virus, cytomegalovirus en herpes simplex virus 1) gerelateerd is aan het ervaren van depressieve gevoelens of een toekomstige depressie diagnose onder 1057 adolescenten. Uit de resultaten bleek dat in deze relatief jonge groep

deelnemers virusinfecties niet geassocieerd zijn met depressieve gevoelens of het later hebben van een depressie twee jaar later. Echter, dit sluit niet uit dat virusinfecties wel degelijk een invloed kunnen hebben op het ontstaan van een depressie. Virussen kunnen latent aanwezig blijven in bij aan depressie gerelateerde hersengebieden. Onder invloed van stress kunnen deze latente virussen gereactiveerd worden en daarmee schade aanrichten in deze hersengebieden. Mogelijk is er bij de onderzochte groep adolescenten nog geen (frequente) reactivatie van de virussen geweest en daarmee nog geen schade aangericht die depressie kan veroorzaken. Dit suggereert dat een relatie tussen depressie en virussen mogelijk pas in de volwassenheid tot uiting komt. Toekomstig onderzoek zal hier meer duidelijkheid over moeten verschaffen.

Conclusie

Het overkoepelende doel van deze thesis was om meer inzicht te verkrijgen in de relatie tussen depressie en inflammatie gedurende de levensloop. Op basis van de resultaten kan er geconcludeerd worden dat er een relatie is tussen depressie en inflammatie. Deze relatie is al aanwezig in de adolescentie en wordt sterker naarmate men ouder wordt. Daarnaast is gebleken dat vooral de recidiverende depressieve klachten voorspellend zijn voor latere inflammatie, maar dat inflammatie geen (recidivirende) depressieve klachten voorspelt. Ook is naar voren gekomen dat de somatische symptomen geassocieerd zijn met inflammatie bij volwassen, mogelijk als reflectie van ziektegedrag. Tevens hebben we aangetoond dat inflammatie niet alleen samenhangt met depressie, maar ook met angst. Een belangrijke en consistente bevinding was dat een ongezonde leefstijl in belangrijke mate de relatie tussen zowel depressie als angst en inflammatie verklaart. Concluderend kan er gesteld worden dat een combinatie van (recidiverende) depressie of angst en een ongezonde leefstijl hogere inflammatie tot gevolg hebben. Deze groep mensen heeft daarmee mogelijk een verhoogd risico op toekomstige hartproblemen. Het behandelen van de ongezonde leefstijl van depressieve- of angstpatiënten zou mogelijk een positief effect kunnen hebben op de inflammatie en daarmee het risico op hart- en vaatziekten kunnen verlagen.

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List of publications

Published

Duivis HE, de Jonge P, Penninx BW, Na BY, Cohen BE, Whooley MA. Depressive symptoms, health behaviors, and subsequent inflammation in patients with coronary heart disease: Prospective findings from the Heart and Soul Study. American Journal of Psychiatry, 2011;168(9):913-920.

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Duivis HE, Vogelzangs N, Kupper HM, de Jonge P, Penninx BW. Differential association of somatic and cognitive symptoms of depression and anxiety with inflammation: Findings from the Netherlands Study of Depression and Anxiety (NESDA). Submitted

Duivis HE, Kupper HM, Penninx BW, Bosch N, Riese H, Oldehinkel T, de Jonge P. Trajectories of depressive symptoms and subsequent inflammation in pre-adolescence: a latent class approach: Data from the Tracking Adolescents' Individual Lives Survey (TRAILS). Submitted

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Duivis HE, Meijer A, Kupper HM, Rosmalen J, Penninx BW, de Jonge P. Depression and inflammation in patients with acute and stable coronary heart disease: a meta-analysis. Submitted

Jonker I, **Duivis HE**, Rosmalen J, Yolken RH, Schoevers R, Klein H. Exposure to HSV-1 reduces cognitive functioning in a general population of adolescents. The TRAILS study. Submitted

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Hester Duivis was born on November 19, 1978 in Amstelveen, The Netherlands. After she graduated from high school at the Keizer Karel College in Amstelveen she completed her bachelor in social work at the Hogeschool van Amsterdam. Subsequently, she continued studying and her obtained her bachelor's and master's degree in psychology at VU University, Amsterdam, with a specialization in clinical neuropsychology. After working as a research assistant at GGZIngeest, Hester started her PhD research at Tilburg University in 2008. In 2009, as part of her PhD project, she worked at GGZIngeest, Amsterdam, for a research collaboration under the supervision of Prof. dr. Brenda Penninx and dr. Nicole Vogelzangs. In that same year, she stayed in Groningen for two months at the University Medical Center of Groningen to do fieldwork for the Tracking Adolescents' Lives Survey (TRAILS) study. In 2010 Hester went to San Francisco for two months to work on two of her papers under the supervision of Prof. Mary Whooley at the VA Medical Center. During her PhD research, she studied epidemiology at VU University from which she will graduate at the end of 2012. Currently Hester works as a policy advisor research/PhD coordinator at the Tilburg School of Social and Behavioral Sciences and as a teaching assistant at the department of developmental psychology, both at Tilburg University.