THE INFLAMMATION HYPOTHESIS OF DEPRESSION: CROSS SECTIONAL ASSOCIATIONS, TEMPORAL RELATIONSHIPS AND THE CONFOUNDS OF COMORBIDITY

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BPsych (Hons)

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The thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository**, subject to the provisions of the Copyright Act 1968.

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Table of Contents

Synopsis

Depression is prevalent and causes substantial personal and economic burden. Yet, the cause of depression is unclear and existing serotonergic pharmacological treatments have limited effectiveness in some individuals. The inflammatory hypothesis of depression posits that subacute inflammation may cause depression. Thus, inflammation may be a target for intervention through pharmacological and behavioural means, and inflammatory markers may be appropriate biomarkers of disorder onset and resolution. Support for this theory includes experimental evidence where administering inflammation-inducing agents leads to depressive-like signs and symptoms in animal models and humans with clinical illnesses. Epidemiological studies provide necessary concurrent evidence for experimental effects. This thesis explores the epidemiological evidence for the inflammatory hypothesis of depression. The chapters employ meta-analysis to re-interpret existing data and generate new summary information, and explore primary cohort data to uncover new knowledge.

The bulk of epidemiological evidence for the inflammatory hypothesis of depression is cross-sectional. Given that these studies have been undertaken with various participant and measurement characteristics, meta-analysis provides a unique opportunity to explore whether these characteristics moderate the strength of the relationship between inflammatory markers and depression. The meta-analysis described in Chapter 2 highlights that although the inflammatory marker

interleukin(IL)-6 is clearly elevated in people with depression, study-level factors such as not verifying a clinical depression diagnosis, community cohort sampling, cardiovascular disease comorbidity and not matching participants on key variables were associated with smaller effect sizes. This study provides the most extensive moderator analysis in the literature to date.

Chapters 3 and 4 examine temporal associations between inflammation and depression via meta-analysis of treatment effects: whether treating depression with antidepressants is associated with changes in inflammatory markers, and whether taking a medication with anti-inflammatory effects – statins – is associated with changes in depression. Despite a limited number of studies and evidence of heterogeneity, there was preliminary evidence that antidepressant treatment is associated with a reduction in IL-6 and marginally significant reductions in C-reactive protein (CRP) and IL-10. Use of statins in randomised controlled trials was also associated with lower depressive symptoms and fewer occurrences of depression, compared with placebo.

Chapter 5 explores whether elevations in inflammatory markers precede depressive symptoms in a cohort of older people, to add to the evidence evaluating whether inflammatory markers may be useful biomarkers. Chapter 5 investigates this question in a primary cohort of older persons. Analysis of primary data allows for more refined research questions, which in this case, concerns evaluating the contribution of aspects of unhealthy lifestyle that have inflammatory consequences. In this

study, aspects of unhealthy lifestyle were important confounders, where adjusting for them rendered the relationships between inflammatory markers and later depression non-significant. However, in examining the relationship between baseline lifestyle factors and later depression, IL-6 was observed as a significant mediator, in the first analysis of its kind. Consequently, aspects of unhealthy lifestyle may be a source of elevated inflammatory markers observed in depression.

The inflammatory hypothesis proposes that one of its benefits is that it can explain high comorbidity between depression and other illnesses. The study in Chapter 6 sought to verify this. It explores whether the elevated inflammatory markers observed in depression have consequences for another prevalent and burdensome condition: cardiovascular disease. In a cohort of older persons, IL-6 was a mediator of the relationship between depression and later hospitalisations for cardiovascular events. As such, reducing levels of inflammation may be a way to address both depression and cardiovascular disease.

The thesis concludes with reflections on the implications of the data presented for the inflammatory hypothesis of depression and management of depression (Chapter 7). The original contribution of knowledge that this thesis provides includes summarising key epidemiological cross sectional and treatment data to verify that inflammation is a plausible associate with depression, and demonstrating the mediation effects of inflammatory markers in relationships between lifestyle and depression, and between depression and cardiovascular disease. This thesis highlights the

importance of unhealthy lifestyle and physical comorbidity to interpreting the inflammation hypothesis of depression. Inflammation-reducing lifestyle management may present a novel strategy to prevent and manage depression.

1. Introduction

1. Introduction

1.1 Depression and inflammation

Depression: A common mental disorder

Depression is common, with 12 month prevalence between 4 and 10% and lifetime prevalence between 10 and 20% (Australian Bureau of Statistics, 2007; Begg et al., 2007; Grant et al., 2004; Kessler et al., 2003; Kessler, Chiu, Demler, & Walters, 2005; Paykel, Brugha, & Fryers, 2005). It is consistently ranked as one of the top causes of burden of disease, is the leading cause of years lost to disability world-wide, and is associated with substantial economic costs (Australian Institute of Health and Welfare, 2008; Luppa, Heinrich, Angermeyer, König, & Riedel-Heller, 2007; Paykel, et al., 2005; Üstün, Ayuso-Mateos, Chatterji, Mathers, & Murray, 2004; World Health Organization, 2008). Its burden stems from its contribution to work and education absenteeism, family and career disruption, and hospitalisations and suicide. Furthermore, depressive disorders often co-occur with other psychological disorders and physical illnesses such as anxiety disorders, substance use disorders, coronary artery disease and cancer, leading to under-diagnosis and complications in health management (Clarke & Currie, 2009; Rush, 2007; Rush et al., 2005).

Depressive disorders including major depression and dysthymic disorder are currently diagnosed based on the individual's phenomenological reports. The symptoms characteristically impair daily functioning and

include daily depressed mood and anhedonia, and a series of other cognitive and behavioural aspects such as significant changes in appetite or sleep, psychomotor retardation or agitation, difficulty concentrating, excessive guilt and suicidal ideation (American Psychiatric Association, 2000). Validation studies have identified symptom clusters in the diagnostic criteria of cognitive (e.g., depressed mood, loss of interest, feeling worthless and guilty, and suicidal ideation) and neurovegetative (e.g., change in appetite or weight, change in sleep, psychomotor agitation or retardation, and fatigue) symptoms (Lux & Kendler, 2010). Although there are limitations of a symptom-based classification system, no current biomarkers can accurately, specifically and sensitively diagnose depression (Lakhan, Vieira, & Hamlat, 2010; Spitzer & First, 2005).

Despite the increasing health and economic salience of depressive disorders, the cause of depression remains unclear (Krishnan & Nestler, 2008). This is one reason that appropriate management often remains elusive for many people with depression, such that up to 54% do not reach remission after initial psychotherapy or pharmacotherapy (Casacalenda, Perry, & Looper, 2002) and 30% do not reach remission after multiple treatment trials and even if they do, have a high rate of relapse (Rush et al., 2006). Consequently, there is a great need to identify novel pathophysiologic pathways relevant to depression in order to develop new treatments as well as identify biomarkers to assist in precise initial diagnosis and assessment of treatment response (Miller, Maletic, & Raison, 2009).

There are several strong psychosocial and biological contenders to explain the cause of depression, with the true cause generally assumed to be an interaction of factors (particularly biological diatheses and psychosocial stressors). In terms of biological aspects of depression, the monoamine hypotheses are frequently cited, fuelled by evidence of low levels of monoamine neurotransmitters in depression and the therapeutic effect of monoamine-based antidepressant medications (such as monoamine oxidase inhibitors and selective serotonin re-uptake inhibitors) (Krishnan & Nestler, 2008). There is also evidence that neuroendocrine and neurotrophin changes may be involved, as people with depression often have hyperactivity of the hypothalamic-pituitary-adrenal axis (Pace, Hu, & Miller, 2007; Pariante & Miller, 2001) and reduced expression of brain-derived neurotrophic factor leading to impaired neuronal and synapse support (Hashimoto, Shimizu, & Iyo, 2004; Sen, Duman, & Sanacora, 2008). Finally, inflammatory and oxidative processes may be involved (Maes et al., 2009; Miller, et al., 2009). The inflammation hypothesis proposes an interrelationship between inflammation and mood and have the potential to draw together much of the psychological phenomenology and biological correlates of depression (Miller, et al., 2009). The epidemiological evidence for inflammatory hypotheses of depression is the subject of this thesis.

The inflammation hypothesis of depression

Inflammation is an important part of the innate immune response (Playfair & Bancroft, 2004). Endogenous and exogenous triggers such as

injury and pathogens activate the innate immune system via the biochemical cascade of inflammatory mediators including acute phase reactants such as C-reactive protein (CRP) and pro-inflammatory cytokines including interleukin (IL)-1, tumor necrosis factor and IL-6. In clinical medicine, elevated blood levels of these inflammatory mediators are regarded as evidence of inflammation. The classic "inflammation response" to manage injury or pathogens is characterised by localised redness, warmth, pain, swelling, and loss of function at the site; however, inflammatory mediators have pleiotropic effects across many systems. Inflammatory mediators pass through the blood-brain barrier (through humoral means or neural activation), leading to systemic effects such as fever, malaise and lethargy ("sickness behaviours"), and potentially also affecting brain functioning (Banks & Erickson, 2010; Ek et al., 2001; Perry, 2004).

A mild increase in inflammatory markers without the classic inflammation characteristics may be considered subclinical inflammation. Besides injury and pathogens, other triggers such as psychological stress and excessive adipose tissue may also lead to activation of acute phase reactants and cytokines, with persistent low-grade elevations in inflammatory markers observed in both cases (Maes, et al., 2009; Maury & Brichard, 2010; Park, Park, & Yu, 2005; Shelton & Miller, 2010; Steptoe, Hamer, & Chida, 2007). Furthermore, while inflammatory abnormalities are present in many immune system disorders such as lupus erythematosus and rheumatoid arthritis, subclinical inflammation may also be present in

non-immune diseases including atherosclerosis and depression (Hansson, 2005; Maes, et al., 2009; Miller, et al., 2009; Rocha & Libby, 2009).

The inflammation hypothesis of depression is an evolving theory with new corollaries added with emerging evidence. At the core is that the processes of inflammation are responsible for the psychological and physiological experience of depression. Often cited as the foundation article is Smith's (1991) macrophage hypothesis: that elevated secretion of cytokines from macrophage cells is responsible for depression. In the last 20 years, this theory has expanded beyond macrophage cells to involve other aspects of inflammation and immune processes, including cell mediated immunity, neurodegeneration and oxidative processes (Maes, 2011; Maes, et al., 2009; Miller, et al., 2009).

Inflammation modulates many physiological systems to produce the various aspects of "sickness behaviour", and such behaviours are (*prima facie*) akin to idiopathic depressive symptoms (Dantzer, 2001; Maes et al., 2012). For instance, inflammatory mediators alter appetite and sleep, typical preservation-type behaviours of acute illness and are also perturbed in people with depression. In animal models, administration of cytokines or immune stimulating agents such as lipopolysaccharide leads to a depressive-like state, including reduced preference for pleasurable stimuli, reduced social interaction, and impaired memory and learning (Dantzer, 2001; Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Dunn, Swiergiel, & Beaurepaire, 2005; Song & Wang, 2011). In humans, acute administration of cytokines, lipopolysaccharide or a vaccination leads to

behaviours which resemble depression, including temporary lethargy, appetite suppression, cognitive impairment and dysphoria in both healthy (Reichenberg et al., 2001) and physically ill people (Raison et al., 2009). In more chronic contexts, the primary human evidence is based on the observation that patients undergoing treatment for hepatitis C or some cancers with pro-inflammatory agents such as interferon-α and IL-2 have very high incidence of major depressive disorder (approximately 50%; Capuron et al., 2009; Capuron & Miller, 2004; Myint, Schwarz, Steinbusch, & Leonard, 2009).

To produce these sickness behaviours, inflammatory mediators modulate neurobiological systems, many of which have been observed to be perturbed in depression. Firstly, there is a bi-directional relationship between inflammation and the neuroendocrine stress system (Dunn, 2000). Generally, people with depression tend to show altered baseline levels of hypothalamic-pituitary-adrenal hormones such as glucocorticoids, as well as increased peak and recovery of glucocorticoids following stressors (Burke, Davis, Otte, & Mohr, 2005; Pace, et al., 2007; Pariante & Miller, 2001; Schuld et al., 2003). Under acute conditions, inflammation and glucocorticoids are mutually regulatory: cytokines stimulate the hypothalamic-pituitary-adrenal axis and glucocorticoids suppress inflammatory processes (Dunn, 2000; Miller, et al., 2009). However, under conditions of chronic stress or inflammation, there is a less reliable relationship; each system compensates for the prolonged activation by becoming glucocorticoid resistant and chronic stress can confer risk of

increased inflammatory activity. Resistance may occur via decreases in receptor density, which is a demonstrated effect of cytokines (Pace, et al., 2007). There is additional evidence that acute stressors stimulate inflammation (Segerstrom & Miller, 2004; Zorrilla et al., 2001) and stressful life events also precipitate infection and physical illness (Cobb & Steptoe, 1996; Smolderen, Vingerhoets, Croon, & Denollet, 2007). Pro-inflammatory cytokines can also alter the synthesis, release and re-uptake of neurotransmitters thought to be implicated in mood including the monoamines serotonin, dopamine and norepinephrine (Miller, et al., 2009; Myint & Kim, 2003). For example, cytokines are thought to reduce serotonin availability by activating indoleamine 2,3 dioxygenase which breaks down tryptophan, the precursor amino acid to serotonin. Serotonin is also necessary for cytokine production (Kenis & Maes, 2002). Furthermore, serotonin transporter gene polymorphisms and antidepressant administration influence cytokine-induced depressive-like symptoms (Capuron, Hauser, Hinze-Selch, Miller, & Neveu, 2002; Miller, et al., 2009). Finally, animal literature demonstrates that with prolonged exposure, pro-inflammatory cytokines are associated with decreased neurogenesis, neurotrophic support and neuroglial support in areas important for behaviour and cognition, such as the hippocampus (Khairova, Machado-Vieira, Du, & Manji, 2009; Miller, et al., 2009). This can be halted by the administration of IL-1 receptor antagonist.

A recent review also places the inflammatory hypothesis of depression within the context of evolutionary psychology, indicating that what may be

considered a depressogenic genetic propensity may actually be an immunological survival advantage (Raison & Miller, 2013). An inflammatory reaction to psychological stress may be adaptive in that it prepares the body – both biochemically and behaviourally – for an increased risk of infection after physical endangerment, therefore improving the likelihood of survival. However, in the modern social context of chronic stressors, the same processes are maladaptive, generating subclinical and clinical depression. Thus, the theory is plausible from psychological, biological and evolutionary perspectives.

Epidemiological evidence for the inflammation hypothesis of depression

Cross sectional evidence

Much of the epidemiological evidence for the inflammation hypothesis of depression has been cross sectional. Several recent meta-analyses indicate that levels of circulating inflammatory markers, in particular CRP, IL-6 and tumor necrosis factor, are elevated in people with high depressive symptoms (Howren, Lamkin, & Suls, 2009) or major depressive disorder (Dowlati et al., 2010; Liu, Ho, & Mak, 2012) compared to those without depressive symptoms. Effect sizes are larger for studies of people diagnosed with depression compared to studies which define depression via depressive symptoms (Howren, et al., 2009). Similar relationships have also been demonstrated for ex vivo stimulated cytokines, where blood is taken and treated with immune stimulating agents such as lipopolysaccharide to measure the responsivity of the system to immune challenge (Suarez,

Krishnan, & Lewis, 2003; van den Biggelaar et al., 2007). Although low in sample size, several studies also replicate the relationships observed in the periphery in cerebrospinal fluid (Levine et al., 1999; Lindqvist et al., 2009). In contrast to the meta-analysis results for pro-inflammatory markers, fewer studies exist for anti-inflammatory cytokines (IL-4, IL-10). Although anti-inflammatory cytokines tended to be increased in people with depression, these elevations were not statistically significant (Dowlati, et al., 2010; Liu, et al., 2012), perhaps due to small sample sizes and low circulating concentrations (Simon et al., 2008).

All the published meta-analyses show high levels of heterogeneity, indicative of discrepancies between studies. Only one of the meta-analyses restricted to people with major depression explored sources of heterogeneity (Liu, et al., 2012). Their limited analyses showed that age had a significant negative relationship with effect size for IL-6 and that effect sizes were significant in studies in European but not non-European populations. Several sources of heterogeneity were explored in the meta-analysis by Howren et al. (2009), owing in part to the more liberal inclusion criteria. Typically, controlling for covariates such as use of medications led to larger effect sizes than not controlling for them. However, the opposite was observed for body mass index where there was significant and substantially smaller effect sizes for studies with adjustment compared to those without (standardised mean difference [d] = 0.11 vs. 0.32 for CRP; d = 0.08 vs. 0.50 for IL-6). This means that studies that do not adjust for body mass index greatly overestimate the effect size. Large effect sizes were observed in

studies of clinically depressed patients without physical comorbidities ($d =$ 0.40 for CRP; $d = 0.71$ for IL-6) compared to studies with co-existing coronary artery disease or cancer as well as community samples (all $d <$ 0.18). However, subgroups were not statistically compared, and several other sources of heterogeneity of potential importance were not explored including smoking, residential status (inpatient vs. outpatient), anxiety comorbidity and methodological factors (e.g., blood sample time, assay supplier).

Evidence from antidepressant treatment studies

Although cross sectional data provide corroborative evidence for the association of inflammation in the pathophysiology of depression, they do not imply a temporal direction. Defining temporality is a necessary, although not sufficient, step toward identifying a meaningful causal model. A small number of largely pre-post design depression treatment studies can comment on the potential directionality of the inflammation-depression relationship. These studies aim to test whether the amelioration of mood symptoms (with either pharmacological or psychological treatment) is associated with change in inflammatory markers.

The more extensive literature examines the changes in mood and inflammatory markers following antidepressant medication use. As described in the review by Kenis and Maes (2002), there is mixed evidence as to the effect of antidepressants on circulating and ex vivo stimulated cytokines, with no effect, increases and decreases all observed in both pro- and anti-inflammatory cytokines. One influential factor on the relationship

may be treatment response with demonstrated decreases in IL-6 production for treatment responders and increases for treatment non-responders (Lanquillon, Krieg, Bening-Abu-Shach, & Vedder, 2000; Maes et al., 1997). This indicates that the outcome following successful treatment is "normalisation" of inflammatory markers. The mechanisms of this change could occur in different ways. In vitro, antidepressants are immunosuppressant, meaning that there may be a direct effect on the inflammatory systems (Kenis & Maes, 2002; Kubera et al., 2001; Szuster-Ciesielska, Tustanowska-Stachura, Slotwinska, Marmurowska-Michalowska, & Kandefer-Szerszeñ, 2003). Alternatively, these alterations to inflammation may occur through other studied pathways of antidepressants (Castanon, Leonard, Neveu, & Yirmiya, 2002; Maes, 2001; Maes et al., 1999). For example, extended pharmacotherapy is associated with decreased levels of serotonin and increases in the secondary messenger cyclic adenosine monophosphate, both of which can decrease pro-inflammatory and increase anti-inflammatory cytokine secretion. What is lacking in this literature is clear synthesis. Concurrently while writing this thesis, one meta-analysis was published demonstrating that antidepressants are associated with declines in cytokines IL-1, but not tumor necrosis factor nor IL-6 (Hannestad, DellaGioia, & Bloch, 2011). Yet, like the previous cross sectional meta-analyses, moderator analyses were not undertaken such as treatment responders and non-responders, besides stratification by antidepressant class. Furthermore, acute phase proteins (e.g., CRP) or anti-inflammatory cytokines (e.g., IL-10) were not examined.

Unlike pharmacological treatments, psychological interventions do not involve consumption of a biochemical substance with pleiotropic effects (as with antidepressants). Hence, any improvements in inflammation may be assumed to occur following cognitive improvements in the mood state and changes in the patient's behaviour. An earlier meta-analysis suggested that few studies conclusively support that psychological interventions, including relaxation, stress management and disclosure writing therapy, improve immune functioning (Miller & Cohen, 2001). However, several studies published since indicate that psychological interventions can influence inflammatory parameters, particularly stress management techniques (Irwin, Pike, Cole, & Oxman, 2003; Pace et al., 2009) as well as cognitive behavioural therapy (Carrico et al., 2005; McGregor et al., 2004; Sharpe et al., 2001). However, often these effects are acute without long term influence on inflammatory parameters (Pace, et al., 2009; Sharpe, et al., 2001). Although this type of evidence will not be explored further in this thesis, it is an important literature to note. This evidence provides experimental understanding of the link between alterations in psychological functioning and immune/inflammatory parameters that is less inhibited by the confounding associated with correlational designs.

Evidence from anti-inflammatory treatment studies

Another line of treatment research examines the effects of medications with anti-inflammatory properties on depression outcomes. Recently published is the first evidence from a randomised control trial of the potential for an antagonist of the pro-inflammatory marker tumor

necrosis factor, infliximab, to be a primary treatment for treatment-resistant major depression (Raison et al., 2013). While there was no overall difference in depression rating after treatment in infliximab compared to placebo groups, there was an interaction between the baseline levels of CRP and treatment. People with elevated CRP showed greater reductions in depressive symptoms in the infliximab treatment group, those with lower levels of CRP showing greater improvement in the placebo group. While these results are preliminary they highlight that treating elevated levels of inflammation may be associated with improvement in depressive symptoms for some people. Previous studies have investigated other traditional anti-inflammatory medications such as cyclooxygenase-2 selective inhibitors in adjunct to traditional antidepressants (selective serotonin reuptake inhibitor or norepinephrine reuptake inhibitor) and showed greater mood improvement than antidepressants alone (Akhondzadeh et al., 2009; Muller et al., 2006).

Another medication suspected to have anti-inflammatory properties and effects on psychological functioning are statins. Statins are very commonly prescribed medications aimed at reducing cholesterol (Australian Prescriber, 2010). Although thought to reduce cholesterol by inhibiting HMG-coenzyme A reductase, these medications have other effects (Farooqui, Ong, Horrocks, Chen, & Farooqui, 2007; Shen, 2005). Studies *in vitro* and in various patient populations indicate that use of statins is associated with reductions in inflammatory mediators including CRP and IL-6 (Balk et al., 2003; Craig et al., 2011; Farooqui, et al., 2007; Lee, Lin, &

Chang, 2008; Pancholi, Jain, Saxena, & Deb, 2009; Sano et al., 2011).

Statins may influence the inflammatory response via their interaction with

various cells which release inflammatory mediators (e.g., macrophages and

lymphocytes), as well as their interaction with endothelial cells and smooth

muscle cells, and anti-oxidant effects (Farooqui, et al., 2007; Pancholi, et al.,

2009; Stafford & Berk, 2011). The hypothesis that treatment with statins

may lead to reduced depression opposes the theory that low cholesterol is

associated with depression, which was driven by early studies linking low

cholesterol and mental health problems (Duits & Bos, 1993; Muldoon,

Manuck, & Matthews, 1990). More recent epidemiological studies do not

support a relationship between statin use and increased risk of depression;

rather they indicate that statin use is associated with improvements in

mood or reduced risk of depression (Otte, Zhao, & Whooley, 2012; Pasco,

Jacka, et al., 2010; Young-Xu, Chan, Liao, Ravid, & Blatt, 2003). A recent

meta-analysis suggested that treatment with statins compared with placebo

was associated with improvements in general psychological functioning

scores (O'Neil et al., 2012). However, whether this improvement is

representative of depression specifically is unknown.

Prospective evidence

Temporal relationships may also be examined via prospective

longitudinal design. This type of design minimises the confounds of small

fluctuations across time which may be observed in (short duration)

treatment studies. It can also examine bi-directional associations between

inflammation at baseline and mood disorder at follow-up, and between mood

disorder at baseline and inflammation at follow-up. These designs are useful for evaluating whether a factor may be a useful biomarker for disease onset.

The body of cohort literature showing a relationship between markers of inflammation and depression over time is growing (summarised in Table 1.1). However, this body of literature is limited in that only one previous retrospective study to my knowledge has demonstrated a relationship between inflammatory markers and depression diagnosed by clinical interview (Pasco, Nicholson, et al., 2010). Most studies use a self-report depressive symptom inventory to measure depression as a continuous score. Besides the few studies which show a relationship between depression and later elevated levels of white blood cell count (Duivis et al., 2013) and the inflammatory marker complement 3, C3 (Boyle, Jackson, & Suarez, 2007), studies generally have examined cytokines and CRP.

Evidence from these studies regarding the direction of the depression-inflammation relationship is mixed. Most previous studies examine only one direction of the relationship (either depression to inflammation, or the reverse). The studies which tested the depression to inflammation direction tended to find no support (Kiecolt-Glaser et al., 2003; Matthews et al., 2007), with one study supporting a relationship from depression to later levels of CRP (Hamer, Molloy, de Oliveira, & Demakakos, 2009b). The two studies which only tested the inflammation to depression direction examined the largest array of biomarkers and found support for selected inflammatory markers (Milaneschi et al., 2009; van den Biggelaar, et al., 2007).

Table 1.1. Prospective literature search (see notes below table for search terms).

Citation	Design	Depression inventory	Inflammat-ory marker	Population	N	Follow-up time	Direction tested	Supported	Predictors/confounders and comments
Duivis et al. 2011	Single group cohort	Patient Health Questionnaire	CRP, IL-6, fibrinogen	Heart and Soul Study, established coronary heart disease (mean age 64 years)	667	5 years	Both	Partially: depression → inflammat-ory marker	Association between depression and later CRP not significant after adjusting for demographics and disease characteristics. Association between depression and IL-6 non-significant after additionally adjusting for lifestyle factors (physical inactivity, smoking, and higher body mass index).
Gimeno et al. 2009	Single group cohort	General Health Questionnaire (cognitive symptoms of depression)	CRP, IL-6	Whitehall II study, civil servants, 35-55 years (mean age 50 years)	3353 (CRP), 3070 (IL-6)	Mean 11.8 years, 2 measurements	Both	Partially: inflammat-ory marker → depression	Association between IL-6/CRP and later depression remained significant after full adjustment for demographic, lifestyle factors (e.g., diet, physical activity, smoking, alcohol consumption, body mass index) and physical illness.

| Hamer et al. 2009a | Single group cohort | Centre for Epidemiologic Studies Depression Scale (8 item) | CRP, fibrinogen | English Longitudinal Study of Ageing, nationally representative sample of English households (mean age 63 years) | 4323 | Inflammatory markers taken at 2 year intermediate between baseline and follow-up depression assessment | Inflammatory marker → depression | Yes | Standardised unit increase in logCRP was associated with higher odds of depressive symptomatology at follow-up after multivariate adjustment for age, gender, occupational class, chronic physical illness, smoking and alcohol use (odds ratio = 1.32). Odds of depressive symptomatology for those reporting moderate or vigorous physical activity were 0.71 and 0.58 respectively, after adjusting for baseline depression, age, gender, occupational class, smoking, alcohol, and chronic illness. However, CRP only explains a modest amount of the association between physical inactivity and risk of depression (<5% of variance). |

| Hamer et al. 2009b | Single group cohort | Centre for Epidemiologic Studies Depression Scale (8 item) | CRP, fibrinogen | English Longitudinal Study of Ageing, nationally representative sample of English households (mean age 61 years) | 3609 | 2 years, 2 measurements | Depression → inflammatory marker | Yes | Compared to those without depression at baseline and follow-up, CRP was elevated in those with depressive symptoms at baseline and depressive symptoms at baseline and follow-up, after adjusting for age, gender, socioeconomic status and morbidity. Controlling for age and gender, lifestyle characteristics of weight change, alcohol use, smoking and physical activity were significant mediators of the depression to CRP relationship. Weight change explained the largest proportion of indirect effect. |

| Janicki-Deverts et al. 2010 | Single group cohort | Centre for Epidemiologic Studies Depression Scale | CRP | Coronary Artery Risk Development in Young Adults study, healthy participants, 33-45 years (mean age 40 years) | 2544 | 5 years | Both | Partially: Depression → inflammatory marker | Association between depression and CRP remained significant after adjusting for demographic, biological/medical risk factors, lifestyle factors (smoking, alcohol, physical activity) and level of CRP at baseline. The effect was conditional on race with the effect present in black but not white participants. When exploring Centre for Epidemiologic Studies Depression Scale subscales, depressed affect, positive affect and somatic symptoms were significantly associated with CRP (for black, but not white participants). |

Kiecolt-Glaser et al. 2003	Case control	Beck Depression Inventory	IL-6	Caregivers and non-caregivers, 55-89 years (mean age 70 years)	225	6 years, two samples per year	Depression → inflammat-ory marker	No	Depression did not predict the change slope for IL-6 in caregivers.
Matthews et al. 2007	Single group cohort	Centre for Epidemiologic Studies Depression Scale	CRP	Study of Women's Health Across the Nation, pre-menopausal women, 42-52 years (mean age 46 years)	3239	5 years, annual measurement	Depression → inflammat-ory marker	No	Unadjusted and adjusted analyses were not significant (covariates examined included socio-demographic characteristics, body mass index, smoking and physical illness).

Matthews et al. 2010	Single group cohort	Centre for Epidemiologic Studies Depression Scale	CRP	Study of Women's Health Across the Nation, pre-menopausal women, 42-52 years (mean age 46 years)	1781	7 years, annual measurement	Both	Yes; better evidence for inflammatory marker → depression	Associations remained after adjusting for socio-demographic characteristics, health risk factors and physical illness and some lifestyle aspects (physical activity, smoking, body mass index). However, additional analyses with adjustment for sleep problems indicated that depression was no longer a significant predictor of CRP, although CRP remained a significant predictor of depression.
Milaneschi et al. 2009	Single group cohort	Centre for Epidemiologic Studies Depression Scale	CRP, IL-1ß, IL-1ra, TNF-α, IL-6, IL-6 receptor, IL-18	InCHIANTI study of ageing (mean age 74 years)	550	6 years, 3 measurements	Inflammatory marker → depression	Partially: only IL-6ra → 6 year depression	Association between IL-6ra and depression remained significant after adjustment for socio-demographic characteristics, lifestyle factors (smoking, alcohol use, physical activity), physical functioning and physical illness.

| Pasco et al. 2010 | Retrospective cohort | Major Depressive Disorder via Structured Clinical Interview | CRP | Geelong Osteoporosis Study, no history of depression at baseline, aged 20-84 years (median age 47 years) | 644 | ~9 years | Inflammatory marker → depression | Yes | Increased risk of developing major depression for each standard deviation increase in logCRP (unadjusted hazard ratio = 1.41, 95% CI 1.07-1.86). Adjusting for smoking, non-steroidal anti-inflammatory medication use and weight did not change the result (adjusted hazard ratio = 1.44, 95% 1.04-1.99), nor did further adjustment for lifestyle factors, comorbid physical illness and use of oral contraceptive or hormone therapy. |

| Stewart et al. 2009 | Single group cohort | Beck Depression Inventory-II | CRP, IL-6 | Pittsburgh Healthy Heart Project, healthy community dwelling adults, 50-70 years (mean age 61 years) | 284 | 6 years, 2 measurements | Both | Partially: depression → IL-6 | In path analysis, depression and socio-demographic characteristics explained 18% of the variance in IL-6 change. Adding biomedical risk factors (e.g., cholesterol, fasting glucose and physical illness) and lifestyle factors (smoking, alcohol use and physical activity) explained an additional 7% of variance. In the final model including covariates of anxiety and hostility, baseline depression accounted for 2% of the variance in IL-6 change. Only body mass index was a better predictor than depression of IL-6 change. |

van den Biggelaar et al. 2007	Single group cohort	Geriatric Depression Scale	Circulating CRP; stimulated IL-1ß, IL-6, TNFα, IL-1ra, IL-10	Leiden 85-plus study, no cognitive impairment or depressive symptoms at baseline, 85 years	267	5 years, annual measurement	Inflammat-ory marker → depression	Partially: CRP → depression; IL-1ß → depression; IL-1ra → No depression	Association between inflammatory markers and depression remained significant after adjusting for gender, education, and global cognitive functioning.

Abbreviations: CRP: C-reactive protein; IL: interleukin; ra: receptor antagonist.

Notes: EMBASE search terms used (69 studies retrieved): beck depression inventory, center for epidemiological studies depression scale, cohort analysis, depression, depression inventory, depressive, depressive disorder, disorder, geriatric depression scale, hamilton scale, hospital anxiety and depression scale, immunity, inflammation, longitudinal study, major depression, montgomery asberg depression rating scale, prospective study.

These were the two studies which tested both pro- and anti-inflammatory cytokine relationships and in both studies, high baseline levels of the anti-inflammatory cytokine IL-1 receptor antagonist were protective against later development of depressive symptoms. High levels of CRP and IL-1ß also conferred a risk of later depression onset (van den Biggelaar, et al., 2007).

Arguably the most informative studies are those testing both relationship directions. Matthews et al. (2010) is the only study to have supported a bidirectional relationship between depressive symptoms and CRP, although the evidence was stronger for the baseline CRP to later depression direction. Gimeno et al. (2009) also supported a relationship between baseline CRP/IL-6 and later depressive symptoms. However, other studies in both generally healthy participants (Janicki-Deverts et al., 2010; Stewart, Rand, Muldoon, & Kamarck, 2009) and in those with established coronary artery disease (Duivis, de Jonge, Penninx, Ya Na, & Whooley, 2011) supported the opposite direction, from depressive symptoms to later levels of CRP and/IL-6.

The conflicting results are most likely due to design and participant factors. Firstly, one would expect that increasing the number of measurement points and decreasing the time between baseline and follow-up would lead to greater temporal resolution to be able to more precisely detect a directional relationship. Several of the studies included annual sampling which seemed to assist in detecting relationships in some (Matthews, et al., 2010) but not all studies (Kiecolt-Glaser, et al., 2003;

Matthews, et al., 2007; van den Biggelaar, et al., 2007). Exceptionally long time distances between baseline and follow-up, such as the mean gap of 11.8 years for Gimeno et al. (2009), run the risk of detecting spurious relationships. Large time gaps run the risk of significant alterations in individual level and population level context as well as attrition, which was a major problem across studies (45% in Gimeno, et al., 2009).

Another issue with these prospective studies is how depression is defined. Often studies used conventional cut-offs of continuous scales to dichotomously define suspected depression compared to no depression (e.g., Hamer, et al., 2009b; Milaneschi, et al., 2009), although continuous outcomes are often preferred to improve power. Although clinical interview would be the gold standard for assessing depression, none of the cohorts have used this method, besides Pasco et al. (2010), presumably due to practicality. Even though each of the self-report depression scales has been well used and validated, some are still problematic in terms of content validity of defining depression. Many scales, particularly the General Health Questionnaire used by Gimeno et al. (2009) load heavily or only assess the cognitive symptoms of depression (e.g., dysphoria, suicidal ideation) and ignore the highly important diagnostic criteria of somatic-vegetative symptoms. In terms of the inflammation-depression relationship, this may account for why some studies fail to find an effect as the somatic-vegetative symptoms are associated with depression more strongly than cognitive symptoms, both cross-sectionally and prospectively (Stewart, et al., 2009).

Finally, samples are often limited to small population subsets which differentially strengthen or weaken relationships. For instance, in subsets with often high physical and psychiatric comorbidity including caregivers (Kiecolt-Glaser, et al., 2003), coronary heart disease (Duivis, et al., 2011), and the very old (van den Biggelaar, et al., 2007), residual confounding from uncontrolled factors is a high possibility and so may lead to erroneous conclusions. Conversely, it might be expected that in healthy, representative samples, the relationships would be weak due to restricted range of depressive symptoms. This was generally the case (Milaneschi, et al., 2009; Stewart, et al., 2009). However, Hamer et al. (2009b) had the largest sample size of any study described and thus power to detect small effect sizes. Consequently, this study demonstrated a relationship between CRP and emergent depressive symptoms in general community sample that were depression-free at baseline. Another related issue concerns residual confounding. Adjusting for particular variables can substantially impact statistical significance. The role of unhealthy lifestyle behaviours are of particular interest.

Modifiable lifestyle factors in people with depression and their inflammatory consequences

Many factors are immunomodulatory, including unchangeable factors such as sex and advancing age (Ershler & Keller, 2000; Ferrucci et al., 2005; Howren, et al., 2009; Lang, 2004; Liu, et al., 2012). There is evidence that particular unhealthy behaviours and other risk factors which are potentially

more modifiable are also associated with inflammation (Hamer, Molloy, de Oliveira, & Demakakos, 2009a; Hamer, et al., 2009b; O'Brien, Scully, Fitzgerald, Scott, & Dinan, 2007; O'Connor et al., 2009). People with depression display a range of unhealthy behaviours including smoking, high alcohol use, poor quality of diet (high fat, low fruit and vegetable intake), and low levels of physical activity and other risk factors such as high anxiety, poor sleep, obesity and abdominal fat (Bonnet et al., 2005; Lopresti, Hood, & Drummond, 2013; Strine et al., 2008; van Gool et al., 2007). Interesting from a clinical perspective is how modifiable lifestyle factors potentially drive inflammation and contribute to depression.

In the online resources provided by Australian non-profit depression awareness initiative *beyondblue* (2008), the suggested self-management strategies for depression are to reduce stress, sleep well, reduce alcohol and other drugs, and keep active. Besides that they are readily modifiable and relevant in the clinical context, each of these has a substantial effect on inflammatory mediators cross-sectionally, as already described in relation to stress and stress management (Lopresti, et al., 2013; O'Connor, et al., 2009). As for other factors, sleep dysregulation is a core feature of depression and is associated with increased circulating IL-6 in experimental (Frey, Fleshner, & Wright Jr, 2007) and observational studies (Burgos et al., 2006). Alcohol use and smoking are particularly prevalent in people with depression (Rush, et al., 2005), and each are associated with impaired inflammatory functioning in cross sectional (Edwards, 2009; Garlichs et al.,

2009; Haddad, 2004; Schleifer, Keller, & Czaja, 2006) and longitudinal contexts (Kiecolt-Glaser, et al., 2003).

Perhaps the most consistently mentioned significant covariate in cross sectional inflammation-depression associations are measures of adiposity (Howren, et al., 2009), and the associated contributing behaviours of sedentary lifestyle and diet. Indeed, body mass index at baseline was a stronger predictor of follow-up levels of IL-6 than depressive symptoms (Stewart, et al., 2009). This robust relationship may be because adipose tissue is an important source of pro-inflammatory cytokines (Bulló, Casas-Agustench, Amigó-Correig, Aranceta, & Salas-Salvadó, 2007; Coppack, 2001). While adiposity is the outcome, diet and exercise are the modifiable contributors to obesity. Both diet and exercise independently contribute to inflammation. Diets high in processed foods, trans-isomer fatty acids and omega-6 fatty acids are associated with increased levels of pro-inflammatory cytokines while diets high in whole foods are associated with lower levels (Kiecolt-Glaser et al., 2007; Lopez-Garcia et al., 2005; Nettleton et al., 2006). In some studies reported in a recent systematic review, depression was associated with higher consumption of "Western" dietary patterns and lower consumption of whole food dietary patterns (Quirk et al., 2013). Sedentary lifestyle has also been associated with increased inflammatory mediators (Woods, Vieira, & Keylock, 2009) whereas moderate exercise improves immune functioning (Gleeson, 2007; Woods, et al., 2009). Sedentary lifestyle is regularly observed in people with depression and is predictive of depression onset (van Gool, et al., 2007).

These observations have substantial consequences for assessing epidemiological evidence regarding inflammation-depression relationships. Firstly, it indicates that unhealthy lifestyle factors are likely to be important confounders or mediators of the relationship. This has been shown previously, indicating that controlling for health behaviours, along with sociodemographic information and physical comorbidity, renders observed relationships between inflammatory markers and depression non-significant (Duivis, et al., 2011). Furthermore, these modifiable lifestyle factors may become causative factors in the inflammatory hypothesis; evaluating the contribution of lifestyle factors in prospective studies may help identify what drives increased inflammation. Patient characteristics appear to have a substantial impact on the effect size and statistical significance of results (Howren, et al., 2009; Stewart, et al., 2009) and may account for unexplained heterogeneity in meta-analyses. Most studies have only examined whether patient characteristics are covariates, rather than mediating or causative factors. Hamer et al. (2009b) argue that the implications of statistically covarying for potential confounding factors, such as obesity and smoking, prevents exploration of these factors as intermediate mechanisms of the directional relationship. The ability to statistically model data relationships is one of the major strengths of epidemiological research. So far, only Hamer et al. (2009b) have investigated modifiable lifestyle factors as mediating factors in the depression to inflammation relationship. They found that physical activity provided the largest mediation effect between baseline depressive symptoms

and later levels of CRP, followed by smoking and alcohol, and finally body weight change between baseline and follow-up, which accounted for a very small proportion of mediating effect. This implies that there is a difference between changes in weight and physical activity, and also that there is a potential argument that, clinically, some activities would be more appropriate to modify than others. The design of this study leaves open the question of what happens in the reverse direction: baseline inflammatory markers associated with later depressive symptoms. If modifiable lifestyle factors are driving the observed inflammation to depression relationship, these have a new reason to be targets for public health.

Applications of the inflammatory hypothesis of depression

Beyond the dyad of depression and inflammation, this relationship may have consequences for general physical health, particularly diseases associated with inflammation. Depression is highly comorbid with a range of physical diseases (Knol et al., 2006; Patten et al., 2008). In particular, depression is thought to be an independent risk factor for cardiovascular disease and cardiovascular events including myocardial and cerebral infarction, and depression is also a prevalent occurrence following a cardiovascular event (Aben et al., 2003; Gallagher et al., 2012; Poole, Dickens, & Steptoe, 2011; Thombs et al., 2006; Van der Kooy et al., 2007). Recent evidence, particularly surrounding CRP, indicates that inflammation may be a causal factor in the development of cardiovascular disease, in the formation and dislodgment of atherosclerotic plaques (Hansson, 2005;

Libby, Ridker, & Maseri, 2002). Inflammation may be a common causal

pathway underlying depression and cardiovascular disease. The

inflammation present in a person with depression may be a diathesis for

cardiovascular disease, and the inflammation present in a person with

cardiovascular disease may be a diathesis for depression.

Although separate studies have identified inflammatory markers

(Danesh et al., 2008; Ridker, 2007) and depressive symptoms (Van der Kooy,

et al., 2007) as prospective predictors of cardiovascular events, fewer have

examined depression and inflammatory markers simultaneously and

prospectively in general community samples (Arbelaez, Ariyo, Crum, Fried,

& Ford, 2007; Davidson et al., 2009; Hamer, Molloy, & Stamatakis, 2008;

Ladwig, Marten-Mittag, Lowel, Doring, & Koenig, 2005). Simultaneously

examining these allows the investigation of whether inflammation accounts

for the observed relationship between depression and cardiovascular

disease, or whether these conditions are independent, or potentially

additive, risk factors. Previous studies have tended to find that

inflammatory markers and depression independently predict later

cardiovascular events, although there has also been some evidence of effect

modification (Surtees et al., 2008). In studies of people with existing

cardiovascular disease, similar independence of depression and

inflammatory markers is observed (Vaccarino et al., 2007; Whooley et al.,

2008). Some previous studies have methodological limitations, such as

studying younger people who are generally at low risk for ischemic events.

Furthermore, no previous studies have investigated statistically whether

inflammatory markers mediate the relationship between depression and cardiovascular events.

Inflammation is not only involved in the development of cardiovascular disease, but also many other somatic conditions. Inflammation therefore could be considered as a potential pathophysiological mechanism linking depression to many other somatic conditions such as diabetes, overweight, lung functioning and disease, osteoarthritis, general disability and physical dysfunction, and all-cause mortality.

Conclusions regarding the state of epidemiological evidence for the inflammatory hypothesis of depression

There appear to be at least associative relationships between inflammatory markers and depression. There is less understanding of these relationships over time, in either treatment or prospective cohort contexts. It remains unclear whether the lifestyle factors present in people with depression account for or drive elevations in inflammatory markers. Understanding this temporal evidence will provide evidence toward the greater question as to whether inflammation may be a cause of depression. It will corroborate experimental evidence to indicate whether the relationships appear in naturalistic contexts, highlighting the practical utility of the inflammation hypothesis. Finally, it is important to examine how the inflammatory hypothesis of depression fits into the overall tableau of health, feasibly using cardiovascular disease as a model.

1.2 Overview and aims of the present thesis

It is the aim of this thesis to explore the epidemiological associations between inflammatory markers and depression. This thesis consists of five studies: three meta-analyses and two primary research papers.

Part 1: Meta-analyses

1. <u>Cross sectional</u>: Levels of inflammatory markers in people with and without depression

2. <u>Antidepressant treatment</u>: Change in levels of inflammatory markers after treatment with antidepressants

3. <u>Statin treatment</u>: Change in levels of depressive symptoms and depression events after treatment with statins

Part 2: Primary prospective research papers

1. <u>Inflammatory markers and depression</u>: Baseline inflammatory markers and unhealthy lifestyle factors in predicting later depressive symptoms in the Hunter Community Study

2. <u>Depression and cardiovascular events</u>: Depressive symptoms, CRP and IL-6 in predicting later cardiovascular hospitalisations in the Hunter Community Study

Each paper contributes to the overall aim of the thesis, which is to explore epidemiological proof-of-concept and prospective validation evidence regarding the inflammation hypothesis of depression. Should there be a causal relationship between inflammation and depression, we should see

evidence of a relationship in a variety of cross sectional, prospective and treatment settings. The overarching aim is to firstly highlight that there appears to be a relationship between depression and inflammatory markers and secondly to investigate whether there are any potentially modifiable lifestyle factors which may explain the relationship. The meta-analyses use the rich data already available to answer research questions with more statistical power than individual studies can provide. It also has the potential for greater generalizability by pooling across studies undertaken in different contexts, which is important in mental health fields with such substantial diversity in clinical presentations. The primary studies can ask more refined questions – in this case examining mediation models. The primary studies each use data from the Hunter Community Study, a large prospective cohort study of older people (55-85 years) in the Newcastle region of New South Wales, Australia (McEvoy et al., 2010; Appendix 8.1). In older people, depression is common (3-13%), often overlooked and is associated with poor prognosis, which is why research into this age group is very important (Baldwin, 2000; Cole, Bellavance, & Mansour, 1999; Copeland et al., 2004; Licht-Strunk, van der Windt, van Marwijk, de Haan, & Beekman, 2007; Pirkis et al., 2009; Snowdon & Lane, 2001; Steffens et al., 2000). With the ageing population, the absolute levels of personal and financial burden that depression causes will increase.

This thesis examines the inflammatory markers IL-6, CRP and IL-10. There was an array of markers that could have been considered based on previous research (e.g., tumor necrosis factor, interleukin-1b, interleukin

receptor antagonist). A subset of markers was examined for practical reasons due to the available data and the time available to complete the analyses, although the choice of these particular markers was made strategically. IL-6 and CRP represent general, robust pro-inflammatory markers and IL-10 represents a good candidate for a marker generally regarded as "anti-inflammatory". IL-6 and CRP show similar profiles in response to immune challenge, rising during the acute phase response (Heinrich et al., 1990; Moshage, 1997; Nijsten et al., 1991); however, previous meta-analyses suggest that IL-6 shows a stronger relationship with depression than CRP (Howren et al., 2009) which is why it was selected for the cross-sectional meta-analysis over CRP. Based on the results of the meta-analyses, IL-10 was not pursued as an indicator in the primary prospective studies, instead exploring outcomes in relation to the strong signals of IL-6 and CRP.

Aims of part 1: The meta-analyses

The cross-sectional meta-analysis (Chapter 2) asks the most basic question: Do circulating levels of three key inflammatory markers – IL-6, CRP and IL-10 – differ in people with and without depression (or depressive symptoms)? This question has been answered in a variety of patient populations by many research groups across countries over the last 20 years, so it is fitting to apply meta-analytic methods to pool these data together. Clear elevations in the pro-inflammatory markers IL-6 and CRP in people with high depressive symptoms are expected. IL-10, commonly

regarded as an anti-inflammatory marker, is also expected to be lower in depressed groups. Besides identifying the overall signal, through meta-regression and subgroup analysis I aim to expand on previous meta-analyses by identifying the particular factors which are associated with the strongest signal. For instance, is the signal larger in studies with a clinical diagnosis of depression compared to self-report depressive symptom inventory cut-off scores? Is the signal larger in particular subgroups of medical comorbidities, such as cardiovascular disease? This paper provides the most basic proof-of-concept evidence for an association between inflammation and depression, that people with depression have differences in markers of inflammation to people without the disorder. This paper includes the most extensive moderator analysis in the literature to date.

The antidepressant treatment meta-analysis (Chapter 3) examines whether antidepressant treatment has the capacity to change levels of circulating inflammatory markers, either due to a resolution of depressive symptoms (i.e., the participants essentially become "non-depressed") or through specific anti-inflammatory actions of antidepressants (Castanon, et al., 2002; Tynan et al., 2012). Again, I examine key inflammatory markers IL-6, CRP and IL-10 and pool together what are generally studies with few participants, to increase power to detect a change. This meta-analysis is the first to examine anti-inflammatory cytokines and acute phase proteins, with the one previous meta-analysis (which was published while the meta-analysis presented in Chapter 3 was under review) examining only pro-inflammatory cytokines (Hannestad, et al., 2011). Should these

inflammatory markers change after treatment, through whatever means, it indicates a temporal relationship for inflammatory markers across the different states of depression, whether due to the depressive symptoms resolving, changed health behaviours or direct anti-inflammatory action of the medication.

The final meta-analysis (Chapter 4) moves away from examining inflammatory marker outcomes to investigate whether, in randomised controlled trials of statins compared with placebo, depression outcomes differ between groups. This is the first meta-analysis to specifically assess depression outcomes; a similar meta-analysis published while the current meta-analysis was under review examines general psychological functioning (O'Neil, et al., 2012). Statins were selected for study in this thesis due to their widespread use and the body of evidence amassing that shows that statins, besides having cholesterol-reducing properties, may also have anti-inflammatory properties (Farooqui, et al., 2007; Pancholi, et al., 2009). If a medication with anti-inflammatory properties could lead to reductions in depression events or depressive symptoms, then we would have another line of supporting proof-of-concept evidence that inflammation may be causally involved in depression. Evidence from placebo-controlled randomised controlled trials is preferred to epidemiological evidence as it minimizes risk of bias due to differential lifestyle effects between statin users and non-users, which is why these were pooled for the meta-analysis in this thesis.

Aims of part 2: Primary prospective research papers

The first prospective paper (Chapter 5) focusses on prospective validation and incremental value in using baseline levels of relatively easily measured pro-inflammatory markers CRP and IL-6 to predict depressive symptoms at follow-up. Aside from looking at the overall signal, the aim is to examine whether aspects of unhealthy lifestyle confound the relationship between inflammatory markers and depression. Furthermore, for the first time, I examine whether inflammatory markers mediate relationships between lifestyle and depression. Unhealthy lifestyle is an important risk factor for depression. For the inflammatory hypothesis of depression, this study highlights whether lifestyle may be an important driver of the inflammatory markers in depression. Specific behaviours of interest are measures of adiposity, alcohol consumption, smoking, physical inactivity and high saturated fat consumption.

The final paper of this thesis (Chapter 6) examines potential flow on effects from the inflammation hypothesis of depression to other aspects of health. The inflammation hypothesis proposes to explain the high comorbidity between depression and physical illnesses. This paper uses cardiovascular events as a test case. Cardiovascular events have an established association with independently with both depression and inflammation (particularly CRP) (Pearson et al., 2003; Ridker, Rifai, Rose, Buring, & Cook, 2002; Thombs, et al., 2006; Van der Kooy, et al., 2007). This paper examines whether baseline levels of depressive symptoms, CRP and IL-6 predict later cardiovascular hospitalisations including myocardial

infarction, angina and ischemic stroke. It then expands on previous studies to investigate whether inflammatory markers mediate the relationship between relatively well-established relationships between depressive symptoms and later cardiovascular events.

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2. A meta-analysis of differences in IL-6 and IL-10 between people with and without depression: Exploring the causes of heterogeneity

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See Appendix 8.2 for the PRISMA checklist for the reporting of meta-analyses regarding this Chapter

2.1 Abstract

Epidemiological evidence for the inflammatory hypothesis of depression is largely cross-sectional; people with depression have elevated levels of circulating pro-inflammatory markers compared to people without depression. The limitation of cross sectional research is the potential for extraneous factors to influence observed effects. The purpose of this meta-analysis of cross-sectional studies of interleukin (IL)-6 and IL-10 in people with and without depression is to provide a targeted analysis of potential moderator factors relating to the diagnosis of depression and to physical and psychiatric comorbidity. Electronic searches of Embase and Medline databases were conducted using subject headings "interleukin-6" or "interleukin-10" and those relating to depression. Studies were included if they measured circulating marker levels in serum or plasma in a group of people with and without depressive symptoms (99 studies for IL-6, 19 studies for IL-10). IL-6 was elevated in depressed compared to non-depressed groups ($d = 0.46$, 99% CI 0.34 to 0.58, $I^2 = 85.9\%$). This effect was larger in subgroups where depressive disorders were diagnosed compared to those with only depressive symptoms via standardized inventory, and those who were recruited from inpatient or outpatient settings compared to the general community. The effect was also larger in those who were not selected for a particular comorbidity compared to those selected for cardiovascular disease. IL-10 effect size was not significant ($d = -0.31$, 99% CI -0.95 to 0.32, $I^2 = 94.1\%$) which was not accounted for in subgroup

analyses or meta-regression, indicating there is not a global elevation in cytokines. These data highlight that comorbidity and behavioral aspects of depression need to be measured and controlled in future prospective and experimental research testing the inflammatory hypothesis of depression.

2.2 Introduction

Depressive disorders are common and contribute substantially to disease burden (Kessler et al., 2003; Üstün, Ayuso-Mateos, Chatterji, Mathers, & Murray, 2004). The key to understanding their origins may be inflammation. The theory that inflammation causes depression relies on the idea that cytokines exert central and peripheral effects which cause the psychological and physiological experience of depression (Miller, Maletic, & Raison, 2009). Inflammation is thought to cause clinical depressive disorders by inducing sickness behaviours which are akin to neurovegetative symptoms of depression (e.g., lethargy, changed appetite, changed sleep), activating the hypothalamic-pituitary-adrenal axis and sympathetic nervous system, altering serotonin and dopamine synthesis and reuptake and causing neurodegenerative processes which lead to the phenomenology of depression (for reviews, see Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Irwin & Miller, 2007; Maes et al., 2009; Miller, et al., 2009). Likely sources of depression-inducing cytokines include internal stressors (e.g., organic disease, adipose tissue) and external stressors (e.g., psychological distress, diet).

Animal models and some experimental human studies support the causal nature of this theory, where administration of cytokines or immune stimulants cause depression-like behaviour and symptoms, and also evidence that medical illness and inflammation-based treatments (i.e., interleukin [IL]-2 or interferon alpha treatments for hepatitis C and cancer)

are linked to incidence of depressed mood or depressive disorders (Capuron et al., 2009; Dantzer, et al., 2008). However, the bulk of human literature is cross sectional; people with depression or depressive symptoms have elevated levels of circulating inflammatory markers compared to those without (Dowlati et al., 2010; Howren, Lamkin, & Suls, 2009). It is impossible to infer causality in this context, but these studies do permit examination of the strength of association between inflammatory markers and depressive symptoms/disorder.

Several recent meta-analyses have verified that people with depression show elevated levels of the cytokine IL-6 compared to people without depression in circulating serum or plasma (Dowlati, et al., 2010; Howren, et al., 2009; Liu, Ho, & Mak, 2012). IL-6 is a pleiotropic cytokine associated with inflammation, both acute and chronic, and also produced by adipose cells. Elevations in IL-6 have been associated with increases in several "trait" factors such as advancing age, high body mass index (BMI), smoking and physical comorbidity as well as "state" factors such as fat consumption, acute physical activity, and psychological stress (see O'Connor et al., 2009 for review). As people with depressive disorders frequently exhibit these lifestyle factors, there is a possibility that one or more of these is driving the observed elevations in circulating levels of inflammatory markers. This is supported in prospective studies which show that once BMI, smoking and physical activity are controlled for, the relationship between depression and IL-6 is no longer significant (Duivis, de Jonge,

Penninx, Ya Na, & Whooley, 2011). It is important to assess the contribution of lifestyle factors to the observed associations between depression and IL-6 to more accurately define the role of peripheral, circulating inflammatory markers as markers of an inflammatory depressive state. A second interleukin of potential interest is IL-10, a cytokine with anti-inflammatory effects which is in part stimulated by IL-6 among other factors. IL-10 is also elevated in response to acute immune challenge (Henry, Huang, Wynne, & Godbout, 2009) and may be implicated in depression (Mesquita et al., 2008; Roque, Correia-Neves, Mesquita, Palha, & Sousa, 2009).

The current meta-analysis compares IL-6 and IL-10 in people with and without depression and is the most extensive to date. It includes a broad range of studies with different degrees of "depression" (diagnosed disorder vs. depressive symptoms), different recruitment sources and instances where depressed and control groups are matched for particular physical disease. The aim was to pool the evidence of a cross sectional association between depression and IL-6 and IL-10 in light of moderating factors to investigate whether the elevations in inflammatory markers are attributable to the physical and psychiatric comorbidities of depressive disorders.

<center>2.3 Methods</center>

Inclusion and exclusion criteria

Studies were included if they met the following criteria: (1) primary study comparing adult participants with non-perinatal depression (either diagnosed with major depression/dysthymia or endorsing high depressive symptoms on a standardized inventory) and a control group of people without depressive symptoms; (2) reported mean or median circulating plasma or serum IL-6 or IL-10 for both groups; (3) publication in English in a peer reviewed journal since 1990; (4) provided sufficient information to calculate an effect size, namely the means or medians of the interleukin for the depressed and non-depressed groups, sample size for each, either the standard deviations or range of interleukin levels for each group or at least an exact *p* value. Studies which measured inflammatory markers via *ex vivo* stimulation were excluded from this meta-analysis to minimize measurement error. Studies which sampled from people with a particular physical or psychiatric comorbidity (e.g., heart disease, post-traumatic stress disorder) were eligible for inclusion in this meta-analysis providing the comorbidity was present in both the depressed and non-depressed groups.

Search strategies

A computerized search of Embase and Medline databases was completed in January 2011 using the key words mapped to subject headings for depression (depression, depressive disorder, major depressive disorder,

dysthymic disorder) and "interleukin-6" or "interleukin-10", limited to human and English language literature. The abstract of each article identified in the search was screened for relevance. Full text articles were extracted and assessed for eligibility when the abstracts described measurement of inflammatory markers or when abstracts lacked detail or were ambiguous in methods (Figure 2.1 shows the article extraction process; Section 2.7 *Supplementary references: Studies included in the IL-6 and IL-10 meta-analyses* contains references the 102 included studies).

Coding procedures

Eligible studies were coded for the outcome variable of mean or median circulating IL-6 or IL-10 and the reported dispersion measure (standard deviation, standard error, range), number of participants and, if appropriate, *p* value. Interleukin measures reported graphically were converted to numerical values using Data Thief III, version 1.6 (Tummers, van der Laan, & Huyser, 2008). Potential moderator variables were also coded including participant characteristics (diagnosis, depressive symptom ratings, age, gender ratio, inpatient or outpatient status, BMI, smoking status, presence of physical and psychiatric comorbidities, antidepressant use, whether the participants were matched) and assay procedure characteristics (assay brand, mood assessment and blood collection on the same day, blood samples frozen and thawed once, time of day of blood sampling, intra- and inter-assay variation). Data were extracted and re-checked by a single author (SH).
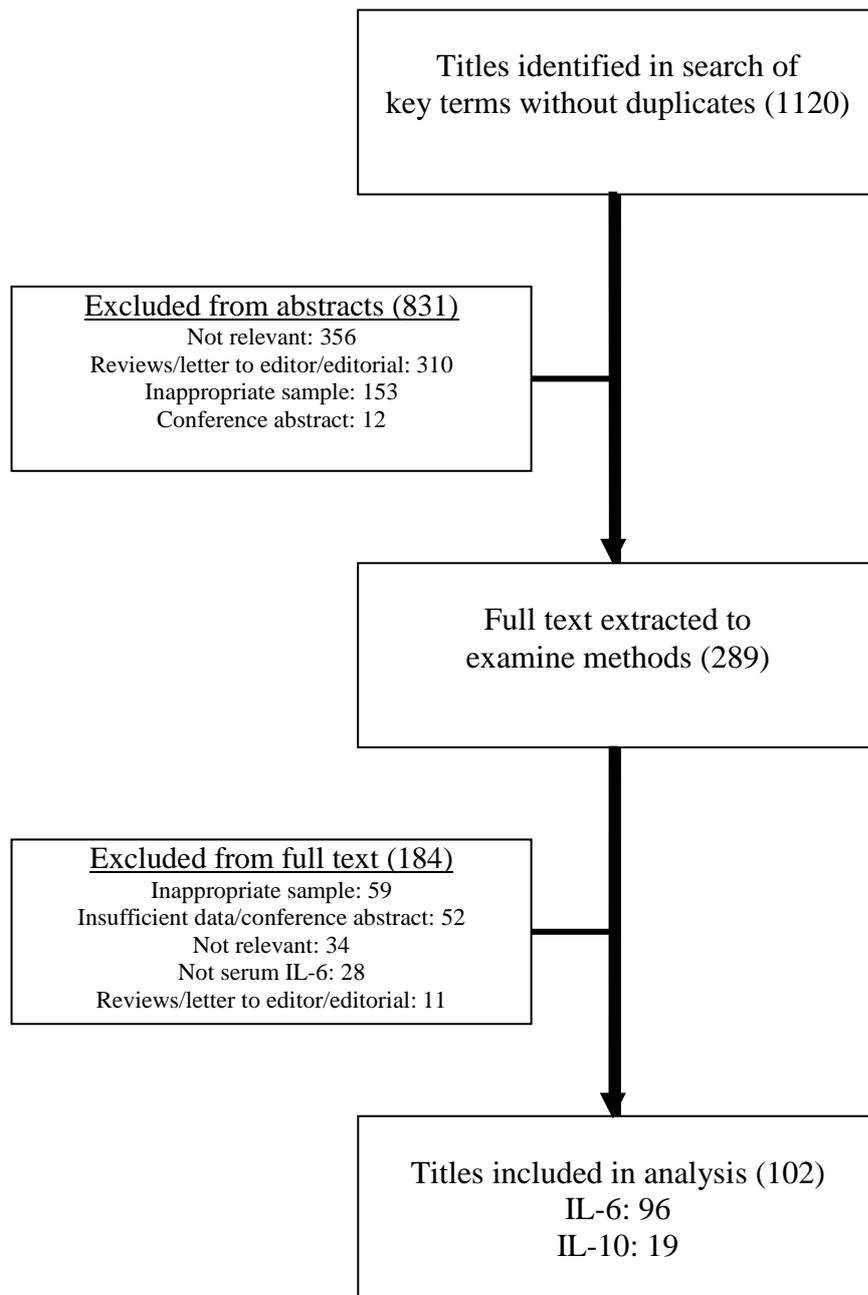
Figure 2.1. Summary of article extraction process and reasons for exclusion for the IL-6 and IL-10 meta-analyses. "Inappropriate samples" includes participants without depression or a control group, participants who are pregnant or undergoing cytokine treatment, and adolescents.

Statistical methods

Statistical analyses were completed using Comprehensive Meta-Analysis II (Biostat, Inc., USA) for study effect size calculations and Stata/SE 11.1 (StataCorp LP, USA) for meta-analysis and meta-regression. Study effect sizes were calculated as Cohen's d standardized mean difference of IL-6 or IL-10 between depressed and non-depressed groups. A positive effect size indicates the depressed group reported elevated interleukin compared to the non-depressed group. If required, the mean was estimated using the median when sample sizes were large ($n > 25$), or estimated using the median and range when sample sizes were small (Pudar Hozo, Djulbegovic, & Hozo, 2005). If required, standard deviation was estimated using range (Pudar Hozo, et al., 2005), and if a study did not report standard deviation or range but otherwise met selection criteria, the study effect size was estimated using the difference in means, sample size and p value.

Most studies only contributed one effect size to the meta-analysis. In studies where participants were followed up or underwent experimental procedure (e.g., stress test, exercise test, antidepressant treatment), the baseline measurement for that study was coded for analysis. In studies where levels of inflammatory markers were reported separately for groups with Major Depressive Disorder (MDD), elevated depressive symptoms and non-depressed controls, only the comparison between MDD and non-depressed controls was coded. For studies which reported inflammatory

markers for subgroups of participants with depression, whole sample effect sizes were imputed by collapsing subgroup means. The three studies which contributed two effect sizes to the meta-analysis reported effect sizes for a depressed and non-depressed group with a particular comorbid condition and without that condition.

Individual study effect sizes were synthesized to generate an overall effect size using a random effects model, weighted by the inverse of variance. We also completed a sensitivity analysis to identify potential outliers by removing each study one by one to examine the individual influence of each study on the overall effect size. To assess publication bias, we computed a funnel plot of effect size versus study standard error and the Egger test of funnel plot asymmetry (Egger, Davey Smith, Schneider, & Minder, 1997) and the fail-safe N (Rosenthal, 1979).

Heterogeneity was assessed using Cochrane's Q and I^2, which calculates the proportion of variation attributed to heterogeneity (Higgins, Thompson, Deeks, & Altman, 2003). To explore sources of heterogeneity, moderator analysis was undertaken via subgroup analysis and residual maximum likelihood meta-regression. Subgroup analysis was completed for categorical variables, comparing pooled random effect size with separate estimates of tau for each subgroup using Z test (Borenstein, Hedges, Higgins, & Rothstein, 2009). Meta-regression included the average magnitude and standardized mean differences of continuous variables

between depressed and non-depressed groups. To control for family-wise error, criterion for significance was set to α = .01 in all analyses.

2.4 Results

Interleukin-6

There were 99 comparisons of IL-6 in depressed and non-depressed groups from 96 publications. The majority of these studies used a diagnostic interview to diagnose depressive disorders, typically a current Major Depressive Episode ($n = 67$). Recruitment source of patients was mixed (inpatient $n = 28$; outpatient $n = 39$; inpatients or outpatients $n = 4$; community sources including cohort studies $n = 19$). In addition to depression, in 35 studies participants were selected for general medical or psychiatric comorbidity including cardiovascular disease ($n = 15$), other DSM-IV Axis I and II disorders ($n = 7$) and renal disease ($n = 6$).

Overall, IL-6 was significantly elevated in the depressed group compared to the non-depressed group, recording a moderate effect size, $d = 0.46$ (99% CI 0.34, 0.58), Figure 2.2. There was evidence of publication bias using Egger's test with a slight positive bias, $b = 2.19$, $SE = 0.399$, $t(97) = 5.49$, $p < .001$, although the fail safe-N was 9145. No outliers were found. There was significantly high heterogeneity, $Q(98) = 693.51$, $p < .001$, $I^2 = 85.9\%$.

| Study name	d (99% CI)	% Weight	Total N
Alesci, Martinez, et al. 2005	0.56 (-0.68, 1.79)	0.60	18
Andrei, Fraguas Jr., et al. 2007	-0.17 (-1.06, 0.71)	0.84	34
Basterzi, Aydemir, et al. 2005	0.54 (-0.23, 1.31)	0.94	46
Berk, Wadee, et al. 1997	5.18 (3.64, 6.72)	0.45	49
Boettger, Muller, et al. 2010	0.14 (-0.80, 1.08)	0.80	30
Bossola, Ciciarelli, et al. 2010	0.72 (0.12, 1.31)	1.10	80
Brambilla and Maggioni 1998	-0.47 (-1.63, 0.70)	0.64	20
Chen, Xia, et al. 2007	-0.26 (-1.06, 0.55)	0.91	48
Cizza, Marques, et al. 2008	4.06 (2.56, 5.57)	0.47	36
Czira, Lindner, et al. 2011	0.20 (0.01, 0.40)	1.43	973
Dervisoglu, Kir, et al. 2008	-0.02 (-0.56, 0.53)	1.15	93
Dhabhar, Burke, et al. 2009	1.60 (0.36, 2.83)	0.60	23
Dimopoulos, Piperi, et al. 2008	1.50 (0.78, 2.21)	0.99	66
Dinan, Clarke, et al. 2008	1.62 (0.53, 2.70)	0.69	30
Dinan, Siggins, et al. 2009	1.25 (0.40, 2.10)	0.87	44
Empana, Sykes, et al. 2005	0.14 (-0.07, 0.35)	1.42	869
Euteneuer, Schwarz, et al. 2011	0.00 (-0.56, 0.56)	1.13	85
Ferketich, Ferguson, et al. 2005	0.30 (-0.62, 1.23)	0.81	32
Fitzgerald, O'Brien, et al. 2006	0.90 (0.02, 1.78)	0.85	38
Fornaro, Martino, et al. 2011	0.01 (-0.90, 0.92)	0.82	32
Forti, Rietti, et al. 2010	0.36 (0.08, 0.64)	1.37	963
Frasure-Smith, Lesperance, et al. 2007	0.10 (-0.14, 0.34)	1.40	602
Frasure-Smith, Lesperance, et al. 2009	0.14 (-0.08, 0.36)	1.41	682
Gabbay, Klein, et al. 2009	0.43 (-0.40, 1.25)	0.90	45
Garcia-Lozano, Capilla-Sevilla, et al. 2008	-0.26 (-0.95, 0.44)	1.01	56
Gill, Luckenbaugh, et al. 2010	3.62 (1.65, 5.59)	0.31	18
Glaser, Robles, et al. 2003	0.14 (-0.33, 0.62)	1.22	119
Gur, Karakoc, et al. 2002	-0.09 (-0.67, 0.48)	1.12	81
Hemingway, Shipley, et al. 2003	0.15 (-0.22, 0.51)	1.31	277
Hope, Dieset, et al. 2011	-0.89 (-1.52, -0.26)	1.07	84
Hung, Hsieh, et al. 2007	0.10 (-0.79, 0.99)	0.84	35
Hung, Wu, et al. 2011	0.69 (0.25, 1.13)	1.25	146
Häfner, Emeny, et al. 2011	-0.00 (-0.14, 0.13)	1.45	700
Janelidze, Mattei, et al. 2011	-0.18 (-1.08, 0.72)	0.83	33
Jehn, Kuehnhardt, et al. 2006	0.61 (0.06, 1.17)	1.14	114
Jehn, Kuhnhardt, et al. 2010	0.42 (-0.13, 0.96)	1.15	114
Jimenez, Sobrino, et al. 2009	-0.01 (-0.58, 0.56)	1.12	134
Johansson, Lesman-Leegte, et al. 2011	0.21 (-0.02, 0.44)	1.40	517
Kagaya, Kugaya, et al. 2001	-0.14 (-1.36, 1.08)	0.61	18
Kahl, Rudolf, et al. 2005	-1.09 (-2.20, 0.02)	0.68	26
Kalender, Dervisoglu, et al. 2007	0.46 (-0.45, 1.38)	0.82	42
Kaminska, Marmurowska-Michallowska, et al. 2002	0.47 (-0.21, 1.15)	1.02	59
Kapczinski, Dal-Pizzol, et al. 2011	-0.04 (-0.85, 0.78)	0.90	40
Kiecolt-Glaser, Belury, et al. 2007	1.10 (-0.08, 2.27)	0.64	43
Kop, Stein, et al. 2010	0.09 (-0.15, 0.33)	1.40	907
Kubera, Kenis, et al. 2000	0.96 (-0.29, 2.21)	0.59	19
Lehto, Niskanen, et al. 2010	0.40 (-0.01, 0.81)	1.27	416
Leo, Di Lorenzo, et al. 2006	1.19 (0.61, 1.77)	1.11	92
Lesperance, Frasure-Smith, et al. 2004	0.08 (-0.37, 0.53)	1.23	481
Leu, Shiah, et al. 2001	1.39 (0.34, 2.44)	0.72	30
Maes, Bosmans, et al. 1995	1.06 (0.46, 1.65)	1.10	13
Maes, Bosmans, et al. 1997	-1.02 (-1.86, -0.19)	0.88	50
Maes, Lin, et al. 1999	-0.16 (-1.60, 1.27)	0.50	90
Maes, Meltzer, Bosmans, et al. 1995	0.47 (-0.07, 1.00)	1.15	99
Maes, Meltzer, et al. 1995	0.44 (-0.44, 1.31)	0.85	41
Mikova, Yakimova, et al. 2001	0.25 (-0.58, 1.08)	0.89	43
Milaneschi, Corsi, et al. 2009	-0.02 (-0.22, 0.18)	1.42	991
Miller, Rohleder, et al. 2005	-0.11 (-0.72, 0.49)	1.09	100
Miller, Stetler, et al. 2002	0.73 (0.20, 1.27)	1.16	72
Moorman, Mozaffarian, et al. 2007	0.28 (-0.19, 0.76)	1.22	129
Motivala, Sarfatti, et al. 2005	0.85 (-0.01, 1.70)	0.87	40
Musselman, Miller, et al. 2001 (cancer group)	0.36 (-0.80, 1.53)	0.64	21
Musselman, Miller, et al. 2001 (non-cancer group)	1.11 (-0.08, 2.29)	0.63	22
O'Brien, Scully, et al. 2006	0.26 (-0.77, 1.29)	0.73	30
O'Brien, Scully, et al. 2007	0.62 (-0.12, 1.35)	0.97	52
O'Connor, Irwin, et al. 2007	0.26 (-0.60, 1.12)	0.86	36
Ortiz-Dominguez, Hernandez, et al. 2007	3.79 (2.39, 5.20)	0.51	43
Pan, Ye, et al. 2008	0.04 (-0.11, 0.19)	1.44	3289
Parissis, Adamopoulos, et al. 2004	0.24 (-0.65, 1.12)	0.84	300
Parissis, Farmakis, et al. 2009	0.17 (-0.14, 0.48)	1.35	35
Penninx, Kritchevsky, et al. 2003	0.20 (-0.02, 0.42)	1.41	3024
Pike and Irwin. 2006	0.74 (-0.01, 1.49)	0.96	50
Pizzi, Manzoli, et al. 2008	0.39 (0.08, 0.69)	1.36	415
Ranjit, Diez-Roux, et al. 2007	0.13 (-0.01, 0.27)	1.45	6778
Rethorst, Moynihan, et al. 2011	0.12 (-0.41, 0.65)	1.16	97
Rief, Pilger, et al. 2001 (non-somatization group)	0.13 (-0.47, 0.73)	1.09	73
Rief, Pilger, et al. 2001 (somatization group)	0.08 (-0.51, 0.66)	1.11	77
Schins, Tulner, et al. 2005	0.11 (-0.40, 0.62)	1.18	103
Selikhova, Kushlinskii, et al. 2002 (Parkinson's group)	1.02 (0.19, 1.85)	0.89	38
Selikhova, Kushlinskii, et al. 2002 (non-Parkinson's group)	0.76 (-0.12, 1.64)	0.84	45
Simic Ogrizovic, Jovanovic, et al. 2009	0.60 (0.13, 1.07)	1.22	128
Simon, McNamara, et al. 2008	0.43 (-0.09, 0.96)	1.17	98
Sluzewska, Rybakowski, Laciak, et al. 1995	0.81 (0.00, 1.62)	0.91	33
Sluzewska, Rybakowski, M. Sobieska, et al. 1995	2.75 (1.75, 3.74)	0.76	64
Sluzewska, Rybakowski, et al. 1996	2.00 (1.11, 2.88)	0.84	54
Sluzewska, Samborski, et al. 1997	2.54 (1.56, 3.51)	0.77	52
Steptoe, Kunz-Ebrecht, et al. 2003	-0.06 (-0.53, 0.41)	1.22	204
Szuster-Ciesielska, Slotwinska, et al. 2008	2.08 (1.24, 2.91)	0.89	59
Tousoulis, Drolias, et al. 2009	0.04 (-0.30, 0.37)	1.33	250
Ushiroyama, Ikeda, et al. 2002	0.74 (0.52, 0.96)	1.41	610
Vaccarino, Brennan, et al. 2008	0.14 (-0.18, 0.47)	1.34	348
Vaccarino, Johnson, et al. 2007	0.27 (-0.06, 0.59)	1.34	356
Weinstein, Deuster, et al. 2010	1.52 (0.42, 2.63)	0.68	28
Whooley, Caska, et al. 2007	-0.15 (-0.35, 0.05)	1.42	984
Yang, Ruan, et al. 2011	0.57 (-0.11, 1.25)	1.02	60
Yang, Xie, et al. 2007	0.63 (-0.08, 1.35)	0.99	56
Yang, Zhang, et al. 2011	-0.03 (-0.56, 0.51)	1.16	100
Yao, Tao, et al. 2004	1.13 (0.38, 1.89)	0.96	60
Yoshimura, Hori, et al. 2009	0.99 (0.47, 1.51)	1.17	81
Overall (I-squared = 85.9%, p = 0.000)	0.46 (0.34, 0.58)	100.00	

NOTE: Weights are from random effects analysis

87

d (99% CI)

Figure 2.2. Forest plot of standardized mean difference IL-6 between depressed and non-depressed groups for each study (Cohen's *d*, 99% CI). A positive effect size indicates that IL-6 is elevated in the depressed compared with the non-depressed group. The pooled effect size is provided as the black diamond at the bottom of the figure.

We completed meta-analysis on more homogenous subgroups to explore sources of heterogeneity (Tables 2.1, 2.2 and 2.3). Many subgroups provided significant effect sizes which differed in magnitude to the overall effect size and differed from other subgroups within their category, although the only subgroups to substantially decrease heterogeneity according to I^2 values (<50%) were subgroups of participants where both depressed and non-depressed groups had cardiovascular disease or cancer.

Effect of depressive disorder

First, we looked at subgroups relating to the measurement of "depression", specifically the extent to which the "depressed" group represented depressive disorder rather than depressive symptoms and the severity of the depressive condition (Table 2.1). When participants in the depression group were diagnosed with a depressive disorder via a diagnostic interview, the effect size was significantly larger than when participants only endorsed high depressive symptoms on a standardized inventory. In terms of specific diagnoses, there were no significant differences in effect size between samples diagnosed with MDD only and samples which included people with a diagnosis of lifetime MDD, people with bipolar disorder in a current depressive phase and other depressive disorders (e.g., minor depression, dysthymia). Furthermore, there was no significant difference in effect sizes between participants recruited from inpatient versus outpatient sources, although a significantly smaller effect size was recorded from those recruited from community samples (e.g., cohort studies)

Table 2.1. Subgroup analyses for IL-6 related to depression, including the number (N) of studies included in each subgroup, subgroup effect sizes (ES; Cohen's d) and the difference in ES between subgroups relative to a reference category (denoted with "Ref").

Subgroups		N	ES (d)	99% CI		I^2 (%)	ES$_{diff}$	99% CI	
Diagnostic interview	Yes	67	0.68***	0.46	0.91	87.9	Ref		
	No	32	0.20***	0.09	0.30	71.8	0.48***	0.23	0.73
Diagnosis	MDD	40	0.60***	0.36	0.84	80.1	Ref		
	No diagnosis	32	0.20***	0.09	0.30	71.8	0.40***	0.14	0.66
	MDD or lifetime MDD	5	0.38	-0.41	1.17	86.7	0.22	-0.61	1.05
	Other depression	12	0.49**	0.08	0.90	82.5	0.11	-0.37	0.59
	Includes people with bipolar depressive phase	10	1.53**	0.12	2.94	95.7	-0.93	-2.36	0.50
Patient source	Inpatient	28	0.65***	0.29	1.01	89.9	Ref		
	Outpatient	39	0.43***	0.24	0.63	78.3	0.22	-0.19	0.63
	Community	19	0.17***	0.05	0.28	67.1	0.48***	0.10	0.86
	Mixed in- & outpatients	4	0.13	-1.04	1.30	83.7	0.52	-0.70	1.74
Depressed group has antidepressant users	Yes	31	0.26***	0.09	0.43	81.8	Ref		
	No	41	0.80***	0.49	1.10	86.8	-0.54***	-0.89	-0.19
Control group has antidepressant users	Yes	16	0.08	-0.05	0.21	67.1	Ref		
	No	45	0.66***	0.40	0.93	84.0	-0.58***	-0.87	-0.29

***<.001, **<.01, *<.05

Note: Other depression includes minor depression, dysthymia, depression not otherwise specified.

Table 2.2. Subgroup analyses for IL-6 related to comorbidity.

Subgroups		N	ES (d)	99% CI		I^2 (%)	ES$_{diff}$	99% CI	
Comorbidity	None	64	0.66***	0.49	0.83	89.0	Ref		
	Cardiovascular	15	0.10*	-0.01	0.20	18.3	0.56***	0.36	0.76
	Renal	6	0.43***	0.01	0.75	67.1	0.23	-0.22	0.68
	Psychiatric	7	0.08	-0.83	0.99	85.0	0.58	-0.35	1.51
	Cancer	3	0.50***	0.13	0.87	0	0.16	-0.25	0.57
	Other	4	0.002	-0.54	0.54	54.7	0.66**	0.09	1.23
People with anxiety in depressed group	Excluded	19	0.51***	0.16	0.87	76.6	Ref		
	Included	9	0.65**	0.01	1.29	88.5	-0.14	-0.87	0.59
BMI	Normal weight	11	0.16	-0.14	0.44	65.3	Ref		
	Overweight/ obese	30	0.26***	0.10	0.43	83.8	-0.10	-0.44	0.24
Smokers	Yes	38	0.29***	0.15	0.42	84.2	Ref		
	No	5	0.33	-0.31	0.98	78.6	-0.04	-0.70	0.62

***<.001, **<.01,*<.05

Table 2.3. Subgroup analyses for IL-6 related to measurement sensitivity.

Subgroup		Subgroup effect sizes					Difference between subgroups		
		N	ES (d)	99% CI		I^2 (%)	ES$_{diff}$	99% CI	
Definition sensitivity	Overall	99	0.46***	0.34	0.58	85.9	Ref		
	MDD diagnosis & no comorbidity	31	0.74***	0.44	1.04	80.3	-0.28*	-0.60	0.04
Matched	Age, gender	15	0.91***	0.35	1.46	91.3	Ref		
	Age, gender, BMI	7	1.10**	0.08	2.11	85.7	-0.19	-1.35	0.97
	Other matching	7	0.99**	0.13	1.85	87.9	-0.08	-1.11	0.95
	No matching	70	0.31***	0.19	0.43	81.8	0.60**	0.03	1.17
ES estimated from median	Yes	27	0.23***	0.10	0.36	74.3	Ref		
	No	72	0.58***	0.39	0.76	87.6	-0.35***	-0.58	-0.12
Mood assessed/blood collected same day	Yes	13	0.30	-0.12	0.73	83.4	Ref		
	No	9	0.41	-0.23	1.05	86.4	-0.11	-0.88	0.66
Samples frozen and thawed once	Yes	21	0.63	0.23	1.02	91.5	Ref		
	Unknown	78	0.43	0.30	0.55	83.1	0.20	-0.22	0.62
Morning sampling	Yes	58	0.59	0.38	0.80	87.4	Ref		
	No	3	0.38	-0.53	1.29	65.8	0.21	-0.72	1.14
Assay suppliers	R&D Quantikine ELISA	36	0.29***	0.14	0.43	82.0	Ref		
	Eurogenetics, Sandwich ELISA	12	0.78	0.04	1.52	90.5	-0.49	-1.25	0.27

***<.001, **<.01,*<.05

Note: other matching includes age, gender, ethnicity, BMI, other demographics.

compared with inpatient settings, indicative of some degree of variation in effect size according to severity of illness. There was also a significantly larger effect size in studies where the depressed group did not include antidepressant users compared with those that included antidepressant users, consistent with evidence that antidepressants may reduce pro-inflammatory markers (Hiles, Baker, de Malmanche, & Attia, 2012). Finally, there was a significantly smaller effect size when the control group included participants who used antidepressants, indicating the potential presence of people in the control group with a depressive disorder without current depressive symptoms who may be diluting the difference in inflammatory markers between depressed and "non-depressed" groups. Meta-regressions on the minority of studies that reported depressive symptom inventory scores were not significant between IL-6 effect size and Hamilton Depression Rating Scale (Hamilton, 1960) score in the depressed group (36 studies) or the standardized difference in depression inventory score between depressed and non-depressed groups (32 studies).

Effect of lifestyle factors

Second, we examined subgroups related to comorbidity (Table 2.2). The only significant differences were smaller effect sizes in studies where all participants had cardiovascular disease or "other" comorbidity (e.g., Parkinson's disease, fibromyalgia), compared to those with participants not selected for a particular comorbidity. Studies that did not select for a particular comorbidity were not significantly different in effect size to those

that selected for renal disease, psychiatric comorbidity (e.g., post-traumatic stress disorder, borderline personality disorder) or cancer. Effect size did not significantly differ depending on whether participants were normal weight or overweight/obese, or studies included or excluded smokers, and meta-regression of these as continuous variables were also not significant. However, in a restricted sample of studies where participants were not selected for particular comorbidities, there was a trend association between BMI and IL-6, where higher BMI in the depressed than the non-depressed group was associated with larger differences in IL-6 between groups, $b = 0.23$, $SE = 0.11$, $a = 0.35$, $t(19) = 2.12$, $p = .048$, $R^2 = 18.36\%$. In the entire sample, meta-regression between IL-6 effect size and the variables percentage males or average age across groups were not significant. However, there was a significant positive relationship between IL-6 effect size and the standardized difference of ages; where IL-6 effect size increased as the age in the depressed group became larger than the age in the control group; $b = 0.39$, $SE = 0.16$, $a = 0.42$, $t(80) = 2.47$, $p = .016$, $R^2 = 10.82\%$.

Sensitivity analyses

Finally, we examined the sensitivity of our analyses (Table 2.3). Compared with the overall analysis with broad inclusion criteria, the effect size was significantly larger for studies where participants were diagnosed with MDD and not selected for comorbid conditions (similar sample to previous meta-analyses; Dowlati, et al., 2010; Liu, et al., 2012). There was a significantly smaller effect size in those studies which did not match for any

variables compared with those that matched for age and gender, and studies where effect size was estimated from the median compared to the mean. There were no significant differences in several other factors related to the sensitivity of measurement, including whether the study collected mood and blood measures on the same day, reported freezing and thawing blood samples once, or sampling blood in the morning. There was also no significant difference in effect size between two common assay suppliers. No meta-regression related to IL-6 measurement sensitivity was significant.

Interleukin-10

19 studies of IL-10 were available. As with IL-6, most of these studies diagnosed depressive disorder via diagnostic interview ($n = 13$), although these studies tended to recruit patients from outpatient sources ($n = 14$; inpatient $n = 3$; mixed inpatients and outpatients $n = 1$; community $n = 1$). Few studies selected for comorbidities (cardiovascular $n = 2$; renal $n = 2$; other $n = 2$). Blood was sampled in the morning. The effect size between depressed and non-depressed groups was non-significant, $d = -0.31$ (99% CI -0.95, 0.32), Figure 2.3. However, there was no evidence of publication bias via Egger's test, $t(18) = 0.23$, $p = .82$. There was significant heterogeneity, $Q(18) = 306.02$, $p < .001$, $I^2 = 94.1\%$. In the subgroup analyses, there were some subgroups with no statistical heterogeneity according to I^2, although these had only 2-3 studies; these included studies which reported whether they included or excluded people with anxiety disorders and those studies where we used the median to estimate the effect size (subgroup data not

Study name	d (99% CI)	% Weight	Total N
Boettger, Muller, et al. 2010	-0.10 (-1.04, 0.84)	5.13	30
Dhabhar, Burke, et al. 2009	-0.95 (-2.09, 0.19)	4.88	23
Garcia-Lozano, Capilla-Sevilla, et al. 2008	0.12 (-0.57, 0.81)	5.42	56
Hernandez, Mendieta, et al. 2008	2.70 (1.71, 3.68)	5.08	53
Hosseini, Azad, et al. 2007	0.39 (-0.49, 1.27)	5.21	36
Huang and Lee. 2007	0.65 (0.06, 1.23)	5.52	82
Kaminska, Marmurowska-Michallowska, et al. 2002	-1.03 (-1.74, -0.32)	5.39	59
Kapczinski, Dal-Pizzol, et al. 2011	0.67 (-0.17, 1.51)	5.26	40
Ko, Kim, et al. 2010	-3.56 (-4.48, -2.64)	5.16	81
Kubera, Kenis, et al. 2000	1.30 (-0.04, 2.63)	4.60	18
Lehto, Niskanen, et al. 2010	0.03 (-0.38, 0.44)	5.65	416
Li, Xiao, et al. 2010	-3.62 (-4.76, -2.48)	4.88	54
O'Brien, Scully, et al. 2006	0.41 (-0.63, 1.44)	5.02	52
O'Brien, Scully, et al. 2007	0.01 (-0.71, 0.73)	5.39	30
Parissis, Adamopoulos, et al. 2004	-1.89 (-2.95, -0.83)	4.99	35
Parissis, Farmakis, et al. 2009	-0.61 (-0.93, -0.30)	5.71	300
Simic Ogrizovic, Jovanovic, et al. 2009	0.10 (-0.36, 0.56)	5.62	128
Simon, McNamara, et al. 2008	0.31 (-0.22, 0.83)	5.57	98
Song, Halbreich, et al. 2009	-1.03 (-1.62, -0.43)	5.51	98
Overall (I-squared = 94.1%, p = 0.000)	-0.31 (-0.95, 0.32)	100.00	

NOTE: Weights are from random effects analysis

d (99% CI)

Figure 2.3. Forest plot of standardized mean difference IL-10 between depressed and non-depressed groups for each study (Cohen's d, 99% CI). A positive effect size indicates that IL-10 is elevated in the depressed compared with the non-depressed group.

96

shown). The only significant subgroup at $\alpha = .01$ level was the overweight/obese subgroup where this effect was negative ($d = -2.15$ [99% CI -4.17, -0.14]), and significantly different to the positive effect size for the normal weight subgroup (normal weight subgroup $d = 1.07$ [99% CI -0.80, 2.94]; effect size difference = 3.22 [99% CI 0.47, 5.97]). No meta-regression was significant.

2.5 Discussion

In this updated literature search we found a moderate and significant effect size with IL-6 elevated in depressed compared to non-depressed groups, consistent with the magnitude and direction of IL-6 effect size in previous meta-analyses (Dowlati, et al., 2010; Howren, et al., 2009; Liu, et al., 2012). However, there was high heterogeneity, such that interpretation of the overall effect size is done with caution, and this heterogeneity was only partly explained by subgroup analyses. Effect size was larger in the subgroup of studies with diagnosed MDD and no known comorbid conditions (similar to criteria of Dowlati, et al., 2010; Liu, et al., 2012) than for the broader inclusion criteria. We expanded on subgroup analyses reported previously (Howren, et al., 2009), demonstrating that aspects related to the operational definition of depression and comorbid conditions affected the magnitude of the effect size. The findings of the subgroup analyses not only help explain past discrepancies between individual study results but also

have implications for the design and interpretation of future studies into depression and inflammation.

First, differences in IL-6 between depressed and non-depressed groups appear to be magnified when studies more closely approximate the complexity of the DSM-IV criteria for depression through a diagnostic interview (i.e., verification that behavioral and cognitive criteria are met, differential diagnoses examined) or more severe experiences of depression obtained through inpatient or outpatient rather than community recruitment settings. This is not unlike studies which show that patients with melancholic features (emphasis on somatic symptoms, often regarded as a more severe form of depression) have different immune profiles to those with non-melancholic features (Maes, Mihaylova, Kubera, & Ringel, 2012; Rothermundt et al., 2001). However, these relationships are not always apparent (Marques-Deak et al., 2007). Furthermore, modest relationships have been observed between acute psychological stress or negative mood induction (possibly mediated via neuroendocrine stress and sympathetic nervous system activity) and increased pro-inflammatory markers, and between acute immune challenge and temporary depressive mood (Reichenberg et al., 2001; Steptoe, Hamer, & Chida, 2007). Subgroup analysis also showed that effect size is smaller when there is evidence that the "non-depressed" control groups contains participants who may have recent/current experience of a depressive disorder, but are currently not reporting depressive symptoms due to antidepressant treatment.

Furthermore, effect size is smaller when the depressed group contains antidepressant users, who may be subject to the anti-inflammatory properties of antidepressants or have fewer emotional and behavioral aspects of depression which would otherwise be contributing to their pro-inflammatory state (Hiles, et al., 2012; Tynan et al., 2012). These findings are consistent with the expectation that "loose" definitions of case and control would tend to bias towards the null, indicating the need to get "tight" case and control definitions in order to best identify any differences. Consistent reporting of depressive symptom inventory scores and perhaps separate reporting of neurovegetative and cognitive-emotional may lead to a clearer relationship between symptom severity and effect size particularly in a more homogenous sample of studies.

Second, depression is substantially comorbid with physical and psychiatric conditions (Evans et al., 2005; Rush et al., 2005; Van der Kooy et al., 2007), most of which also have pro-inflammatory associations. For instance, adiposity is thought to considerably contribute to levels of circulating IL-6, vascular inflammation is thought to cause atherosclerosis, and anxiety is associated with increased levels of pro-inflammatory markers most likely via neuroendocrine and nervous system stress activation (O'Donovan et al., 2010; Pitsavos et al., 2006; Rocha & Libby, 2009; Shelton & Miller, 2010). Compared to studies that did not select for a particular comorbidity, presence of cardiovascular disease, renal disease, cancer and other psychiatric conditions reduced the effect size between depressed and

non-depressed groups, significantly so for cardiovascular disease. These results suggest that inflammatory cytokines are influenced by many disease processes and these obscure the association of cytokines with depression. Studies that match for age and gender had a significantly and substantially larger effect size than those that did not. Age matching is important, as highlighted through the meta-regression where IL-6 effect size increases as the difference in age between the depressed group and control group increases. This is again consistent with the expectation that removing confounding influences on cytokine levels from other factors, such as age, allows the association with depression to be more clearly detected. However, there is also some preliminary evidence that as BMI becomes higher in the depressed group compared to the non-depressed group, IL-6 effect size increases; this raises the possibility that there may be some synergy or interaction between depression and other disease processes in raising cytokine levels.

One final issue examined in our subgroup analyses was sensitivity of IL-6 measurement. As expected, we observed a smaller effect size when effect size was estimated from the median, rather than the mean, but importantly, each effect size was positive and significant. No significant differences in effect size were observed in any other sensitivity analyses or meta-regression (regarding mood assessed the same day as blood collection, samples frozen and thawed once, time of blood collection, assay supplier, intra- and inter-assay variation). These may reflect genuine indifferences of

effect size to these measurement issues, or perhaps that they require routine description in this literature to more accurately identify their role and ensure that effect sizes are more likely to represent genuine between-group differences.

Despite clear evidence that the pro-inflammatory marker IL-6 is elevated across most studies, to date, IL-10 has given conflicting signals in people with and without depression. There was no significant effect size for IL-10 which could not be accounted for in subgroup analyses or meta-regression. At this stage, the reason for disparity across human studies is unclear. Generally, IL-10 and IL-6 have some association, with IL-6 alone or with other cytokines increasing production of IL-10 (for instance, via STAT3 in Th17 cells which are pathogenic in several autoimmune disorders) and IL-10 regulating and inhibiting pro-inflammatory responses in various cells (Dantzer, et al., 2008; Ouyang, Rutz, Crellin, Valdez, & Hymowitz, 2011; Saraiva & O'Garra, 2010). Animal research indicates there may be a relationship between IL-10 and depressive behavior such as helplessness and changes in sleep patterns (Mesquita, et al., 2008; Toth & Opp, 2001) and research in human populations has indicated that in MDD there may be a deregulation of the association between IL-6 and IL-10 as shown via lack of correlation between these cytokines in depressed but not non-depressed samples (Dhabhar et al., 2009). Absolute levels of cytokines may therefore be less important than relative levels (Dantzer, et al., 2008). Perhaps a greater number of homogenous studies are required for a clear pattern to

emerge or relative levels, or ratios of cytokines, might be more relevant in future meta-analyses.

Heterogeneity was not substantially reduced or explained in any of our subgroup analyses or meta-regression, including those related to measurement considerations. While this high heterogeneity caused by the non-restrictive inclusion criteria means the size of the effect size estimates may be unreliable, the criteria has helped satisfy our aim of developing a set of exploratory subgroup analyses and meta-regression to explore the circumstances under which effects are strongest and weakest. It is possible that combinations of factors or other untested factors may explain the high heterogeneity.

There are other behavioral aspects of depression that we could not capture in this meta-analysis, but are important to consider for the primary researcher. One feature of DSM-IV-TR criteria for Major Depressive Episode (American Psychiatric Association, 2000) missed in this meta-analysis is sleep. Pro-inflammatory cytokines generally help regulate the sleep-wake cycle, although in high levels they can promote somnolence as would occur during acute inflammation and deprivation of sleep can also increase some pro-inflammatory markers (Kapsimalis et al., 2008). A relationship between difficulty initiating sleep and elevated IL-6 in people with MDD has been observed (Motivala, Sarfatti, Olmos, & Irwin, 2005). Pro-inflammatory cytokines have been independently associated with other behaviors related to DSM-IV-TR criteria such as fatigue (Raison, Lin, & Reeves, 2009),

suicidal behavior (Janelidze, Mattei, Westrin, Träskman-Bendz, & Brundin, 2011; Kim et al., 2008), transient guilt (Dickerson, Kemeny, Aziz, Kim, & Fahey, 2004), and cognitive problems and psychomotor slowing (Brydon, Harrison, Walker, Steptoe, & Critchley, 2008; Reichenberg, et al., 2001). Understanding the role of each is important for developing a comprehensive inflammation-based theory of depression.

The pooled effect seen in this meta-analysis of cross-sectional studies does not shed light on the directionality of causation between depression and inflammation. It is consistent with the idea that cytokines influence a variety of behavioral aspects including appetite, sleep and fatigue let alone mood symptoms and concentration difficulties; therefore a larger pro-inflammatory cytokine response would provide a broader depressive symptom profile. It is also consistent with the idea that the behavioral aspects of depression occur prior to (or simultaneously with) and cause the observed elevated pro-inflammatory markers. This is impossible to verify in the cross sectional context, but it highlights that the pattern of depressive symptoms is important. Prospective research has focused on depressive symptoms as the operational definition of depression, demonstrating that elevations in pro-inflammatory markers both precede and follow depressive symptoms (Hamer, Molloy, de Oliveira, & Demakakos, 2009; Matthews et al., 2010). The few that have been completed using diagnosis have shown that levels of inflammatory markers can predict *de novo* depressive disorder (Pasco et al., 2010). Additionally, often in the context of general medical

illness, randomized controlled trials have indicated that psychological or antidepressant treatment for depression may reduce pro-inflammatory markers and improve immune functioning, administration of antidepressants can reduce depression induced by treatment with pro-inflammatory cytokines such as interferon, and administration of anti-inflammatory medications such as etanercept and celecoxib can reduce depressive symptoms (Andersen et al., 2004; Kraus et al., 2008; Muller et al., 2006; Pizzi et al., 2009; Tyring et al., 2006). This meta-analysis informs the design of future necessary prospective and human experimental literature in how to operationally define depression, recruit patients and control for comorbidity, in the context of methodological and measurement rigor.

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3. Interleukin-6, C-reactive protein and interleukin-10 after antidepressant treatment in people with depression: A meta-analysis

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See Appendix 8.3 for the PRISMA checklist for the reporting of meta-analyses regarding this Chapter

3.1 Abstract

Objective: Cross sectional studies support an association between depression and inflammatory markers. However, little is known of their relationship in the context of antidepressant treatment. Our aim was to explore via meta-analysis whether antidepressant treatment is associated with a reduction in three inflammatory markers associated with depression.

Methods: A computerised search of Embase, Medline, PsycINFO and Cochrane Library databases was completed using subject headings for depression and either interleukin-6, C-reactive protein or interleukin-10, selecting studies which reported circulating levels of inflammatory markers before and after antidepressant treatment for people with depression. Outcome and moderator variables were coded for analysis, including inflammatory marker change, depression severity change, age, gender ratio, assay brand, treatment response and weight change.

Results: Pooled effect sizes showed a significant decrease in interleukin-6 (N = 14, d = -0.42, p = .02), marginally significant decrease in C-reactive protein (N = 8, d = -0.57, p = .05) and a non-significant decrease in interleukin-10 (N = 3, d = -0.45, p = .14) after treatment. High levels of heterogeneity were observed, which may be associated with clinical variations between the studies such as weight gain, anxiety, incomplete remission and other individual differences and comorbidities.

Conclusions: The findings of this meta-analysis indicate that there may be a normalization of overactive inflammatory processes following antidepressant treatment.

3.2 Introduction

The comorbidity of depression and inflammation-related physical illnesses, particularly cardiovascular disease, has raised the possibility of a shared, underlying inflammatory pathway involving central and systemic responses (Irwin & Miller, 2007). Proposed bi-directional mechanisms for how inflammatory processes may induce depressive mood, and vice versa, incorporate evidence that prolonged exposure to inflammatory mediators can impair the regulation of neuroendocrine stress, influence the availability of monoamine neurotransmitters, and decrease neurogenesis and neurotropic support (Maes et al., 2009; Miller, Maletic, & Raison, 2009).

Much of the evidence supporting inflammatory theories of depression is cross-sectional with many studies demonstrating that, compared to people without depressive symptoms, people with high depressive symptoms show elevated levels of inflammatory markers (including cytokines, chemokines and acute phase proteins) in peripheral serum and cerebrospinal fluid (Dowlati et al., 2010; Lindqvist et al., 2009; Zorrilla et al., 2001). These differences have been observed in both medically healthy people with depressive symptoms and those with co-occurring medical comorbidities such as cardiovascular disease, renal disease and cancer (Bossola et al., 2010; Howren, Lamkin, & Suls, 2009). Fewer studies examine the association between depression and inflammatory markers over time, although this can help develop the case for whether elevated levels of

137

inflammatory markers may be a cause or simply a consequence of depression.

This meta-analysis is one of the first to estimate change in inflammatory markers before and after antidepressant treatment. Through narrative review and meta-analytic moderator analysis, we also explore sources of heterogeneity related to individual differences and comorbidity. The cytokine interleukin (IL)-6 and an acute phase protein, C-reactive protein (CRP), were selected as prototypical pro-inflammatory markers, robustly associated with systemic inflammatory response, and IL-10 was selected as an important anti-inflammatory marker. These are repeatedly shown to be elevated in people with depression, compared to people without depression (Dowlati, et al., 2010; Howren, et al., 2009) and assuming there is an association, we would expect these to decrease as depressive symptoms decrease in association with antidepressant treatment.

3.3 Method

Systematic search

Studies were included in the meta-analyses if they met the following criteria: (i) participants were adults either diagnosed with major depression/dysthymia or endorsing high depressive symptoms on a standardized inventory; (ii) patients were not undergoing cytokine treatment (e.g., interferon or IL-2); (iii) the study explicitly reported

antidepressant treatment in the methods; (iv) mean or median resting levels of IL-6, CRP or IL-10 in circulating plasma or serum was reported before and at least once after starting antidepressant treatment; (v) either a pre-test-post-test design or a randomized controlled trial design was used; (vi) publication was in English in a peer reviewed journal; and (vii) enough information to calculate an effect size was reported. Studies of people with depression and a comorbid general medical condition were included providing the methods stated that the disease and medications were stable for the duration of the study.

A computerized search of Embase, Medline, PsycINFO and Cochrane Library databases was completed in March 2011 using two different search strategies: (i) the key terms mapped to subject headings for depression (depression, depressive disorder, major depressive disorder, dysthymic disorder) and either "interleukin-6", "C-reactive protein" or "interleukin-10"; and (ii) depression subject headings and "cytokine/interleukin" or "acute phase protein" and "antidepressants". Both searches were limited to human and English language literature. The abstract of each article identified in the search was screened for relevance. If the abstracts mentioned antidepressant use and measurement of inflammatory markers, or the abstracts lacked detail, full text articles were extracted and compared against selection criteria for eligibility. The reference lists of included studies and relevant review articles were screened for additional articles.

Eligible studies were coded and blindly checked by one author (SH) for the sample size and the outcome variable of mean (with standard deviation/standard error) circulating IL-6, CRP or IL-10 before and after treatment. Means and standard errors of inflammatory markers reported only graphically were converted to numerical values using Data Thief III, version 1.5 (Tummers, van der Laan, & Huyser, 2008). Moderator and mediator variables were also coded, including: diagnosis, mean group depressive symptom rating at baseline and follow-up, mean age, proportion of males, treatment duration, inpatient or outpatient status, proportion of treatment responders and type of antidepressant. Where not reported, these variables were coded as missing values.

Statistical methods

Statistical analyses were completed using Comprehensive Meta-Analysis II (Biostat, Inc., USA). For the antidepressant treatment arm in each study, individual study effect sizes for change in inflammatory markers and depressive symptoms were calculated as repeated-measures Cohen's *d* standardized mean difference using the pre- and post-test means, standard deviation of the difference calculated from the pre- and post-test standard deviations and an estimation of the pre- and post-test correlation (Borenstein, Hedges, Higgins, & Rothstein, 2009). If the mean was not reported, the median was used as an estimate because sample sizes were sufficiently large (Pudar Hozo, Djulbegovic, & Hozo, 2005). We used a

conservative correlation of 0.5 as an estimate for the correlation between pre- and post-treatment inflammatory marker but also re-tested the meta-analysis model using other correlations ($r = 0.4, 0.6, 0.7$), with none producing a large difference in result. For one study which failed to report standard deviations/errors but otherwise met selection criteria (Dawood et al., 2007), the effect size was calculated using the difference in means, sample size and paired p value. For the few studies that included more than one follow-up measurement of IL-6, CRP or IL-10 (Hernandez et al., 2008; Kubera et al., 2000; Mackay et al., 2009), we calculated a study effect size for the follow-up point closest to the mean duration of the remaining studies for that inflammatory marker, so as to reduce the variability that too large a range of treatment duration might cause. For studies reporting data for subgroups only, whole sample data points were imputed by collapsing subgroup means (Leo et al., 2006; Sluzewska et al., 1995; Yoshimura et al., 2009). A positive effect size indicates that there was an increase in the inflammatory marker over time.

Individual study effect sizes were synthesized to generate an overall effect size using a random effects model, weighted by the inverse of variance. We also completed a sensitivity analysis to identify potential outliers by removing each study one by one to examine the influence of each individual study on the overall effect size. The Egger test of funnel plot asymmetry (Egger, Davey Smith, Schneider, & Minder, 1997) and fail-safe

N (Rosenthal, 1979) were calculated to assess publication bias.

Heterogeneity in the meta-analysis was assessed using Cochrane's Q and I^2

which calculates a proportion of variation attributed to heterogeneity

(Higgins, Thompson, Deeks, & Altman, 2003). Moderator analysis was

undertaken to explore sources of heterogeneity. Subgroup analysis was

completed by comparing pooled inflammatory marker effect size in

subgroups of categorical moderator/mediator variables, while method of

moments meta-regression was completed to explore the relationship

between effect size and continuous variables.

3.4 Results

There were 22 studies relevant to these meta-analyses (N = 14 for IL-

6, N = 8 for CRP and N = 3 for IL-10; article extraction process

summarized in Figure 3.1, methods and relevant findings summarized in

Table 3.1). Most study designs were of a single group of people with

depression, measured before and after (or during) antidepressant treatment

(n = 15). Seven studies were randomized control trials (RCTs) comparing an

antidepressant treatment condition and either a non-antidepressant

treatment or antidepressant in conjunction with non-antidepressant

pharmacological treatment. Many studies included a control condition of

people without depression who were measured for inflammatory markers at

a single time point (n = 17) to infer whether levels of inflammatory markers

in the depression group change to a level comparable to a non-depressed control group. Every study reporting depressive symptoms showed a significant group reduction in depressive symptoms after antidepressant treatment.

Titles identified in search of key terms
$N = 2083$

IL-6 $N = 789$
CRP $N = 684$
IL-10 $N = 274$
"antidepressant" strategy $N = 336$

Potentially relevant: Full text extracted to examine methods
$N = 291$

Excluded:
Not antidepressant or unclear treatment $N = 119$
Not depressed/inappropriate sample $N = 52$
Not relevant $N = 43$
No serum inflammatory marker $N = 34$
Insufficient data to calculate effect size $N = 10$
Review $N = 7$
Not in English $N = 3$
Animal study $N = 1$

Titles included in analysis
$N = 22$

IL-6 $N = 14$
CRP $N = 8$
IL-10 $N = 3$

Figure 3.1. Summary of article extraction process after two systematic search strategies either based on search for "depression" and either the specific mediator interleukin (IL)-6, C-reactive protein (CRP) or IL-10, or "antidepressants", "cytokine/interleukin" and "acute phase protein". Reasons for exclusion are listed.

143

Table 3.1. Key characteristics of the studies included in the meta-analysis regarding changes in interleukin (IL)-6, C-reactive protein (CRP) and IL-10 following antidepressant treatment.

Study	Population				Intervention		Design/comparator		Outcome		Change after treatment				
	Clinical description	Age M (SD)	% male	N^+	Drug (dose)	Length (weeks)	Design	Healthy control group	Depression measure	Blood measure	Dep	Resp (%)	IL-6	CRP	IL-10
Maes et al 1995	Inpatients, DSM-III-R major depressive episode	39 (9.4)	53	17	Clinician decided: fluoxetine (20mg) or tricyclic antidepressants	≈ 12	Pre-test-post-test	✓ (N = 38) IL-6 higher in depression than control group	Schedule for Affective Disorders and Schizophrenia, Ham-D	Plasma, sandwich ELISA (Eurogenetics) Overnight fast, 15 min rest, collected at 0845	↓	.	→	.	.
Sluzewska et al 1995	Inpatients, DSM-III-R MDD – group with elevated IL-6 at baseline	42.5 (6.4)	13	6	Fluoxetine (20mg)	8	Pre-test-post-test Compared changes in IL-6 for groups with elevated and non-elevated IL-6 at baseline	✓ (N = 11) IL-6 higher in depression than control group	Ham-D	Serum, ELISA	.	.	↓	.	.

	Inpatients, DSM-III-R MDD – group without elevated IL-6 at baseline	40.6 (2.5)	13	16	Fluoxetine (20mg)	8	Pre-test-post-test Compared changes in IL-6 for groups with elevated and non-elevated IL-6 at baseline	✓ (N = 11) IL-6 no different between depression and control group	Ham-D	Serum, ELISA	.		→	.	.
Maes et al 1997	Inpatients, DSM-III-R MDD	47.5 (15)	54	25	Clinician decided: trazodone (100mg), trazodone (100mg) + pindolol (7.5mg) or trazodone (100mg) + fluoxetine (20mg)	5	Pre-test-post-test	✓ (N = 15) IL-6 higher in depression than control group	Semi-structured interview for DSM-III-R, Ham-D	Serum, sandwich ELISA (Eurogenetics) Collected between 0730 and 0800	↓	60	→	.	.
Sluzewska et al 1997	Inpatients with refractory MDD ($N=$ 19) and bipolar ($N=$ 13)	44 (11)	19	32	Existing various antidepressants + lithium carbonate (500 – 1500 mg)	4	Pre-test-post-test	✓ (N = 20) CRP lower in depression than control group	Ham-D	Plasma, rocket immunoelectrophoresis Overnight fast, collected at 0730	.	75	.	↓	.

Study	Sample	Age	%	N	Treatment	Duration	Design	Findings	Measure	Assay					
Kubera et al 2000	Inpatients, DSM-IV recurrent MDD	47.3 (3)	44	9	Clinician decided: antidepressant	6 (blood also drawn at 2)	Pre-test-post-test	✓ (N = 11) IL-6 and IL-10 no different between depression and control group	Ham-D	Serum, sandwich ELISA (Eurogenetics) 10hr fast, 12hr abstain caffeine, nicotine, alcohol, collected between 0730-0830	↓	.	→	.	→
Lanquillion et al 2000	Inpatients, MDD	53.5 (15.2)	38	24	Amitriptyline (150 - 250mg)	6	Pre-test-post-test	✓ (N = 15) CRP higher in depression than control group	SCID, Ham-D	Routine methods Collected at 0800	↓	.	.	↓	.
Kagaya et al 2001	DSM-III-R MDD (N = 9) or dysthymia (N = 3)	31.1 (8.2) [++]	75	8	Clinician decided: antidepressant, mainly clomipramine	4	Pre-test-post-test	✓ (N = 12) IL-6 no different between depression and control group at baseline	Ham-D	Plasma, ELISA (BioSource International, Camarillo, CA) Collected between 1100-1400 in EDTA	↓	.	→	.	.

146

Study	Population	Age mean (SD)	N	N	Treatment	Weeks	Design	Findings	Measures	Blood/Assay					
Mikova et al 2001	Inpatients, DSM-IV major depressive episode, Ham-D ≥ 18	47.3 (11.3)	18	14	Clinician decided: paroxetine or tricylic antidepressants	6	Pre-test-post-test	✓ (N = 15) IL-6 no different between depression and control group at baseline	Ham-D	Serum, ELISA, (Eurogenetics, Tessenderlo, Belgium) Collected between 0800-0900, no additive	↓	64	→	.		.
Tuglu et al 2003	Inpatients major depressive episode via SCID DSM-IIIR	39.4 (14.6)	58	30	Clinician decided: SSRI	6	Pre-test-post-test	✓ (N = 17) CRP no different between depression and control group at baseline/ follow-up	SCID DSM-IIIR, Ham-D, BDI	Serum, routine methods. Overnight fast, collected between 0700-0800, no anticoagulant	↓	92	.	↓		.

Yao et al 2004	Inpatients, depression according to ICD-10 and CCMD-3 criteria, Ham-D > 17	37 (8)	33	40	Clinician decided: SSRI or Venlafax-ine	4	Pre-test-post-test	✓ (N = 20) IL-6 higher in depression than control group at baseline, no different between depression and control group at follow-up	Ham-D	Serum, ELISA (Tianjing Jierui Biological Product Company) Collected on second morning after admission to hospital	↓	.	↓	.		.
Basterzi et al 2005	DSM-IV MDD or MDD-recurrent	33.8 (12.8)	13	23	Clinician decided: SSRI	6	Pre-test-post-test	✓ (N = 23) IL-6 no different between depression and control group at baseline	Structured clinical interview, Ham-D	Serum, Cytelisa sandwich ELISA (Cytimmune Sciences, Maryland) Collected 0830-1000, no anticoagulant	↓	74	↓	.		.
Leo et al 2006	MDD (first episode) – sertraline group	34.9 (5.9)	40	20	Setraline (100mg)	6	RCT (sertraline vs. citalopram)	✓ IL-6 higher in depression than control group	Ham-D	Quantikine High Sensitivity Immunoassay (R & D Systems, MN)	↓	.	↓	.		.

Study	Sample	Age	% male	N	Medication	Weeks	Design	Cross-sectional finding	Depression measure	Assay / collection					
	MDD (first episode) – citalopram group	34.9 (5.9)	50	20	Citalopram (20mg)	6	RCT (sertraline vs. citalopram)	✓ IL-6 higher in depression than control group	Ham-D	Quantikine High Sensitivity Immunoassay (R & D Systems, MN)	↓	.	↓	.	.
O'Brien et al 2006	DSM-IV MDD with melancholic features, Ham-D > 17	37.9	0	20	Clinician decided: fluoxetine (20mg), paroxetine (20mg) or sertraline (50mg)	3	Pre-test-post-test	✗	Ham-D	Serum, immunoturbimetric assay (Olympus). Collected between 0900-1100	.	90	.	↓	.
Dawood et al 2007	Outpatients recruited from the community, DSM-IV MDD, 84% recurrent	45 (11)	42	24	Clinician decided: SSRI (citalopram 40mg, sertraline 200mg, fluvoxamine 200mg, fluoxetine 40mg)	12	Pre-test-post-test	✓ (N = 15) CRP higher in depression than control group	MINI, CIDI, Ham-D	hsCRP particle-enhanced immunoturbidimetric assay (Roche Diagnostics, Mannheim, Germany). Collected in the morning after 10 mins of supine rest, 12 hours free from caffeine and tobacco	↓	88	.	↑	.

Study	Diagnosis	Age (SD)			Treatment	Weeks	Design	Control	Measures	Sample/Assay					
Hernand-ez et al 2008	DSM-IV MDD, 52% recurrent, BMI ≤ 25	32 (9.4)	29	10	Clinician decided: SSRI	52 (blood also drawn at 5, 20 and 36)	Pre-test-post-test	✓ (N = 22) IL-10 higher in depression than control group at baseline, lower at 52 week follow-up	MINI, Ham-D, BDI	Serum, DuoSet ELISA (R & D Systems) Collected between 0800-0900	↓	100	.	.	↓
Mackay et al 2009	DSM-IV depression – fluoxetine group only	39.0 (2.4)	18	19	Fluoxetine (20mg)	18 (blood also drawn at 6, 12)	RCT (fluoxetine vs. fluoxetine + T_3 vs. counselling)	×	Ham-D, CDS, BDI, MADRS, SF-36	Unclear	↓	.	.	→	.
Pizzi et al 2009	Coronary heart disease, mild to severe depression (BDI ≥ 10)	57.4 (8.7)	47	100	Sertraline (50mg, dose increased weeks 6 - 13)	20	RCT (sertraline vs. placebo	×	BDI	CRP: Latex/BN II (Dade Behring, Marburg, Germany) IL-6: Quantikine ELISA (R & D Systems, MN). Overnight fast, after 30min rest, collected in EDTA.	↓	55	↓	↓	.

Study	Diagnosis				Drug	Weeks	Design	Findings	Measures	Sample/Assay					
Song et al 2009	DSM-III MDD	34 (13)	42	24	Fluoxetine (20mg)	6	RCT (fluoxetine + sham EA vs. placebo + EA vs. placebo + sham EA)	✓ (N = 30) IL-10 lower in depression than control group at baseline and follow-up.	SCID, Ham-D, Clinical Global Impression scale	Serum, ELISA (Gene May, San Diego, USA) Collected between 0700 – 0900	↓	.		.	.	→
Yoshimura et al 2009	DSM-IV MDD (first episode) – treatment responder group	40 (9)	40	31	Clinician decided: SSRI or SNRI	8	Pre-test-post-test	✓ (N = 30) IL-6 higher in depression than control group	Ham-D	Plasma, quantitative sandwich enzyme assay technique (R & D Systems, Minneapolis, MN), measured from standard curve Collected between 0800-1000 before breakfast into heparinised tubes	.	100	↓	.	.

Study	Population	Age mean (SD)	N	N	Treatment	Weeks	Design	Control	Measure	Assay/Sample					
	DSM-IV MDD (first episode) – treatment non-responder group	40 (9)	42	20	Clinician decided: SSRI or SNRI	8	Pre-test-post-test	✓ (N = 30) IL-6 higher in depression than control group	Ham-D	Plasma, quantitative sandwich ELISA (R & D Systems, MN) Collected between 0800-1000 before breakfast, heparinised tubes	.	0	→	.	.
Chen et al 2010	Inpatients, DSM-IV MDD (first episode), BMI ≤ 25, 20-25 years	23.3 (2.6)	100	43	Maprotiline, fluoxetine, venlafaxine, mirtazapine (titrated).	4	RCT (maprotiline vs. fluoxetine vs. venlafaxine vs. mirtazapine) Note: results were reported as a whole group, not these subgroups.	×	Ham-D	CRP: Latex hs-assay (Roche Diagnostics GmbH, Mannheim, Germany) IL-6: hs-ELISA (Diaclone Research, Besancon Cedex, France) 10 hr fast, serum/plasma *	↓	.	↑	→	.

Study	Diagnosis	Age mean (SD)	n+	n++	Antidepressant	Weeks	Design	Control group	Assessment	Assay					
Jazayeri et al 2010	DSM-IV MDD – fluoxetine group	37 (8.5)	29	14	Fluoxetine (20mg)	8	RCT (fluoxetine vs. EPA vs.fluoxetine + EPA)	×	Ham-D	Serum, ELISA (Bender MedSystems, Austria) Fasting, collected at 0800	.	.	→	.	.
Fornaro et al 2011	DSM-IV MDD via SCID	51.1 (11)	25	16	Duloxetine (60mg)	6	Pre-test-post-test	✓ (N = 16) Non-depressed control group also received antidepressant treatment. IL-6 no different between depression and control group at baseline or follow-up.	SCID, Ham-D	Serum, ELISA (Bender MedSystems, Burlingame, CA) Collected between 0730 – 1000	↓	56	→	.	.

+people in the study who received antidepressants after drop outs/non-measurement.

++whole sample, not subset who completed treatment.

<u>Key</u>:

✓ present

× absent

↓ significant decrease after antidepressant treatment

↑ significant increase after antidepressant treatment

→ no significant change after antidepressant treatment

. measurements not reported

<u>Abbreviations</u>:

BDI: Beck Depression Inventory

BMI: body mass index

CCMD: Chinese Classification of Mental Disorders

CDS: Carroll Depression Scale

CIDI: Composite International Diagnostic Interview

CRP: C-reactive protein

Dep: depression

DSM: Diagnostic and Statistical Manual

EA: electroacupuncture

ELISA: enzyme-linked immunosorbent assay

EPA: eicosapentaenoic acid

Ham-D: Hamilton Rating Scale for Depression

hs: high sensitivity

ICD: International Classification of Diseases

IL: interleukin

MADRS: Montgomery–Åsberg Depression Rating Scale

MINI: Mini-International Neuropsychiatric Interview

MDD: major depressive disorder

Resp: responders (>50% reduction in depressive symptoms)

RCT: randomised controlled trial

SCID: Structured Clinical Interview for the Diagnostic and

Statistical Manual

SF-36: Short Form 36 Health Survey

SNRI: serotonin-norephinephrine reuptake inhibitor

SSRI: selective serotonin reuptake inhibitor

T3: tri-iodothyronine

Most of the included studies had similar exclusion criteria: excluding people with DSM-IV axis I disorders besides major depressive episodes or dysthymic disorder ($n = 12$; except Sluzewska et al. (1997) who additionally included people with a lifetime diagnosis bipolar disorder in a current depressive episode), people on psychotropic medication in the two weeks before blood collection ($n = 22$), people with general medical illness ($n = 15$), people with recent allergic reactions ($n = 7$) and pregnant women ($n = 7$). The study where patients had stable coronary heart disease were not acutely ill and had been stable on medication (Pizzi et al., 2009). Most studies collected blood in the morning.

Interleukin-6 and C-reactive protein

Although in two studies, administration of the serotonin reuptake inhibitor (SSRI) fluoxetine resulted in no significant change in IL-6 (Jazayeri et al., 2010) or CRP over multiple time-points (Mackay, et al., 2009), most of the remaining studies that administered SSRIs reported significant reductions in IL-6 or CRP, including other studies of fluoxetine (significant reduction only observed in people with elevated IL-6 at baseline) (Sluzewska, et al., 1995), sertraline (Leo, et al., 2006; Pizzi, et al., 2009) and citalopram (Leo, et al., 2006).

Few studies examined non-SSRI antidepressants. No significant changes in IL-6 were observed in studies administering a serotonin-norepinephrine reuptake inhibitor (duloxetine) (Fornaro, Martino,

Battaglia, Colicchio, & Perugi, 2011), nor a serotonin antagonist and reuptake inhibitor (trazodone) either alone or augmented with pindodol (Maes et al., 1997). Significant reductions in CRP were observed after administration of the tricyclic antidepressant, amitriptyline, in people with and without 50% reductions in depressive symptoms (treatment responders and non-responders, respectively) (Lanquillon, Krieg, Bening-Abu-Shach, & Vedder, 2000). People with higher levels of CRP after treatment also recorded significantly higher levels of trait anxiety than people with lower levels of CRP. Finally, Chen et al. (2010) reported a significant increase in IL-6, but no change in CRP, in young, normal weight males who were maintained on a strict diet with restricted alcohol and coffee intake while in an inpatient facility. While participants were randomized to four different SSRI and non-SSRI antidepressant conditions, the authors only reported summary statistics for the entire study sample, as the increase in IL-6 was comparable across conditions. The authors reported an increase in weight in two of the four conditions which while not statistically significant, may still be clinically meaningful in contributing to elevated IL-6.

In the remaining studies, a clinician decided case-by-case which antidepressant was administered. In the two studies where clinicians were unrestricted in their choice of antidepressant, neither demonstrated a significant change in IL-6 (Kagaya et al., 2001; Kubera, et al., 2000). No significant change in IL-6 was observed in two studies where participants

were administered either a tricyclic antidepressant or a particular SSRI [fluoxetine (Maes et al., 1995) or paroxetine (Mikova, Yakimova, Bosmans, Kenis, & Maes, 2001)]. However, significant decreases were observed in IL-6 and CRP after administration of various other antidepressants from SSRI classes (Basterzi et al., 2005; O'Brien, Scott, & Dinan, 2006; Tuglu et al., 2003). In contrast, Dawood et al. (2007) demonstrated a significant increase in CRP, with only 5 from 24 patients recording a decrease in CRP after treatment. Two studies that selected from SSRI and serotonin-norepinephrine reuptake inhibitors also reported significant reductions in IL-6, but not for treatment non-responders (Yao et al., 2004; Yoshimura, et al., 2009). Sluzewska et al. (1997) also reported a significant reduction in CRP for both treatment responders and non-responders in participants with a refractory major depressive episode receiving various antidepressants and lithium carbonate. Neither change in depressive symptoms nor change in CRP differed significantly between people with lifetime bipolar disorder or major depressive disorder admitted to the study during a depressive episode.

Interleukin-10

No significant changes in IL-10 were observed after administration of fluoxetine, although levels were initially lower than controls (Song, Halbreich, Han, Leonard, & Luo, 2009). The remaining studies reported that initially elevated levels of IL-10 reduced over treatment to a level below

the controls at baseline (Hernandez, et al., 2008; Kubera, et al., 2000).

Additionally, Hernandez et al. (2008) reported that while IL-10

measurements at 5, 20, 36 and 52 weeks showed decreasing trend,

participants reached clinical remission for depression by 20 weeks.

Antidepressants vs. non-antidepressant treatment conditions

There were only two RCTs with placebo treatment arms. Pizzi et al.

(2009) found no change in IL-6 or CRP after 20 weeks of placebo treatment.

Furthermore, treatment with sertraline led to significant reductions in IL-6

and CRP compared to placebo at follow-up (between-subjects IL-6 d = -0.74,

95% CI [-1.16, -0.32]; CRP d = -1.00, 95% CI [-1.43, -0.56]). Song et al. (2009)

also showed no significant decline in IL-10 after placebo treatment;

however, there was no significant difference in IL-10 between placebo and

active treatment groups at follow-up, as IL-10 did not significantly decline

in the active treatment groups (fluoxetine and sham electroacupuncture or

anti-depression sequence of electroacupuncture and placebo capsules). The

null result may possibly be due to non-specific treatment effects of sham

electroacupuncture. In both studies, there was no significant decline in

depressive symptoms in the placebo treatments, but significant declines in

the active treatments.

Two studies randomized participants to antidepressants and other

anti-depressive treatments. Neither study showed that IL-6 or CRP

significantly declined within the active treatment groups over time nor

significantly differed between the active treatment groups at follow-up (IL-6 after treatment with fluoxetine, eicosapentaenoic acid and fluoxetine and eicosapentaenoic acid combined for Jazayeri et al. (2010); and CRP after treatment with fluoxetine, fluoxetine with tri-iodothyronine and counselling groups for Mackay et al. (2009)). One final study treated a depressed and non-depressed control group with duloxetine, and found that IL-6 did not change in either group, although depressive symptoms declined in the depressed but not the control group (Fornaro, et al., 2011).

Meta-analysis

Firstly, we examined whether depression significantly declined over the course of antidepressant treatment. Several studies were excluded from this analysis as they did not report sufficient information (Jazayeri, et al., 2010; Mikova, et al., 2001; O'Brien, et al., 2006; Sluzewska, et al., 1995; Sluzewska, et al., 1997; Yoshimura, et al., 2009). Meta-analysis of the depression severity scores (chiefly the Hamilton Rating Scale for Depression; Hamilton, 1960) revealed a significant decrease in the studies which investigated IL-6 ($N = 10$, $d = -1.82$, 95% CI [-2.36, -1.28], $I^2 = 85.4$), CRP ($N = 7$, $d = -1.93$, 95% CI [-2.59, -1.27], $I^2 = 90.1\%$) and IL-10 ($N = 3$, $d = -4.77$, 95% CI [-8.13, -1.41], $I^2 = 92.0\%$).

Secondly, we analysed changes in inflammatory markers after antidepressant treatment. Meta-analysis revealed a significant decrease in IL-6 ($N = 14$, $d = -0.42$, 95% CI [-0.78, -0.06], Z = 2.30, $p = .02$, Figure 3.2).

There was a marginally significant decrease in CRP ($N = 8$, $d = -0.57$, 95% CI [-1.140, 0.005], Z = 1.94, $p = 0.052$, Figure 3.3). In both instances, there was high heterogeneity (IL-6: $I^2 = 88.2\%$, $Q(13) = 110.23$, $p < .001$; CRP: $I^2 = 93.2\%$, $Q(7) = 103.66$, $p < .001$). There was no evidence of publication bias via Egger's test (IL-6: $t(13) = 0.42$, $p = .68$; CRP: $t(7) = 1.53$, $p = .24$). The fail safe N was 148 for IL-6, which means 148 extra studies would be required to bring the p value to greater than .05. Fail safe N was 82 for CRP.

Based on the very few studies and a fail-safe N calculation of 5, the meta-analysis for IL-10 should be considered exploratory and considered a description of currently available data, rather than a true representation of the effect. There was a non-significant decrease in IL-10 following antidepressant treatment ($N = 3$, $d = -0.45$, 95% CI [-1.03, 0.14], Z = 1.49, $p = .14$, Figure 3.4). Significantly high heterogeneity was also observed, $I^2 = 77.3\%$, $Q(2) = 8.81$, $p = .01$. There was no significant publication bias according to Egger's test although this test lacks power when there are few studies, $t(2) = 0.76$, $p = .59$. Sensitivity analyses revealed no extreme influence of any single study in the IL-6, CRP or IL-10 analyses.
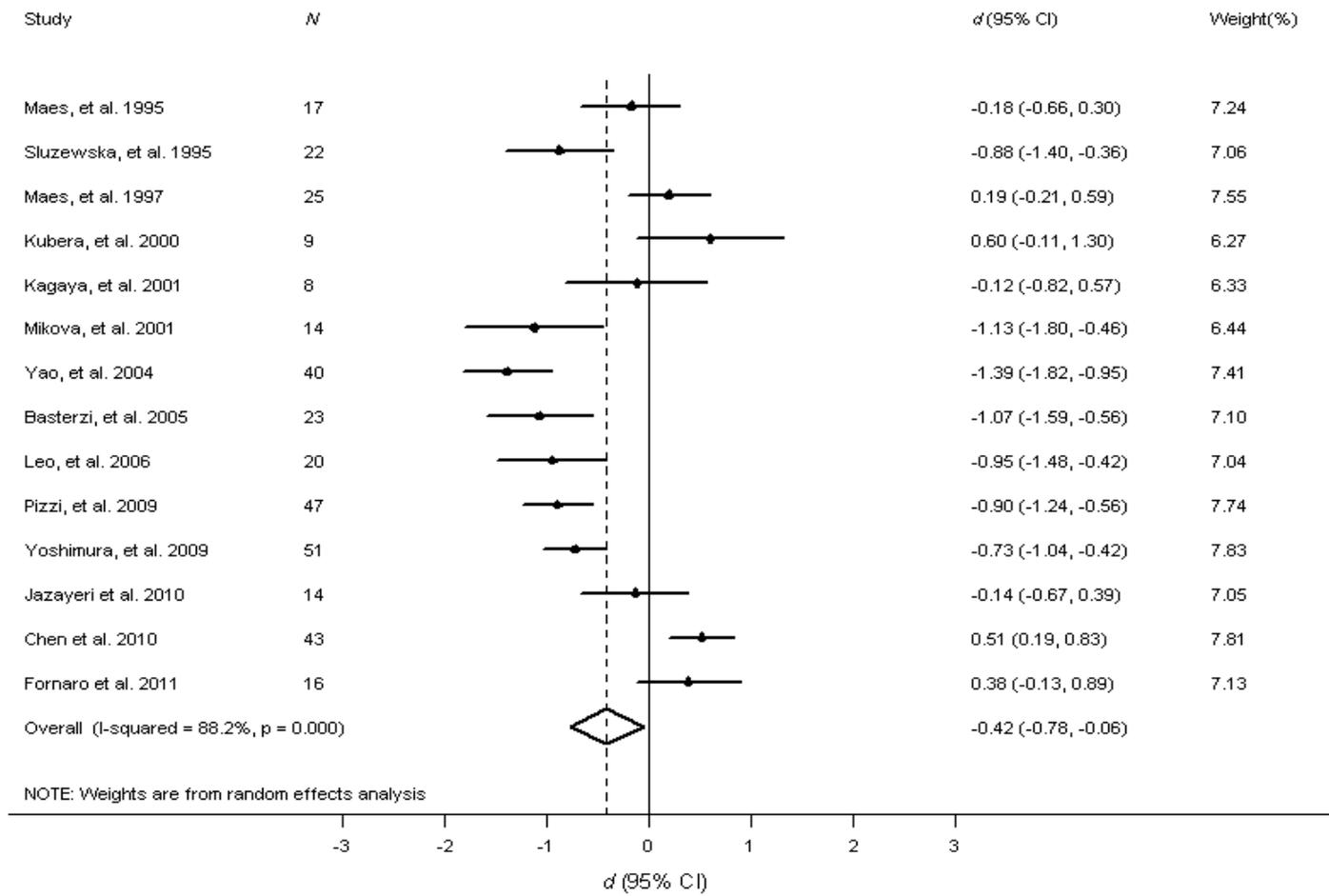
Figure 3.2. Forest plot for change in IL-6 after antidepressant treatment including study name identifier, total number of

participants, the standardized paired difference in IL-6 (*d*, 95% CI) and the relative weight that each study contributes to the

overall pooled estimate of effect. The diamond at the bottom of the effect size plot represents the overall pooled effect size for

standardized change in IL-6 (d, 95% CI) and the I^2 measure of heterogeneity. Negative effect sizes represent a decrease in IL-6

following antidepressant treatment.
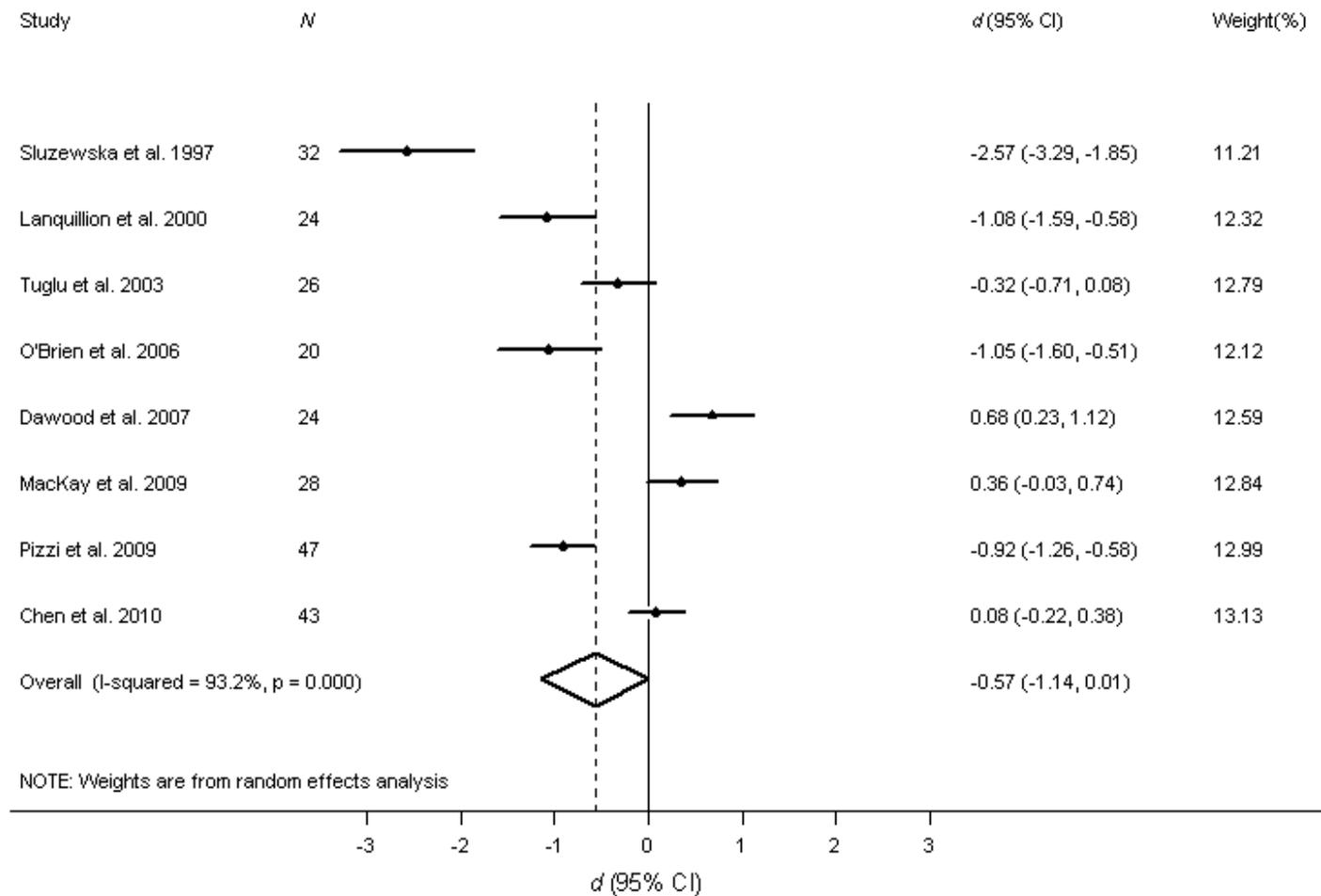
Figure 3.3. Forest plot for change in CRP after antidepressant treatment, details as per Figure 3.2.
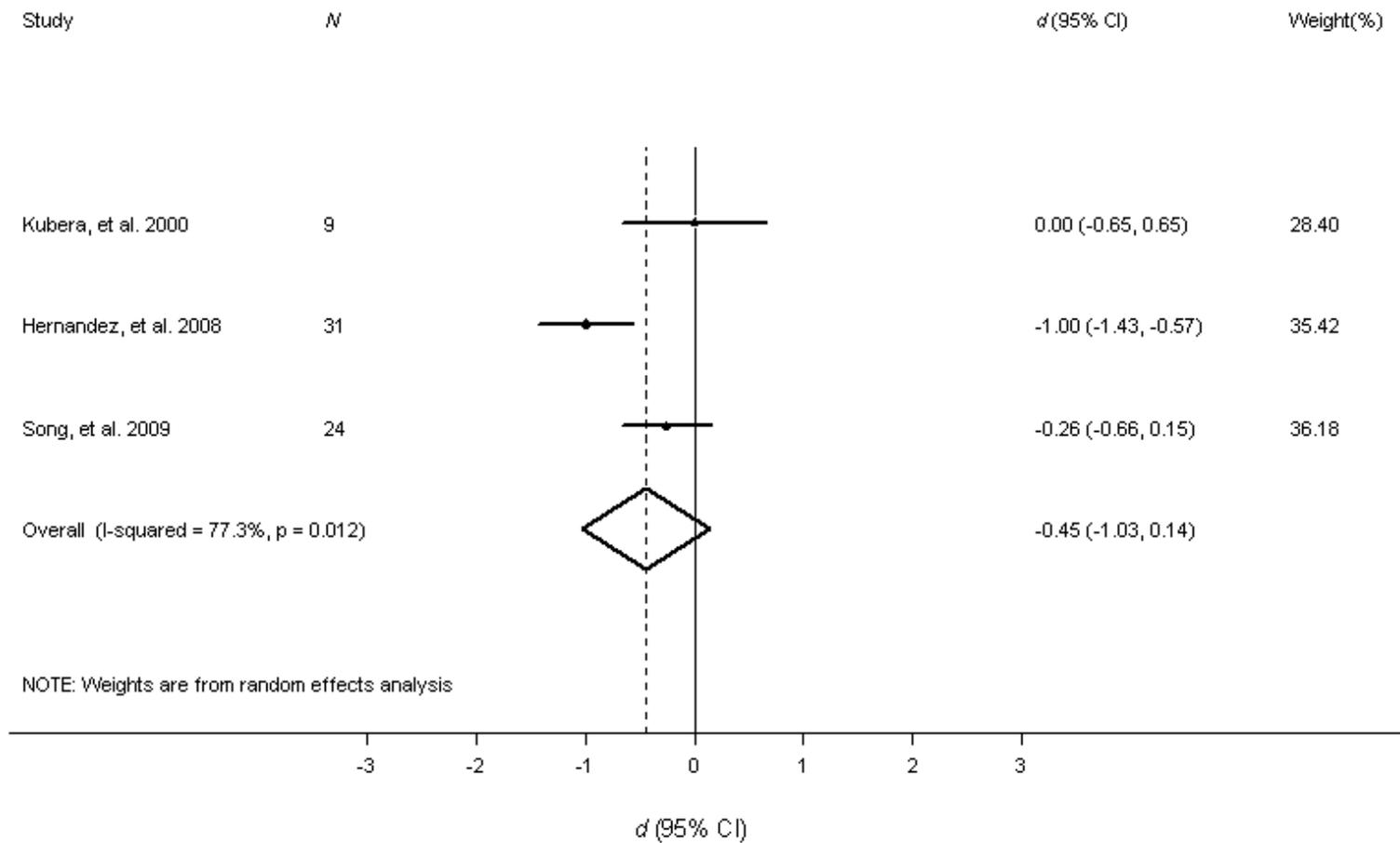
Figure 3.4. Forest plot for change in IL-10 after antidepressant treatment, details as per Figure 3.2.

Moderator analysis

Moderator analysis was completed on the IL-6 and CRP studies (no analysis was undertaken for IL-10 as there were too few studies). Table 3.2 shows the results of random effects analysis on smaller subgroups of the included studies (whether or not a formal diagnosis was completed, whether it was a uniform or tailored antidepressant administration, whether participants were inpatients or outpatients, and IL-6 assay brand). Only the subgroup of outpatients for IL-6 resulted in a strong significant pooled effect size ($p < .01$), recording a larger effect size than in the overall meta-analysis. There was no substantial reduction in I^2 in each subgroup, suggesting that none of these factors alone were responsible for the heterogeneity.

Meta-regression compared the standardized changes in IL-6 or CRP and continuous variables of standardized change in depression severity score, weeks in treatment, age, percentage males, and percentage of responders, for studies in which these variables were reported. Meta-regression revealed significant, positive associations between standardized change in IL-6 and percentage of males ($N = 17$, $\beta = -0.02$, SE = 0.006, b = -1.12, $p = .03$). No other associations were significant ($p > .05$). Finally, meta-regression of the standardized change in depressive symptoms against the baseline levels of IL-6 and CRP showed a negative, although non-significant, relationship (IL-6: $N = 10$, $\beta = -0.14$, SE = 0.15, b = -1.44, $p = .39$; CRP: $N = 6$, $\beta = -0.26$, SE = 0.25, b = -1.58, $p = .35$).

Table 3.2. Cohen's d (95% CI) pooled effect sizes under a random effects model for the change in CRP or IL-6 following antidepressant treatment for subgroups of studies within the overall meta-analysis, with k number of studies and I^2 measure of heterogeneity.

	Subgroup	k	Effect size (d)	95% CI	I^2 (%)
IL-6	Overall	14	-0.42*	-0.78, -0.06	88.2
Formal diagnosis					
	Yes	13	-0.41*	-0.79, -0.04	87.1
	No	1	-0.90***	-1.24, -0.56	.
Treatment type					
	Clinical decision	8	-0.50*	-0.96, -0.04	85.9
	Uniform administration	6	-0.32	-0.91, 0.27	90.8
Patient type					
	Inpatient	7	-0.32	-0.93, 0.29	91.5
	Outpatient	7	-0.53**	-0.90, -0.16	78.3
CRP	Overall	8	-0.57	-1.14, 0.01	93.2
Formal diagnosis					
	Yes	7	-0.52	-1.56, 0.12	93.3
	No	1	-0.92***	-1.26, -0.58	.
Treatment type					
	Clinical decision	2	-0.66	-1.38, 0.06	78.2
	Uniform administration	5	-0.78	-1.58, 0.01	94.7
Patient type					
	Inpatient	4	-0.93*	-1.85, -0.003	94.3
	Outpatient	3	-0.53	-1.44, 0.38	93.1

* p < .05; ** p < .01; *** p < .001 (based on a single study)

3.5 Discussion

This study is the first to provide pooled estimates of the change in resting, circulating IL-6, CRP and IL-10 in people with depression after antidepressant treatment. Meta-analysis indicated that after various antidepressant treatment durations there was a significant pooled reduction in IL-6, marginally significant reduction in CRP, and non-significant decrease in IL-10. There was good support for the reliability of the pooled reduction in IL-6 and CRP, with no evidence of publication bias and a high fail safe N, meaning it would take many unpublished, or yet to be published, studies to negate this pooled estimate. The few studies and low fail safe N for the IL-10 meta-analysis means that this observed pooled reduction should be interpreted with caution and considered preliminary. There was high heterogeneity in all the pooled estimates that could not be explained by meta-regression and subgroup analysis. Narrative review identified several issues which may help clarify unexplained sources of heterogeneity regarding comorbidity and individual differences.

In cross sectional studies, levels of IL-6, CRP and IL-10 are elevated in people with high compared to low depressive symptoms (Dowlati, et al., 2010; Howren, et al., 2009). It is fitting that these inflammatory markers decrease as depressive symptoms decrease in association with antidepressant treatment. There may be a difference in the sensitivity of CRP and IL-6 for recording a change after treatment, with some studies

demonstrating a change in IL-6 but not CRP (Chen, et al., 2010) and the pooled decrease only marginally significant for CRP. Nevertheless, the association between antidepressant treatment and reduction in inflammatory markers was demonstrated with different antidepressants, in a depressive episode irrespective of lifetime diagnoses of major depressive disorder or bipolar disorder (Sluzewska, et al., 1997), and to a limited extent, over time (Hernandez, et al., 2008; Kubera, et al., 2000; Mackay, et al., 2009).

IL-6, CRP and IL-10 play a regulatory role in the acute phase of inflammation, with IL-6 and CRP being primarily pro-inflammatory and IL-10 being inhibitory (Gabay & Kushner, 1999; Moore, de Waal Malefyt, Coffman, & O'Garra, 2001), yet the role of each of these markers in depression is unclear. Indeed, there may be a problem in balancing pro- and anti-inflammatory agents, and the relative concentrations of the pro- and anti-inflammatory markers may be more important than the absolute levels. At a broad level, elevations in pro- and anti-inflammatory markers during depressive illness may represent a generalized over-activation of the inflammatory system during the acute emotional state, which normalizes following alleviation of depressive symptoms. This is supported in studies which showed no significant difference between levels of inflammatory markers in the depression group post-treatment and in the healthy control group (Yao, et al., 2004). However, the relationship between depression and

inflammatory markers over time is not necessarily linear. For example, the longest study with multiple measurements demonstrated constant reductions in IL-10 at weeks 5, 20, 36 and 52, although depressive symptoms remained relatively consistent from week 20 (Hernandez, et al., 2008). These results require replication due to the methodological limitations of this study, including that only 35% of participants were retained by 52 weeks.

Although no outliers were detected and there was no evidence of publication bias for the IL-6 and CRP meta-analyses, there are limitations to the interpretation of these pooled effect sizes. Firstly, it is likely that the magnitude of the decrease in these inflammatory markers is slightly inflated due to regression to the mean (Bland & Altman, 1994). This problem is characteristic of single group pre-test-post-test designs, highlighting the need for further placebo-controlled RCTs. The available placebo-capsule RCT demonstrated that sertraline use was superior to placebo at reducing inflammatory markers (Pizzi, et al., 2009). Furthermore, the high levels of heterogeneity observed in each meta-analysis make interpreting the overall pooled estimates difficult. This level of heterogeneity was expected because liberal inclusion criteria were employed to maximally canvass the literature. The liberal inclusion criteria are both a weakness and strength of this meta-analysis. While studies of low methodological quality and studies with diverse samples were included, it

has also identified potential sources of heterogeneity to be considered in future research.

The main drivers of the high heterogeneity in the IL-6 and CRP data were not identified using subgroup analysis or meta-regression. This implies that there is likely a cumulative effect of patient characteristics on the degree of change in pro-inflammatory markers, and factors not reported in the primary studies that may more precisely account for unexplained heterogeneity. For instance, many studies lacked potentially useful prognostic information, such as compliance information, to consider whether declines are associated with the biochemical effects of antidepressants compared to decline in depressive symptoms over time. In the subgroup analyses, the only highly significant effect size was for IL-6 in outpatients, which only slightly decreased the level of heterogeneity and generated a slightly larger pooled decrease in IL-6. The only significant positive linear association in the meta-regression analyses was that having fewer males in a study was associated with a larger standardized decrease in IL-6. The reason for this is unclear but may be because females tend to have higher response rates to particular antidepressant treatments (particularly SSRIs) in clinical trials (Khan, Brodhead, Schwartz, Kolts, & Brown, 2005).

Narrative review identified several other potential sources of heterogeneity in comorbidity and individual differences. Changes in weight variables were rarely reported despite the comorbidity between depression

and obesity, and the relationship between antidepressant use and weight gain (Evans et al., 2005; Serretti & Mandelli, 2010). Studies which recorded an increase in IL-6 indicated that there were increases in fat distribution (Chen, et al., 2010), and adiposity is significantly associated with inflammatory changes, particularly increases in IL-6 (Park, Park, & Yu, 2005). Furthermore, people with higher levels of CRP after treatment had significantly higher levels of trait anxiety than people with lower levels of CRP (Lanquillon, et al., 2000). This finding may explain continued elevations in CRP or IL-6 in other studies which often fail to measure anxiety, despite its high comorbidity with depression (Rush et al., 2005) and independent association with inflammatory markers (O'Donovan et al., 2010).

Major depressive disorder is a clinically heterogeneous disorder and these differences may extend to differences in inflammation. Sluzewska et al. (1995) highlighted that only certain people with depression may exhibit elevated levels of IL-6, and only these people decreased their levels of IL-6. Disregarding issues of regression to the mean, this study highlights that certain people with depression may be more susceptible to elevations in pro-inflammatory markers. For instance, some research suggests that people with depression with melancholic features may exhibit a different inflammatory profile to those without melancholic features (Rothermundt et al., 2001). Depression with melancholic features is associated with a

decrease in CRP in serum following treatment (O'Brien, et al., 2006) and there are indications that levels of stimulated cytokines decline in melancholic but not other depression (Rothermundt, et al., 2001), implying that perhaps the inflammatory state is more closely associated with organic rather than cognitive symptoms of depression.

Furthermore, there is a possibility that baseline levels of inflammatory markers may identify those who may respond to treatment. Meta-regression in the current review showed no significant association between baseline IL-6 or CRP and change in depressive symptoms, although the pattern across studies was that higher baseline IL-6 and CRP were related to larger decreases in depressive symptoms. In individual studies, there is evidence in support of this, with higher baseline levels of CRP in treatment responders (Sluzewska, et al., 1997), and evidence to the contrary that lower stimulated levels of IL-6 at baseline (but not baseline serum CRP) in treatment responders compared to non-responders (Lanquillon, et al., 2000). Additionally, meta-regression showed no significant relationship between percentage of individuals who responded to treatment and inflammatory marker change, perhaps because it was infrequently reported and thus the meta-regression was restricted in range. At the individual study level, there was evidence of decreases in IL-6 for treatment responders, but not treatment non-responders (Yoshimura, et al., 2009), although two studies demonstrated a significant decrease in inflammatory

markers in both responders and non-responders (people with and without a 50% reduction in depression, respectively) (Lanquillon, et al., 2000; Sluzewska, et al., 1997). It is possible that even with small decreases in depressive symptoms, substantial changes to inflammatory markers may occur. Future studies should verify whether differences exist in inflammatory marker change and baseline inflammatory markers for treatment responders and non-responders, to support whether improvement in depressive symptoms is associated with a normalization of inflammatory markers and provide evidence for whether inflammatory markers can act as a biomarker of treatment response.

On the whole, the evidence presented in this meta-analysis is consistent with the inflammation theory of depression; with a reduction in depressive symptoms, there is a co-occurring reduction in inflammatory markers. At an illustrative level, these data support the idea that the causal chain is "depression driving inflammation", because treatment for depression also has the capacity to change inflammatory markers. Yet at the same time, antidepressants may have a direct anti-inflammatory effect, thus potentially causing the reductions in depressive mood. In vitro studies demonstrate that administration of antidepressants, particularly SSRIs, produces anti-inflammatory effects in the blood of both people with depression and healthy volunteers, decreasing pro-inflammatory markers including IL-6, IL-8 and tumor necrosis factor and increasing anti-

inflammatory markers including IL-10 (Janssen, Caniato, Verster, & Baune, 2010; Kenis & Maes, 2002). The observed anti-inflammatory effects may occur through antidepressants increasing glucocorticoid receptor-mediated negative feedback of the hypothalamic-pituitary-adrenal axis or increasing intracellular cyclic adenosine monophosphate (for reviews see Carvalho, Garner, Dew, Fazakerley, & Pariante, 2010; Carvalho & Pariante, 2008; Janssen, et al., 2010; Maes, 2001). Alternatively, studies into the effect of anti-inflammatory medications on depressive symptoms would provide evidence for the "inflammation driving depression" causal chain, and if supported the idea of an underlying common cause would be persuasive. To further explore the directionality, more longitudinal and prospective measurement of depression and inflammatory markers is necessary. It would also be of benefit to investigate changes in other immunomarkers after antidepressant treatment to provide the context of the changes in the few inflammatory markers reported in this study, as cross sectional evidence suggests that depression is associated with many markers of cell-mediated immune activation (Zorrilla, et al., 2001).

3.6 References

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4. Statins and risk of depression: A meta-analysis

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See Appendix 8.4 for the PRISMA checklist for the reporting of meta-analyses regarding this Chapter

4.1 Abstract

Background: Inflammation may be involved in the pathogenesis of depression and cardiovascular disease. Cholesterol-reducing medications, statins, may be anti-inflammatory, and therefore could lead to reductions in depressive symptoms.

Aims: Conduct a meta-analysis of depression outcomes in randomized controlled trials (RCT) comparing any statin with placebo.

Method: Five databases were searched using terms relating to statin, depression and RCT. Two authors assessed the eligibility of 1393 identified studies. 13 were relevant.

Results: There was a small, significantly greater reduction in depressive symptoms in participants treated with statins compared with placebo, $d = -0.11$ (95%CI -0.20, -0.02), $p = .02$, $I^2 = 0$. There was also evidence that statins may be protective against reported depression events, $RR = 0.90$ (95%CI 0.81, 1.00), $p = .05$, $I^2 = 0$. Our results likely underestimate the true effect as depression was a secondary outcome in these trials, and thus results are biased toward the null.

Conclusions: Statin treatment may benefit beyond cholesterol management. The findings support the inflammatory hypothesis of depression.

4.2 Introduction

Depression is common, with 12 month prevalence between 6 and 10% (Australian Bureau of Statistics, 2007; Kessler, Chiu, Demler, & Walters, 2005). It is consistently ranked as one of the top causes of burden of disease and is the leading cause of years lost to disability world-wide (World Health Organization, 2008). The causes of depression remain unclear. Recent biological theories of depression have shifted from serotonin-focused theories toward inflammation-based theories, due to observed associations between depression and inflammation (Maes et al., 2009). Circulating inflammatory mediators are elevated in people with depression, seem to decrease after antidepressant treatment, and may even predict later onset of depression (Dowlati et al., 2010; Hiles, Baker, de Malmanche, & Attia, 2012a, 2012b; Pasco, Nicholson, et al., 2010). Recent evidence from a randomized controlled trial also indicates that use of an inhibitor of the pro-inflammatory cytokine tumor necrosis factor, infliximab, is associated with improved depressive symptoms in people with depression who have elevated baseline levels of inflammatory markers (Raison et al., 2013). A causal relationship is biologically plausible. Inflammatory mediators affect several of the key biological correlates of depression – neuroendocrine stress activity, neurotransmitter activity, and neurotrophic support and neurogenesis – which has implications for triggering many of the observable symptoms of depression including altered appetite and sleep, fatigue, anhedonia, and stress reactivity (Maes, et al., 2009; Miller, Maletic, & Raison, 2009). The source of the elevated inflammatory mediators is

uncertain, whether from internal stressors (including adipose tissue), insults (including vascular insults) or lifestyle factors (Mikhailidis & Press, 2007; Miller, et al., 2009); it is also unclear whether inflammation is causative of depression or an epiphenomenon of other causative processes. Nevertheless, inflammatory markers seem to be closely related to the experience of depression.

Depression is particularly prevalent in people with medical illnesses, particularly cardiovascular disease (CVD). Almost a quarter (22.3%) of people with a history of CVD report lifetime depression compared to 15.1% in people without CVD (Fan, Strine, Jiles, & Mokdad, 2008). Meta-analysis indicates that depression is associated with an increased risk of developing CVD, including myocardial infarction, coronary heart disease and stroke (Fan, et al., 2008). It is possible that inflammation underlies this CVD-depression relationship (Grippo & Johnson, 2009; Joynt, Whellan, & O'Connor, 2003; Panagiotakos et al., 2004). There is evidence that CVD, in particular atherosclerosis, has some inflammatory basis, with leukocytes and pro-inflammatory cytokines involved in the early formation of atherosclerotic plaques, and inflammation promoting the instability of plaques which cause acute coronary events such as myocardial infarction and stroke (Karakas & Koenig, 2009; Libby, 2002; Libby, Ridker, & Maseri, 2002). Furthermore, the inflammatory marker C-reactive protein is a risk marker for CVD, with levels predicting future coronary events (Buckley, Fu, Freeman, Rogers, & Helfand, 2009; Karakas & Koenig, 2009; Ridker, 2007; Ridker et al., 2009).

An important aspect of the prevention of CVD is reducing cholesterol, often achieved by taking cholesterol-lowering medication such as statins (HMG-CoA reductase inhibitors). The primary action of statins is to lower low-density lipoprotein and cholesterol by inhibiting the enzyme HMG-CoA reductase, reducing levels of cholesterol precursor and depleting intracellular cholesterol; however, there is evidence that they have other functional effects including anti-inflammatory effects (Farooqui, Ong, Horrocks, Chen, & Farooqui, 2007; Kinlay, 2007; Libby, et al., 2002; Pancholi, Jain, Saxena, & Deb, 2009; Shen, 2005). Studies *in vitro* and in various patient populations indicate that use of statins is associated with reductions in inflammatory mediators including C-reactive protein and interleukin-6 (Balk et al., 2003; Craig et al., 2011; Farooqui, et al., 2007; Lee, Lin, & Chang, 2008; Pancholi, et al., 2009; Sano et al., 2011). Randomized designs have also demonstrated that use of statins leads to a lower post-operative peak in inflammatory mediators such as interleukin-6, interleukin-8 and tumor necrosis factor (Chello et al., 2007). Reductions in C-reactive protein are similar across different types of statins and at different doses (Balk, et al., 2003). Statins may influence the inflammatory response (and reduce atherosclerosis) via their interaction with endothelial cells, smooth muscle cells, macrophages and lymphocytes (Pancholi, et al., 2009). Furthermore, the anti-inflammatory effects of statins may be associated with their inhibition of reactive oxygen species which are closely linked to inflammation (Farooqui, et al., 2007; Stafford & Berk, 2011). The immunomodulatory effects of statins may be observed in the brain

(Farooqui, et al., 2007). Taken *in toto*, this evidence indicates a novel

mechanism by which statins may improve CVD and, possibly, depression.

Many of the early case studies and even prospective literature linking

statins and mental health suggested that low levels of cholesterol may be

associated with depression and suicide (Duits & Bos, 1993; Muldoon,

Manuck, & Matthews, 1990). However, more recent prospective literature

indicates this may not be the case (Pasco, Jacka, et al., 2010; Young-Xu,

Chan, Liao, Ravid, & Blatt, 2003). Furthermore, evidence from animal

models suggests that statins may potentiate the effects of antidepressants,

enhancing the improvement in depression-like behaviors (Renshaw et al.,

2009; Santos et al., 2012). The suspected anti-inflammatory effect of statins

offers a potentially opposing prediction to low-cholesterol hypotheses,

instead indicating that use of statins may be associated with a reduction in

depression and depressive symptoms. The current meta-analysis pools

evidence from randomized controlled trials (RCTs) comparing any type of

statin with placebo to examine whether after treatment, there is a

difference between groups in severity of depressive symptoms measured via

a standardized inventory, or self-reported depression events.

4.3 Methods

Search strategy and coding procedure

Studies were included if they met the following criteria:

1. Participants were adults (≥18 years). There were no restrictions on

   the type of participants in terms of medical or psychiatric

comorbidity, age group or residential setting (i.e., inpatient or outpatient).

2. Design was an RCT where participants were allocated to treatment with any type of statin or placebo. Participants in the placebo or statin treatment arms could not be randomized to receive differential medication or psychosocial intervention. Participants could be on concurrent stable medication or all receiving psychosocial intervention (including diet intervention/advice).

3. Treatment duration was at least 4 weeks.

4. The study measured depressive symptoms on a standardized inventory (self-report or clinician rated) or "depression" adverse events (new instances of self-reported depressive episodes after commencing statin or placebo treatment). This excluded measurement of psychological stress and negative mood states.

5. Publication of the primary study was in a peer reviewed journal in any year.

In May 2012, electronic searches of Embase (1947+), Medline (1946+), Cochrane Library (1994+), PsycInfo (1806+) and Web of Science databases (1956+) were conducted. Search terms were "$statin" or "HMG-CoA reductase inhibitor", terms relating to depression ("depress$" or "distress$" or "psychosocial" or "sadness" or "mood" or "affect$" or "major depression" or "self-report depression rating scale") and terms relating to RCT ("controlled clinical trial" or "randomi$ed controlled trial" or "placebo" or "clinical trial" or "random$" or "drug therapy" or "assign$" or "allocat$" or "double-blind" or

"blind"; in accordance with terms identified in the Cochrane handbook (Higgins & Green, 2011)). The reference list of relevant papers and reviews uncovered during the search were checked for additional relevant articles.

Assessment of potentially relevant abstracts was completed in duplicate by two authors (SH and TH). When abstracts were absent, unclear on methods or otherwise did not meet criteria for exclusion, full-text articles were extracted and compared against inclusion criteria (see Figure 4.1 for article extraction process). For non-English language publications identified in the search that were considered potentially relevant based on the abstract, the method was translated via Google Translate. None were deemed eligible based on the method (not statin vs. placebo RCT) so were not fully translated. The two authors had complete, independent agreement in identifying eligible studies from full-text articles. These authors then independently extracted information from relevant studies into a specific spreadsheet form devised for this study, including participant numbers, mean depressive symptoms before and after treatment (and standard deviation/error), type of statin, weeks of treatment, various participant characteristics (average age, gender ratio and medical comorbidity) and Jadad scale for study-level methodological quality (Jadad et al., 1996), resolving discrepancies by consensus. Means presented in figures were extracted with Data Thief III software (Tummers, van der Laan, & Huyser, 2008).
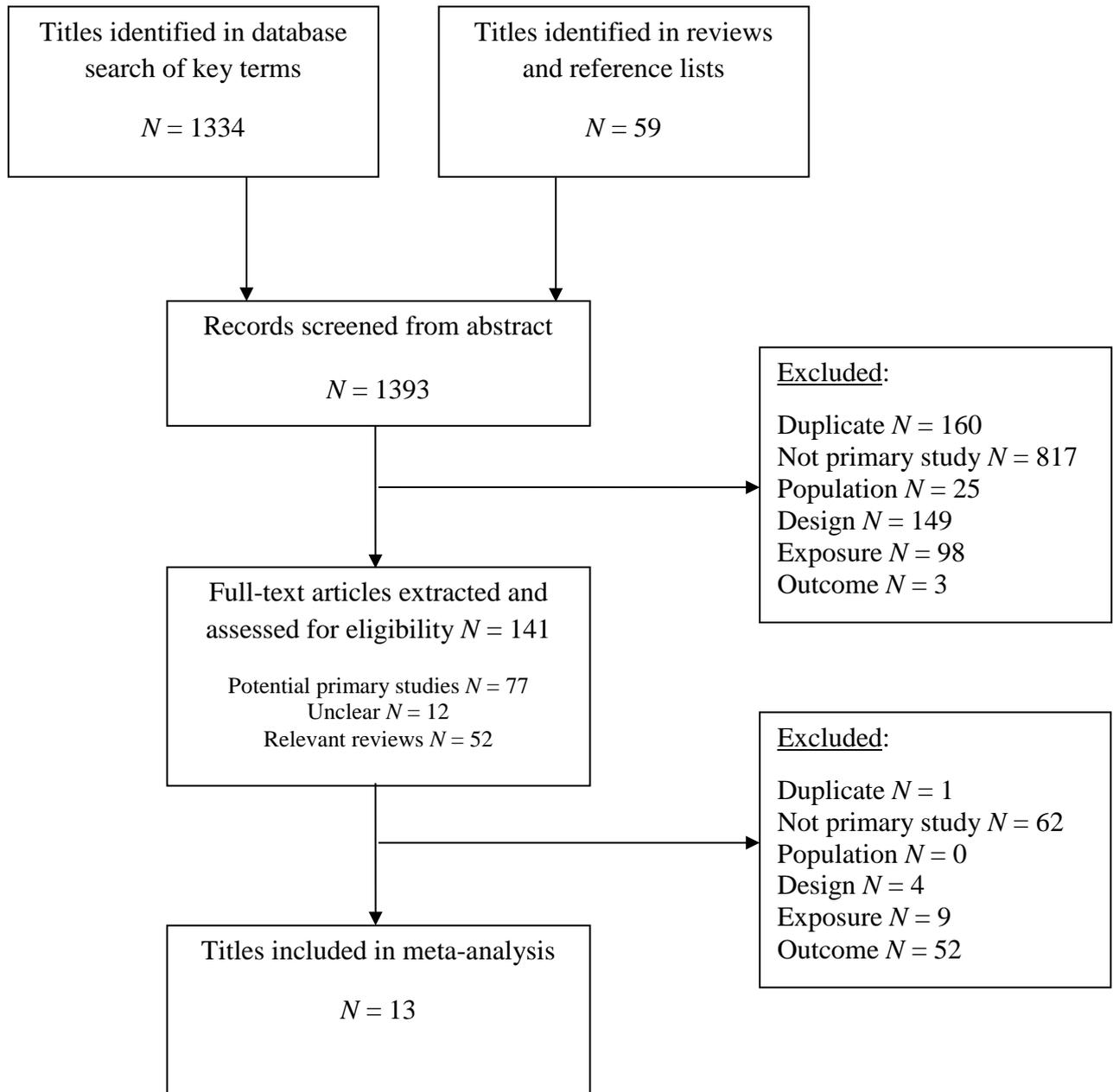
Figure 4.1. Flow diagram of the article extraction process, with the number of studies (N) excluded or included at each stage.

Statistical methods

Analyses were conducted using Comprehensive Meta-Analysis (Biostat, Inc., USA) and Stata SE/11 (StataCorp, LP, USA). In studies where participants were randomized to different types or dosages of statins (in addition to placebo), the mean effect size across the different statin groups within studies were imputed using the methods of Borenstein et al. (2009) to examine the overall effect of any statin versus placebo on depressive symptoms. For continuous depressive symptom scores, Cohen's d study-standardized mean differences were calculated in two ways. The first method compared post-treatment mean scores of depressive symptoms in statin and placebo groups. A negative effect size indicates that depressive symptoms were lower in the statin compared with placebo group. The second method calculated effect size using the mean change in depressive symptom score during treatment (post-score minus pre-score) compared between statin and placebo groups. A negative effect size indicates that there was a larger reduction in depressive symptoms in the statin compared with placebo group. This latter method is more powerful in that it takes into account the pre-treatment differences. For binary depression event outcomes, risk ratios were calculated using the number of reported depression events, with a risk ratio less than 1 indicating fewer depressive events in the statin treatment group. Study effect sizes were pooled using a random effects model, separately for continuous and binary outcomes. Publication bias was assessed via inspection of contour-enhanced funnel plots (Peters, Sutton, Jones, Abrams, & Rushton, 2008), Egger's test of

funnel plot asymmetry (Egger, Davey Smith, Schneider, & Minder, 1997), trim and fill (Duval & Tweedie, 2000) and fail-safe N (Rosenthal, 1979). Sensitivity analysis was completed via a cumulative meta-analysis to examine whether any extreme effect sizes were present, with none apparent. Subgroup analyses by statin type (hydrophilic or lipophilic) were also conducted.

<div align="center">4.4 Results</div>

There were 13 RCTs relevant to these meta-analyses (total of 1858 participants from 6 studies for continuous measures, 36262 from 7 studies for binary measures; see Table 4.1 for comparison of participant samples and methodological quality). Most studies reported sufficient information to be considered as having sound methodological quality, although at an outcome level, depression outcomes were generally secondary outcomes. Each study reported a significant reduction in cholesterol following statin treatment. There was no significant difference in post-treatment depressive symptom severity scores between statin and placebo treated participants, d = -0.05 (95% CI -0.14, 0.05), p = 0.36, I^2 = 8.7% (study level data not shown). However, when taking into account pre-treatment levels of depressive symptoms available in five studies, the overall pooled effect size represented a small but significantly greater reduction in depressive symptom severity over the course of statin compared with placebo treatment, d = -0.11 (95% CI -0.20, -0.02), p = .02, Figure 4.2. Some caveats apply in that there was clear clinical diversity with a large variety in types of statins, treatment

Table 4.1. Characteristics of the randomized controlled trials included in the meta-analyses. All studies had a significant decline in cholesterol in the statin compared with placebo treatment group.

									Randomized	-described/appropriate	Double-blind	-described/appropriate	Withdrawals/dropouts described
									Methodological quality (Jadad, et al., 1996)				
					Study description								
Study citation	Patients	Mean age	Males (%)	Design	Statin type	Treatment duration (weeks)	Depression measure	Total N					
Gentile et al. (2000)	Outpatients with type II diabetes; 50-65 years; LDL >160mg/dL and triglyceride <400mg/dL	59	67	Open label RCT	Atorvastatin (10mg), simvastatin (10mg), pravastatin (20mg), lovastatin (20mg)	24	Reported depression (binary)	409	1	0	0	0	1
Harrison & Ashton (1994)	Healthy volunteers; 20-31 years	24	68	Cross-over RCT	Pravastatin (40mg) and simvastatin (40mg)	4	HADS depression subscale* (continuous)	25	1	0	1	0	1

Hsia et al. (2011)	Generally healthy (no history of cardiovascular disease or diabetes); men >50 years and women >60 years; LDL <130mg/dL, CRP >2mg/L	66	62	RCT	Rosuvastatin (20mg)	104[†]	Reported depressed mood, depression, suicidal ideation or attempt (binary)	16304	1	0	0[‡]	0	0
Morales et al. (2006)	Healthy volunteers; ≥65 years; high normal/mildly elevated serum cholesterol; total cholesterol 160-240mg/dL	71	49	RCT	Simvastatin (up to 20mg)	15	CES-D "case" score >16 (binary)	80	1	0	1	1	1
Muldoon et al. (2000)	Generally healthy with hypercholesterolemia; 24-60 years; LDL >160mg/dL	46	54	RCT, age stratified	Lovastatin (20mg)	26	HDRS (continuous)	194	1	0	1	1	1
Plotkin et al. (2002)	Women with regular menstrual cycles in the last year; 25-41 years; LDL 130-250mg/dL	36	0	RCT	Simvastatin (40mg)	16	Reported depressive disorder (binary)	86	1	0	1	1	1

Study	Population			Design	Drug		Outcome							
Sano et al. (2011)	Probable Alzheimer's Disease; >50 years; mild to severe impairment on MMSE; LDL >80mg/dL, triglycerides <5000mg/dL	75	41	RCT	Simvastatin (20mg for 6 weeks, increased to 40mg)	78	Reported depression (binary)	406	1	1	1	1	1
Santanello et al. (1997)	Generally healthy, >65 years; LDL 159 to 221mg/dL	71	31	RCT	Lovastatin (20mg or 40mg)	26	CES-D (continuous)	376	1	0	1	0	1
Sparks et al. (2005)	Probable or possible Alzheimer's Disease; >51 years, mild to moderate impairment on MMSE	79	63	RCT	Atorvastatin (80mg)	52	GDS (continuous)	46	1	1	1	1	1
Stewart et al. (2000)	Acute MI/hospitalisation for unstable angina in prior 3-36 months; 30-71 years; fasting serum cholesterol 4-7mmol/L	--	80	RCT	Pravastatin (40mg)	26 (also at 52, 104, 208)	GHQ (continuous)	1130	1	0	1	0	0

| Tonelli et al. (2005) | Pooled from WOSCOPS (high risk men who had not experienced a MI and had fasting LDL <155 mg/dL after 4 weeks of cholesterol restricted diet), CARE and LIPID studies (men and women with previous acute coronary syndromes and average cholesterol levels at baseline <240 and 155-271 mg/dL, respectively) | 59 | 87 | 3 RCTs | Pravastatin (40mg) | 260 | Reported depression (binary) | 18555 | 1 | 0 | 1 | 0 | 0 |
| Visseren et al. (2001) | Outpatients with type II diabetes on insulin; LDL >4.1mmol/L after diet intervention, HbAlc <8% | 61 | 37 | RCT | Fluvastatin (40mg) | 12 | ZSDS (continuous) | 87 | 1 | 1 | 1 | 1 | 1 |

| Wardle et al. (1996) | People at high risk of coronary heart disease (history of MI, angina, stroke, TIA, peripheral vascular disease, diabetes or hypertension); 40-75 years; total cholesterol >3.5mmol/l | 64 | 85 | RCT | Simvastatin (20mg or 40mg, randomized to either) | 152 | Reported depression, weekly clinics (binary) | 621 | 1 | 0 | ? | 1 | 1 |

*Baseline scores not available, follow-up only.

†Treatment duration provided as median follow-up time.

‡Double blind status was not reported in this follow-up publication, only presented in earlier publication (Ridker, et al., 2009).

Abbreviations:

CARE: Cholesterol and Recurrent Events study (Sacks et al., 1996)

CES-D: Center for Epidemiologic Studies Depression Scale

CRP: C-reactive protein

GDS: Geriatric Depression Scale

GHQ: General Health Questionnaire

HADS: Hospital Anxiety Depression Scale

HbAlc: glycolated haemoglobin

HDRS: Hamilton Depression Rating Scale

LDL: low-density lipoprotein cholesterol

LIPID: Long-term Intervention with Pravastatin in Ischemic Disease study (LIPID study group, 1998)

MI: myocardial infarction;

MMSE: Mini-Mental State Examination

RCT: randomized controlled trial

TIA: transient ischemic attack

WOSCOPS: West of Scotland Coronary Prevention Study (Shepherd et al., 1995)

ZSDS: Zung Self-Rating Depression Scale.
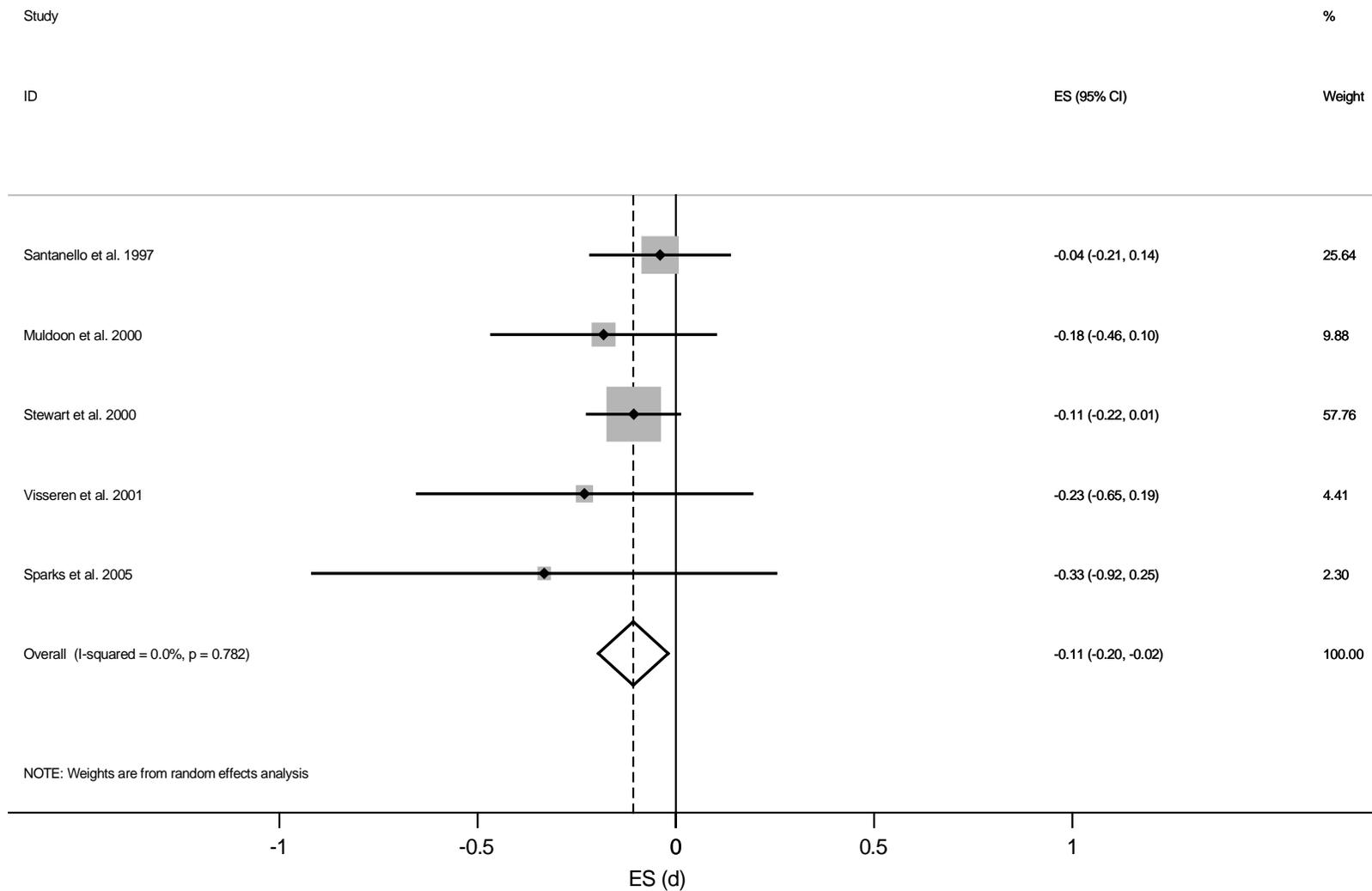
Figure 4.2. Forest plot of effect sizes (ES, Cohen's *d*) for the reduction in depressive symptom severity over the course of statin

compared with placebo treatment, taking into account pre-treatment levels of depressive symptoms. Negative effect sizes represent

an advantage for the statin treatment group (larger reduction in depressive symptoms during treatment).

lengths and patient types between studies, although no significant statistical heterogeneity, $Q(4) = 1.75$, $p = .78$, $I^2 = 0\%$. Subgroup analysis was not possible due to the small number of studies. The contour-enhanced funnel-plot indicated that effect sizes all fell within the $p > 10\%$ range, Figure 4.3a. While this indicates that bias due to suppression of non-significant studies is unlikely, unpublished null-effects to the right of the mean may alter the observed effect size (Peters, et al., 2008). However, there was no evidence of publication bias according to Egger's test ($t(3) = 1.84$, $p = .16$), and trim and fill indicated no trimming was performed and therefore, no change in effect size. Fail-safe N was very low ($N = 4$) indicating that only a few unpublished or yet-to-be-published studies would render the observed effect size non-significant.

Meta-analysis of reported depression events indicated that statin treatment may be protective against depressive events compared with placebo, $RR = 0.90$ (95% CI 0.81, 1.00), $p = .05$, Figure 4.4. There was no evidence of statistical heterogeneity, $Q(6) = 3.50$, $p = 0.74$, $I^2 = 0\%$. The contour-enhanced funnel plot again demonstrated effect sizes in the $p > 10\%$ range, Figure 4.3b. However, there was no evidence of publication bias according to Egger's test, $t(5) = 1.08$, $p = .33$. Trim and fill analyses indicated little change in the observed effect size, with the addition of only one study, adjusted $RR = 0.90$ (95% CI 0.81, 0.99), $p < .04$. Given the statistical significance, fail-safe N was 0.
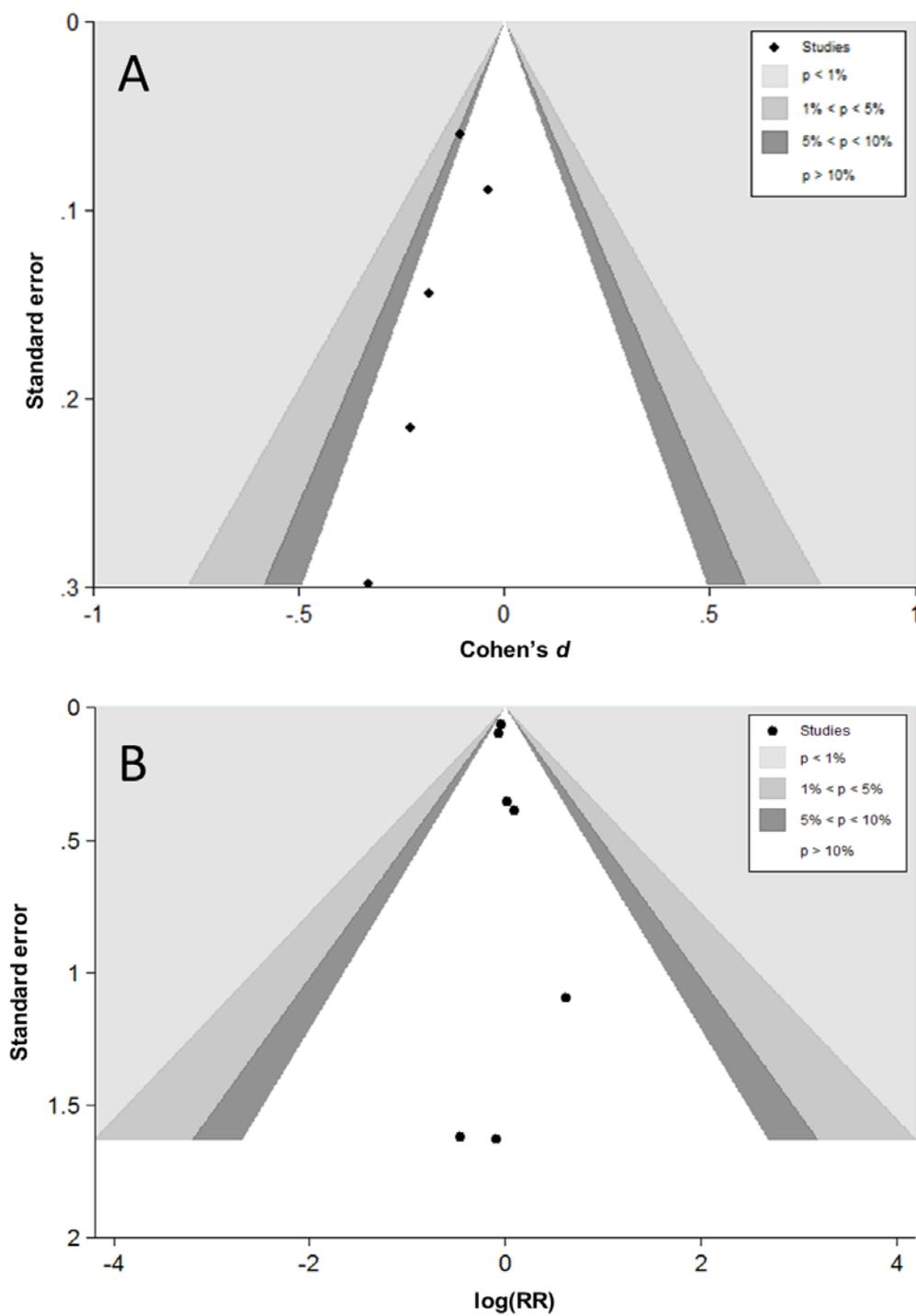
Figure 4.3. Contour-enhanced funnel plot of the relationship between effect size estimate and standard error for continuous depressive symptom outcomes after statin treatment (A) and logarithm of binary depression events after statin treatment (B). Contour lines indicate milestones of statistical significance for the observed effect sizes.
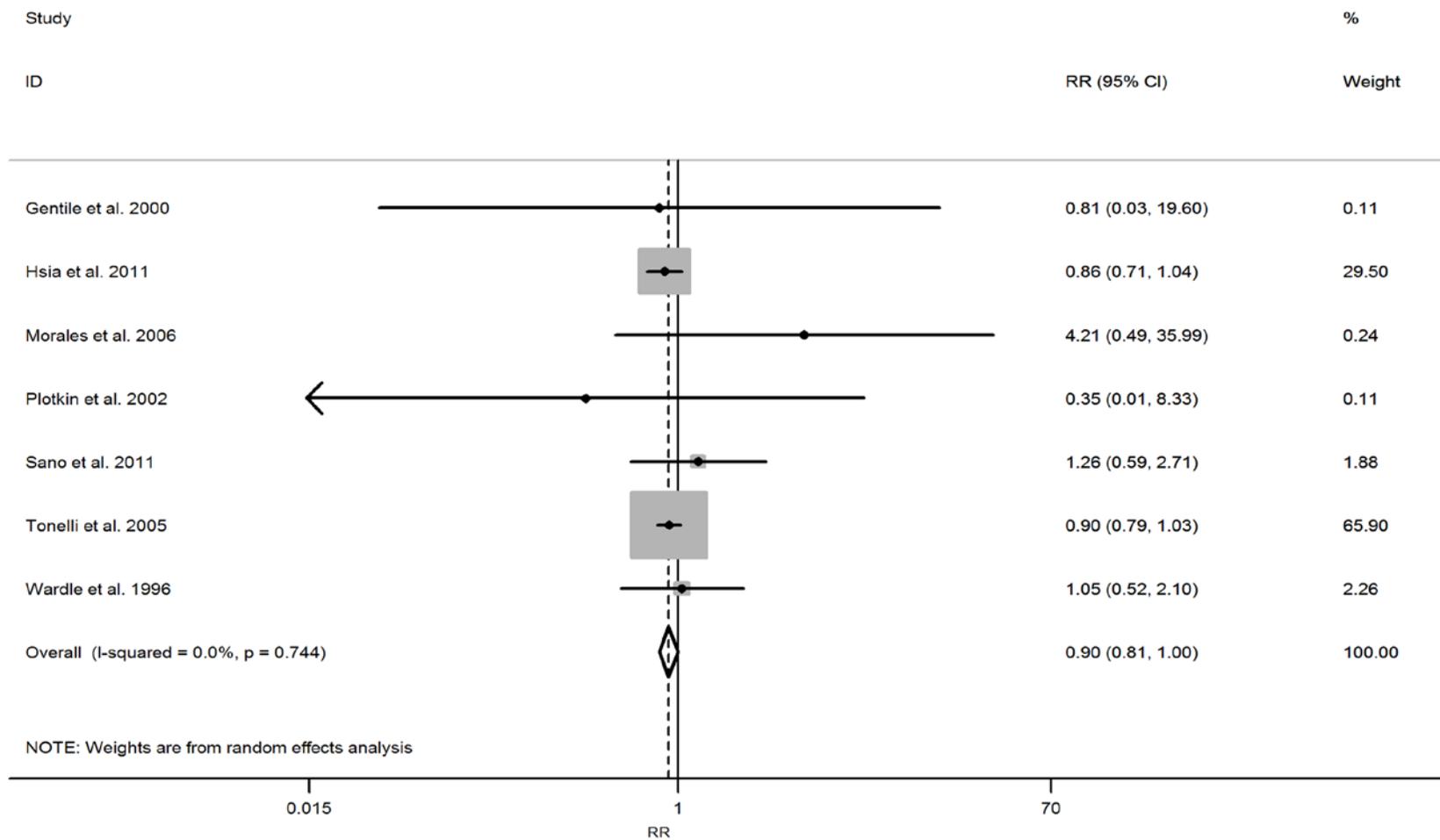
Figure 4.4. Forest plot of risk ratio (RR) effect sizes for the number of reported depression adverse events in the statin compared with placebo groups. Risk ratios below 1 indicate fewer instances of reported depression in the statin group.

Subgroup analyses by statin type (hydrophilic or lipophilic) revealed no significant difference in depressive symptoms in either subgroup of statin type versus placebo, or for depressive events between lipophilic statins and placebo. There were significantly fewer depressive events in the hydrophilic statins compared with placebo, $RR = 0.89$ (95% CI 0.80, 0.99), $p = .03$, $I^2 = 0\%$.

4.5 Discussion

There was a significant reduction in depressive symptoms as measured by standardized inventory following treatment with statins compared with placebo. There were also significantly fewer adverse events of depression in the statin compared with placebo treatment. While these conclusions are limited to the few, diverse published studies, they are likely to underestimate the true effect. Depression was a secondary outcome in these trials which would bias results toward the null. Furthermore, most participants in these studies were without depressive symptoms at baseline, and so could not show additional improvements in symptoms. These results are consistent with previous large pooled databases of placebo and active-controlled RCTs of fluvastatin which demonstrate non-significantly fewer depression adverse events in subgroups of patients under 65 and with creatinine clearance < 50mL/min (Bruckert, Verpilleux, Dejager, & Isaacsohn, 2005; Holdaas, Wanner, Abletshauser, Gimpelewicz, & Isaacsohn, 2007).

Early research hypothesized that there was a link between low cholesterol and mental illness, driven in particular by early studies indicating increased risk of death by suicide after taking statins (Muldoon, et al., 1990). For instance, people who were exposed to simvastatin and hypolipidemic diet, as opposed to other diets or medication such as beta-blockers, had increased prevalence of absenteeism due to depression, although this was not the case for new incidence of absenteeism due to depression (Boumendil & Tubert-Bitter, 1995). Yet, this study only controlled for gender and professional status. Epidemiological studies which demonstrate an association between low total cholesterol and suicide or depression tend to not control for confounding factors such as weight loss, alcohol, smoking, diet and physical activity (Brunner, Parhofer, Schwandt, & Bronisch, 2002; Strandberg & Tilvis, 1995). Such a problem of differences in behaviour between groups is not present in placebo-controlled RCTs. Indeed, in a later meta-analysis of statin RCTs and mental health outcomes, rates of suicide were no different in cholesterol lowering treatments including statins and dietary intervention compared with control (Muldoon, Manuck, Mendelsohn, Kaplan, & Belle, 2001).

The results of the current meta-analysis of RCTs mirrors results observed in more recent prospective observational studies where treatment with statins confers no risk for depression (Agostini et al., 2007; Feng, Yap, Kua, & Ng, 2010), and may even be protective of depressive episodes (Otte, Zhao, & Whooley, 2012; Pasco, Jacka, et al., 2010; Young-Xu, et al., 2003). For instance, patients discharged from hospital after a coronary event had

high rates of statin use, and after 9 months statin use was associated with a 79% reduction in the likelihood of depression (Stafford & Berk, 2011). The benefits for depression may be specific to statins. Whilst there were reduced odds of depression (as well as anxiety and hostility) in cardiology clinic outpatients using statins, this was not the case for other non-statin cholesterol lowering drugs (Young-Xu, et al., 2003). This effect was observed irrespective of achieved cholesterol level. Similar results have been observed in naturalistic practice. In a nested case control study of computerized general practitioner records in the United Kingdom, there was a lower risk of depression in current statin users compared with non-users after adjustment for smoking, body mass index, stressful life events, general practitioner visits, peripheral vascular disease and history of psychiatric disorders (Yang, Jick, & Jick, 2003). The protective effect was primarily in patients treated for over a year. Likewise, in a review of a database of adverse drug reactions in Italy, simvastatin users had a significantly lower rate of many of the symptoms present in people with depression (such as agitation, somnolence, anxiety, anorexia and impotence) (Tuccori et al., 2008). One limitation of cohort studies is that those individuals who are compliant and continue to use statins during follow-up may exhibit healthier behaviours (and therefore, less depression). In the context of RCTs, this threat is reduced as both treatment and controls face the same risk.

Statins seem to have effects beyond cholesterol reduction, perhaps the most robust being anti-inflammatory and immunomodulating actions,

although there is also evidence of anti-oxidant, anti-thrombotic, and anti-arrhythmic effects (Pancholi, et al., 2009; Shen, 2005). Cross sectional evidence largely indicates no clear association between depression and cholesterol, and therefore, there is the potential that one of the other mechanisms of statins – such as anti-inflammatory actions – may be responsible for the observed reductions in depressive symptoms. One other possible competing reason for reduction in depressive symptoms in statin users is that participants had improved quality of life through decreases in disease-related symptoms, which may lead to improvements in mood. However, the participants in these RCTs were generally healthy upon enrolment in the study and hyperlipidemia is generally asymptomatic.

In contrast to the results observed in the current meta-analysis, some recent epidemiological evidence continues to identify a cross-sectional association between low cholesterol and depression (Tedders et al., 2011). The effects were observed only in the most severely depressed young people who are seldom present in the RCTs included in the current review. Furthermore, the effect was absent or reduced when taking into account demographic and lifestyle factors. If there were a link between cholesterol and mental health problems, it is proposed to be mediated via serotonin (Zhang, 2011). Levels of serotonin transporter, which removes serotonin from the synaptic cleft, may increase in the first two months after the onset of statin treatment and return to normal levels after a year, possibly due to compensatory mechanisms) (Vevera et al., 2005). Therefore, there may be a short window of sensitivity for developing depression which is not present

during the longer treatment durations mostly captured in the current meta-analysis. Alternatively, depression may be associated with low cholesterol in cross sectional studies via decreased appetite or other pathways (e.g., decreased dietary intake of polyunsaturated fatty acids or vitamins) (Zhang, 2011).

Using RCTs to empirically test the hypothesis that reductions in inflammatory markers are responsible for reductions in depressive symptoms in statin users is warranted. Such RCTs are particularly indicated in clinically depressed populations with co-occurring hyperlipidemia. The purported effects of inflammation in depression are most likely mediated centrally. Therefore, lipophilic statins which tend to more easily permeate the blood-brain barrier (e.g., atorvastatin, fluvastatin, lovastatin, simvastatin) may exert greater psychiatric effects than the more hydrophilic statins which have low permeability (e.g., pravastatin, rosuvastatin). In outpatients from a cardiology clinic, the reduction in depression was only observed in those taking lipophilic and not hydrophilic statins (Young-Xu, et al., 2003). Although subgroup analysis in the current meta-analysis was unable to detect this difference, should one exist, it is an important avenue for future research. Indeed, recent research indicates that simvastatin in particular accesses the brain most efficiently and so may be the best candidate for future studies in this area (Sierra et al., 2011).

Regarding limitations, study-level risk of bias is possible, although all studies were of moderate to high methodological quality. Although the outcome of self-reported depressive symptoms is adequate and important

from a phenomenological perspective, future research will benefit from including independently diagnosed clinical depression as a primary outcome. There is also the potential threat of regression to the mean through analysis of the gain score, rather than the post-treatment comparisons. However, the participants were not chosen based on higher depression scores, making regression to the mean less likely. The discrepancy between the results for the continuous outcomes adjusting and not adjusting for baseline values may be puzzling but the former method is more powerful in that it takes into account the pre-treatment differences observed. The three small studies that showed an effect in favour of placebo without baseline adjustment reverse their direction when adjusted. This may be a function of the smaller sample sizes and the increased benefit of adjusting for baseline in this setting. Indeed, Santanello et al. (1997) observed a selective increase in depressive symptoms in the placebo group, compared to the reduction observed in the statin group. At a review level, there was a low fail-safe N and few studies retrieved. Incomplete retrieval of published literature is unlikely, although there is the chance that additional unpublished studies exist, particularly demonstrating a null result. Nevertheless, Egger's test and trim and fill analyses did not indicate publication bias, nor was there evidence of publication bias due to suppression of non-significant results. The likelihood that additional studies in the opposite direction exist is relatively unlikely, given the early context of a supposed relationship between low cholesterol and mental health problems. The study criteria were made deliberately broad to increase the

number of eligible studies, as the focus was on whether there would be broad changes to depressive symptoms after any statin intervention. Future studies in more homogeneous subgroups will answer questions of whether the observed reductions in depression are strongest in particular patient groups, statin types or treatment durations. Furthermore, examining studies of longer treatment duration would demonstrate whether the effects are enduring.

While the reduction in depressive symptoms is small in magnitude, it nevertheless represents a potentially substantial population effect (Horwath, Johnson, Klerman, & Weissman, 1992). At a clinical level, it suggests that statin use may be particularly advantageous for individuals at high risk of developing depressive symptoms, such as those with CVD or dementia (Fan, et al., 2008; Shahnawaz et al., 2013). The meta-analysis highlights that research into depression and CVD needs to take into account statin use, as statins seem to have a significant impact on both disease processes. The observation that treatment with a medication with anti-inflammatory properties is associated with reductions in depressive symptoms is highly important for providing proof-of-concept evidence supporting the causal nature of the inflammation hypothesis of depression.

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5. Inflammatory pathways to depression: Unhealthy behaviours as confounders or causes

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5.1 Abstract

Background: Depression and inflammatory markers have a reliable cross-sectional association although less is known about the prospective relationship. The current study aims to investigate whether pro-inflammatory markers are associated with later depression, and whether aspects of unhealthy lifestyle may account for this association.

Methods: Participants were drawn from the Hunter Community Study, a community-dwelling cohort of individuals aged 55-85 years. At baseline, participants completed physiological assessment, depressive symptom and other health-related questionnaires, and underwent blood draw for the analysis of inflammatory markers, C-reactive protein (CRP) and interleukin (IL)-6. Participants completed the same depressive symptom questionnaire again after 3.5 to 5.5 years. Depression outcomes at follow-up were analysed dichotomously using established scale cut-off scores, and continuously as a "residual score", representing the variation in follow-up depressive symptoms not explained by baseline symptoms and age. Analyses were conducted on males and females separately.

Results: At baseline, indicators of unhealthy lifestyle, depressive symptoms and inflammatory markers were associated. For males, there were no relationships between inflammatory markers and follow-up depression outcomes. In females, IL-6, but not CRP, was significantly associated with residual score and dichotomous depression. IL-6 was no longer significant when taking into account confounding from lifestyle factors. However, IL-6

was also a significant mediator of the association between waist-to-hip ratio and residual score, and current smoking and residual score.

Conclusions: The results support the inflammatory hypothesis of depression, although females may be more vulnerable to effects. The findings raise the possibility that unhealthy lifestyle may drive this inflammation and subsequent depressive symptoms.

5.2 Introduction

There is increasing investigation into whether inflammation may have a causative role in depression. The inflammatory hypothesis of depression is supported by observations of depressive-like behaviour following cytokine administration in animals and humans, and idiopathic major depressive disorder in patients treated with cytokines such as interferon-alpha or interleukin (IL)-2 (Anisman, Merali, Poulter, & Hayley, 2005; Capuron et al., 2009; Dantzer, O'Connor, Lawson, & Kelley, 2011; Miller, Maletic, & Raison, 2009; Myint, Schwarz, Steinbusch, & Leonard, 2009; Reichenberg et al., 2001). Emerging evidence from randomised controlled trials suggests that anti-inflammatory medications may improve depression outcomes (Akhondzadeh et al., 2009; Raison et al., 2013). Furthermore, inflammatory mediators interact with key biological systems implicated in depression, including altering neuroendocrine stress activity, neural plasticity, cognitive functioning, reactive oxygen species, and neurotransmitter metabolism and activity (Irwin & Miller, 2007; Miller, et al., 2009). The source of inflammation in people with depression may be related to the autonomic and hormonal stress response to psychosocial stressors, nascent or apparent medical illness including obesity, and/or aspects of lifestyle such as physical activity and diet. Thus, a causal relationship between inflammation and depression is biologically plausible, and the theory suggests that inflammatory mediators may be targets for treatment themselves, or have utility in predicting depression onset or treatment response.

Much of the epidemiological literature regarding inflammatory mediators in depression is cross sectional. Studies show a relatively consistent elevation in inflammatory mediators, including IL-6, C-reactive protein (CRP), and tumor necrosis factor, highlighted in several recent meta-analyses (Dowlati et al., 2010; Hiles, Baker, de Malmanche, & Attia, 2012; Howren, Lamkin, & Suls, 2009). In contrast, there are very few published longitudinal studies addressing whether elevations in inflammatory markers precede or follow depressive symptoms.

Existing prospective studies show mixed evidence regarding the potential temporality of the depression-inflammation relationship. In relation to depression predicting future levels of inflammatory markers, two studies have shown no significant association between baseline levels of depression and later levels of IL-6 (Kiecolt-Glaser et al., 2003) or CRP (Matthews et al., 2007), although significant results have been observed in other studies for IL-6 and CRP (Duivis, de Jonge, Penninx, Ya Na, & Whooley, 2011; Janicki-Deverts et al., 2010; Stewart, Rand, Muldoon, & Kamarck, 2009), and for other inflammatory mediators, such as complement factor 3, C3 (Boyle, Jackson, & Suarez, 2007), and white blood cell count (Duivis et al., 2011). There has been more evidence for the opposite causative pathway. CRP, IL-1β, IL-1 receptor antagonist and IL-6 at baseline have been associated with later depressive symptoms (Gimeno et al., 2009; van den Biggelaar et al., 2007). Levels of CRP have been associated with the development of major depressive disorder (Pasco et al., 2010). In yet another study, IL-6 receptor antagonist was associated with

229

depressive symptoms 6 years later, although a range of other inflammatory markers including CRP, IL-1ß and IL-6 were not (Milaneschi et al., 2009). Only one study has shown bi-directional support, although evidence was stronger for the CRP to depression relationship (Matthews et al., 2010). Two publications on participants from the same cohort also indicate support for relationships in both directions (Hamer, Molloy, de Oliveira, & Demakakos, 2009a, 2009b). Given the mixed evidence, perhaps due to differences in the participant samples and in adjusting for sources of confounding, further exploration of the prospective relationship is warranted, with close consideration of the influence of effect modifiers and confounding.

Some previous prospective studies have selectively examined women (Matthews, et al., 2007, 2010) or men (Boyle, et al., 2007), although gender is generally used as a control variable, rather than an effect modifier. Women experience depressive disorders more frequently, have an earlier onset of disorder, and have high comorbidity between depression and anxiety disorders, whereas men with depression have high comorbidity with substance use disorders (Marcus et al., 2005). Antidepressant treatment effectiveness may also differ by sex (Khan, Brodhead, Schwartz, Kolts, & Brown, 2005). Differences may be due to both social and biological factors. For instance, gender differences are observed in levels of some inflammatory markers and neuroendocrine stress hormones (Edwards, Burns, Ring, & Carroll, 2006; Kudielka & Kirschbaum, 2005; Lakoski et al., 2006; Larsson, Gullberg, Rastam, & Lindblad, 2009; Marriott & Huet-Hudson, 2006; McConnell et al., 2005). Given that gender influences the experience of

depression and seems to modulate inflammatory markers, it may be pertinent to examine the evidence for the prospective relationship between depression and inflammatory markers by gender.

One difficulty with investigating the relationship between inflammatory markers in depression is that aspects of unhealthy lifestyle associated with depression also have inflammatory consequences which may explain observed cross-sectional and temporal associations. These lifestyle factors include central adiposity, low physical activity, poor diet quality, smoking and alcohol use (Hamer, et al., 2009a; Lopresti, Hood, & Drummond, 2013; Milaneschi, et al., 2009; O'Connor et al., 2009). For instance, adipose tissue, particularly hypertrophic abdominal fat, produces inflammatory cytokines and mediators (Bastard et al., 2006; Maury & Brichard, 2010; Odegaard & Chawla, 2013) and it may be this abdominal, and not subcutaneous, fat that is associated with depression (Everson-Rose et al., 2009). Studies that have examined the contribution of unhealthy lifestyle to the depression-inflammation relationship generally find that health behaviours render the relationship between inflammatory mediators and depressive symptoms non-significant (Duivis, de Jonge, Penninx, Ya Na, et al., 2011). Such an approach addresses the question of whether lifestyle confounds the relationship between inflammatory markers and depression. However, an alternative question is whether inflammatory markers may mediate relationships between lifestyle and depression. Previous mediation analysis has shown both significant direct effects of depressive symptoms on later levels of CRP, and significant indirect effects

via smoking, alcohol and physical activity (Hamer, et al., 2009b). To our knowledge, mediation analyses have not been completed examining the reverse direction with baseline levels of inflammatory mediators and later depressive symptoms; namely whether inflammatory markers mediate the relationship between baseline lifestyle factors and later depression. This approach may highlight whether lifestyle may be a source of elevated inflammatory markers observed in people with depression.

The current study explores the relationship between inflammatory markers, depressive symptoms and aspects of unhealthy lifestyle (central adiposity, low physical activity, poor diet quality, smoking and alcohol use). Specifically, the first aim is to explore a practical question from a biomarker perspective: whether baseline levels of inflammatory markers – IL-6 and CRP – are associated with levels of depressive symptoms at follow-up, and whether the effects remain after adjusting for the confounding generated by lifestyle. The second aim is to instead examine lifestyle as the predictor of depressive symptom outcomes at follow-up, and explore whether inflammatory markers mediate this relationship.

5.3 Method

Participants

Participants were drawn from the Hunter Community Study, a study of the health of older persons in the large regional centre of Newcastle, New South Wales, Australia (McEvoy et al., 2010; see Appendix 8.1). Briefly, between December 2004 and December 2007, community-dwelling

individuals from the Newcastle region were randomly selected from the Australian electoral roll and invited to participate in the study. 3318 individuals agreed (44.5% participation rate). The gender and marital status of these participants were similar to national Australian profiles, although they were slighter younger. Participants were re-contacted between January and December 2010 with an invitation to complete follow-up questionnaires. By follow-up, the study team was notified of 132 deaths (4%), 169 people actively withdrew (5%) and 767 (23%) were lost to follow-up with unknown reasons, leaving 2250 who completed follow-up questionnaires. Those who completed follow-up were significantly younger, more likely to be married and less likely to be widowed, yet had the same gender profile as those who did not.

Procedures

At baseline, participants completed self-report questionnaires and a face-to-face clinical assessment to gather information regarding health status, functioning and health behaviours (for detail on measures see McEvoy, et al., 2010; Appendix 8.1). 78% of participants provided a serum blood sample for routine blood testing and for storage for future use, which included analysis of CRP and IL-6. At follow-up, participants completed self-report questionnaires with a focus on mental health.

Measures

Inflammatory markers: 12 hour fasting blood was collected (95% were collected in the morning). Samples were centrifuged at 4°C and 3000g for 10mins, and serum was stored at -80°C until analysis. High sensitivity CRP

was analysed via CRP Flex System on Dimension Vista System immunonephelometry (Siemens Healthcare Diagnostics, Newark, DE, USA). The limit of detection was 0.16mg/L and coefficient of variation was 4.8%. High sensitivity IL-6 was analysed via Access IL-6 magnetic bead/chemiluminescent immunoassay (Beckman Coulter, Fullerton, CA, USA, ref A16369), performed on a Beckman DxI. The lower limit of detection was 0.5pg/mL and coefficient of variation was 12%.

Depressive symptoms: Depressive symptoms were measured at baseline and follow-up using the 20-item self-report Centre for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977). It provides a continuous score in the range of 0-60 based on the frequency of depressive symptoms in the past week. A cut-off score of 16 is used as a marker of at least mild and clinically relevant depressive symptomatology and possible depression (100% sensitivity and 88% specificity for major depression) (Beekman et al., 1997). The scale was designed for use in epidemiological studies and has been validated for use in older samples (Beekman, et al., 1997). For participants missing five or fewer items on the scale, values were imputed based on the average of the items completed.

Lifestyle factors: Five factors were selected as indicators of unhealthy lifestyle: adiposity (body mass index or waist-to-hip ratio), steps per day, percentage of energy intake from saturated fat, smoking status, and alcohol misuse. Measures of adiposity were calculated from measurements taken by trained staff during the physical assessment. Average steps per day as an indicator of physical activity was obtained using measurements from a

pedometer worn over a week. Diet quality, including assessment of saturated fat intake, was assessed using a previously validated semi-quantitative food frequency questionnaire (Smith, Mitchell, Reay, Webb, & Harvey, 1998), from which nutrient intakes were determined using a custom-made nutrient analysis programme based on the NUTTAB 2006 database (Food Standards Australia New Zealand, 2006). Saturated fat was selected as it is promoted as a key nutrient to reduce in public health guidelines (National Health and Medical Research Council and Department of Health and Ageing Australian Government, 2005; U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2010; World Health Organization, 2013) and this particular aspect of diet is associated with elevated inflammatory markers (King, Egan, & Geesey, 2003). Smoking status was self-reported. Alcohol use was also self-reported using a modified timeline follow-back method (Cumming & Mitchell, 1997; Skinner, 1982; Sobell et al., 1979). Based on the number of days when alcohol was consumed and number of standard drinks consumed in the last month, we determined whether the participant drank above contemporaneous Australian alcohol guidelines (>4 standard drinks per day for men, >2 standard drinks per day for women) (National Health and Medical Research Council, 2001).

Data analysis

Analyses were conducted using Stata SE/11 (StataCorp LP, USA). Participants who did not complete follow-up surveys ($N = 1067$), were without a blood sample ($N = 734$), or who reported lupus erythematosus or

use of immunosuppressants ($N = 1$) were excluded. Furthermore, as the focus was on community-dwelling participants without acute illness, participants with high concentrations of inflammatory markers were also excluded from analysis as a conservative indicator of probable acute illness (CRP>10mg/L based on previously published guidelines (Clyne & Olshaker, 1999) and IL-6>23pg/L which represented values more than 3 standard deviations above the mean in this sample; $N = 107$). Those excluded from the analyses were significantly older, and were more likely to be female and not married at baseline.

As an alternative approach to using a clinical guideline of probable acute illness to exclude cases, a secondary sensitivity analysis was conducted where a statistical criterion was used to monitor outlying cases. To indicate influential data points, Cook's distance was calculated for key regression analyses between inflammatory markers and follow-up depression outcomes. Cook's distance values above $N/4$ were excluded (for females $N = 32$ for IL-6, $N = 30$ for CRP; for males $N = 22$ for IL-6, $N = 30$ for CRP).

All analyses were performed separately for females and males, as significant interactions between IL-6 and gender were observed in dummy coded regression analyses predicting baseline continuous CES-D score ($t(1301) = 1.96$, $p = .05$), follow-up continuous score ($t(1319) = 2.19$, $p = .03$) and dichotomous score ($Z = 1.94$, $p = .05$). To explore cross sectional relationships, we examined the association between baseline inflammatory markers, depressive symptoms and lifestyle factors, using age-adjusted and

fully lifestyle-adjusted multiple linear regression models with robust standard errors. Besides the standardised correlation coefficients, semi-partial correlations were calculated to demonstrate the unique relationship between the predictor and the outcome. Robust standard errors were used to account for observed heteroscedasticity and non-normality of residuals via inspection of plotted residuals and Breusch-Pagan/Cook-Weisberg tests.

To test the first study aim, we examined whether inflammatory markers and lifestyle factors were associated with follow-up depressive symptoms. Given the substantial correlation between baseline and follow-up measures of psychological symptoms, instead of using follow-up scores as the outcome variable, a residual score was derived for each participant from a regression model of age and baseline CES-D score predicting follow-up CES-D score. Thus, the outcome for these analyses (henceforth referred to as "residual score") represents the variation in follow-up depressive symptoms not explained by baseline depressive symptoms and age. For residual scores, a high value would represent that the participant had a higher than expected score at follow-up, relative to their baseline score and age. We also completed a logistic regression on the outcome of dichotomised high vs. low depressive symptoms at follow-up, based on the established CES-D cut-off score of 16 (Beekman, et al., 1997), excluding people with high depressive symptoms at baseline (CES-D $\geq$16). In both the linear and logistic regression analyses, potential sources of confounding were identified using a directed acyclic graph (Textor, Hardt, & Knüppel, 2011) (Figure 5.1), and adjusted for in multivariate analysis. This directed model indicated the

237
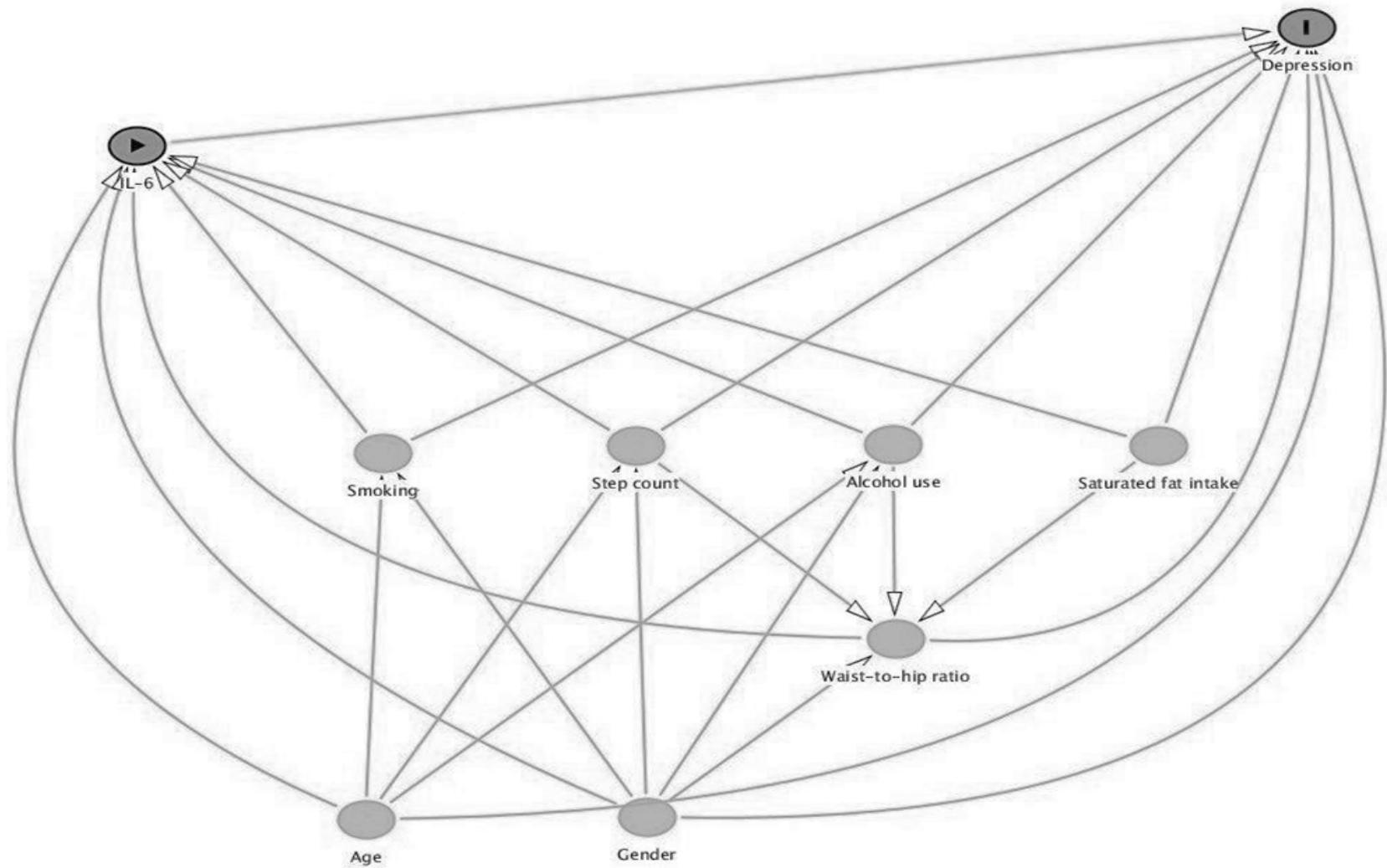
Figure 5.1. Directed acyclic graph demonstrating the relationship between the predictor interleukin (IL)-6, outcome depression and confounding variables age, gender, adiposity (waist-to-hip ratio), smoking, step count, alcohol use, and saturated fat intake.

minimal set of confounders to control for were age, adiposity (waist-to-hip ratio, or body mass index), alcohol use, saturated fat intake, smoking, and physical activity (steps per day). We must acknowledge that it does not represent a complete causal diagram, noting the absence of cognitive/emotional and other physical potential causes of depression in this diagram, due to the constraints of the available dataset.

To explore the second aim regarding whether lifestyle factors may explain the relationship between inflammation and depression, mediation analysis was applied using a model of a lifestyle factor (with significant direct effect $p < .10$) predicting residual CES-D score, mediated via IL-6 or CRP. The lifestyle factors included waist-to-hip ratio, percentage energy from saturated fat, and current smoking. Sobel-Goodman mediation tests were completed and confidence intervals were derived based on Preacher and Hayes (2004) methods, using a bias-corrected bootstrapping approach with case resampling.

5.4 Results

Sample description

The 1410 participants included in these analyses were, at baseline, an average of 65.6 years old ($SD = 7.1$); 771 (50%) were females, 1074 (76%) were married or de facto/living with a partner, and all were living in the community. By follow-up, most were still living in the community, with less than 1% in retirement or hostel facilities. On average at baseline, most participants had few depressive symptoms according to the CES-D ($M = 6.8$,

SD = 7.7, range 0-53). Table 5.1 shows the characteristics for the included participants.

Cross sectional associations between depressive symptoms, lifestyle and inflammatory markers

For females, most aspects of unhealthy lifestyle were significantly associated with IL-6 (Table 5.2; see also Supplementary Table 5.8.1). Only steps per day and current smoking remained significant after adjusting for other lifestyle factors. For males, similar patterns were observed, although only waist-to-hip ratio remained significant after multivariate adjustment. Similar patterns were observed for the association between CRP and lifestyle factors, except that for females, additional factors remained significant after multivariate adjustment (alcohol use, waist-to-hip ratio and steps per day; Table 5.2; Supplementary Table 5.8.2).

Inflammatory markers and most aspects of unhealthy lifestyle were cross-sectionally associated with baseline CES-D scores (Table 5.2; Supplementary Table 5.8.3). For females, IL-6 and waist-to-hip ratio remained significant in the multivariate analysis, with similarly sized independent contributions according to semi-partial correlations of 0.12 and 0.10, respectively. Note that CRP and body mass index were excluded from this multivariate analysis; repeating the multivariate model with CRP instead of IL-6 did not change the pattern of results, and using body mass index instead of waist-to-hip ratio, body mass index was not significant. For males, a largely similar pattern of lifestyle factors was significantly associated with CES-D. In the multivariate analysis, waist-to-hip ratio,

Table 5.1. Baseline characteristics of included participants ($N = 1410$).

Characteristic		Mean (SD)
Age (years)		65.6 (7.1)
Centre for Epidemiologic Studies Depression Scale		6.8 (7.7)
logIL-6		0.6 (0.6)
logCRP		0.6 (0.8)
Waist-to-hip ratio (as %)		89.4 (8.8)
Body mass index		28.5 (4.6)
Steps per day (in thousands)		7.2 (3.1)
% energy from saturated fat		11.5 (3.1)

Characteristic		N (%)
Gender	Male	711 (50.0)
	Female	699 (50.0)
Marital status	Married	1048 (76.0)
	De facto/living with partner	26 (1.9)
	Widowed	132 (9.6)
	Divorced or separated	135 (9.8)
	Never married	37 (2.7)
Depression	Scores ≥ 16	177 (13.4)
	Scores < 16	1146 (86.6)
Current smoking	No	1299 (94.8)
	Yes	71 (5.2)
Alcohol use	No use	236 (17.1)
	Safe use	888 (64.2)
	Hazardous use	139 (10.0)
	Use at unknown quantity	121 (8.7)

Table 5.2. Standardised regression coefficients between baseline lifestyle factors, interleukin(IL)-6, C-reactive protein (CRP), and Centre for Epidemiologic Studies Depression Scale (CES-D), adjusted (adj.) for age, and for age and remaining predictors. Significant results using α = .05 criterion are marked with an asterisk. Further details including unstandardised coefficients and exact p values are included in supplementary files to the chapter (Tables 5.8.1 to 5.8.3).

		Outcomes								
		logIL-6			logCRP			CES-D		
Gender	Predictor variable	Age adj. ß	Lifestyle adj. ß	Semi-partial r	Age adj. ß	Lifestyle adj. ß	Semi-partial r	Age adj. ß	Lifestyle adj. ß	Semi-partial r
Female	logIL-6				0.39*			0.14*	0.13*	0.12*
	logCRP	0.37*						0.13*		
	CES-D	0.14*			0.13*					
	Waist-to-hip ratio (as %)	0.11*	0.06	0.059	0.15*	0.10*	0.10*	0.13*	0.11*	0.10*
	Body mass index	0.27*			0.40*			0.14*		
	Steps per day (in thousands)	-0.24*	-0.22*	-0.21*	-0.21*	-0.19*	-0.19*	-0.03	0.01	0.01
	% energy from saturated fat	0.08*	0.07	0.07	0.08*	0.04	0.05	0.10*	0.07	0.07
	Smoke now (0 no, 1 yes)	0.13*	0.10*	0.10*	0.07	0.05		0.10*	0.07	0.07
	Alcohol use (reference: no use)									
	Safe use	-0.09*	-0.06	-0.05	-0.11*	-0.11	-0.09*	-0.09	-0.03	-0.03
	Hazardous use	-0.01	-0.01	-0.01	0.003	0.02	0.01	0.02	0.01	0.01
	Use at unknown quantity	-0.03	-0.03	-0.03	-0.09*	-0.11	-0.10*	-0.06	-0.03	-0.02

		1	2	3	4	5	6	7	8	9
Male	logIL-6				0.42*			0.04	-0.004	-0.004
	logCRP	0.37*						0.09*		
	CES-D	0.03			0.09					
	Waist-to-hip ratio (as %)	0.18*	0.16*	0.16*	0.21*	0.19*	0.19*	0.13*	0.10*	0.09*
	Body mass index	0.19*			0.33*			0.18*		
	Steps per day (in thousands)	-0.11*	-0.07	-0.07	-0.12*	-0.06	-0.06	-0.12*	-0.09*	-0.08*
	% energy from saturated fat	-0.02	-0.02	-0.02	0.05	0.05	0.05	0.09*	0.09*	0.09*
	Smoke now (0 no, 1 yes)	0.06	0.01	0.01	0.08*	0.06	0.06	0.04	0.04	0.03
	Alcohol use (reference: no use)									
	Safe use	-0.02	-0.03	-0.02	-0.11*	-0.11	-0.07	-0.01	-0.004	0.003
	Hazardous use	0.04	-0.02	-0.02	-0.01	-0.06	-0.04	0.04	0.03	0.02
	Use at unknown quantity	0.01	-0.02	-0.02	0.68	0.01	0.01	0.13*	0.10	0.09

steps per day and energy from saturated fat remained significant, contributing similarly according to semi-partial correlations (all $r \approx 0.08$ or 0.09). Using body mass index instead of waist-to-hip ratio, body mass index was significant, and steps per day and energy from saturated fat were only marginally significant.

Follow-up depressive symptoms and confounding

Fewer factors were significantly associated with CES-D residual scores (i.e., variation in follow-up CES-D not explained by baseline CES-D and age; Table 5.3). For females, significant predictors of residual score were waist-to-hip ratio and energy from saturated fat after multivariate adjustment. Using body mass index instead of waist-to-hip ratio in the lifestyle-adjusted model, energy from saturated fat remained significant. For males, inflammatory markers were no longer significant predictors in the lifestyle-adjusted model (although waist-to-hip ratio approached significance). Adding IL-6 to a multivariate regression model with lifestyle factors did not improve the model for females (R^2 change .002; $F(1,530) = 0.91$, $p = .34$) or males (R^2 change $< .001$; $F(1,542) = 0.001$, $p = .98$). We also examined whether inflammatory markers were associated with dichotomous depression at follow-up, excluding participants with high depressive symptoms at baseline (CES-D ≥ 16; Table 5.4). There were no significant effects in the multivariate lifestyle-adjusted model for females or males.

For the sensitivity analysis using a statistical criterion for outliers, the pattern of results remained the same as in the final analyses included in Table 5.3: for females, significant association between logIL-6 and

Table 5.3. Association of baseline inflamatory markers and baseline lifestyle factors, with the continous outcome of residual scores, which represent the variation in follow-up Centre for Epidemiologic Studies Depression Scale (CES-D) scores not explained by baseline CES-D score and age.

| Gender | Predictor variable | Residuals | | | | Multivariate adjusted residuals* | | | | Semi-partial |
		b	SE	β	p	b	SE	β	p	r
Female	logIL-6	1.18	0.52	0.09	.024	0.57	0.58	0.04	.330	0.04
	logCRP	-0.31	0.42	-0.03	.469					
	Waist-to-hip ratio (as %)	0.13	0.05	0.10	.009	0.12	0.05	0.09	.028	0.09
	Body mass index	0.03	0.06	0.02	.609					
	Steps per day (in thousands)	-0.04	0.12	-0.01	.739	0.09	0.13	0.03	.510	0.03
	% energy from saturated fat	0.33	0.11	0.12	.002	0.29	0.11	0.11	.012	0.10
	Smoke now (0 no, 1 yes)	4.29	2.16	0.11	.048	3.53	2.17	0.10	.104	0.10
	Alcohol use (reference: no use)									
	Safe use	-1.09	0.89	-0.06	.224	-1.63	0.99	-0.09	.101	-0.08
	Hazardous use	1.06	2.77	0.02	.703	-0.83	2.77	-0.02	.764	-0.02
	Use at unknown quantity	1.93	1.65	0.06	.241	0.30	1.81	0.01	.870	0.01
Male	logIL-6	0.18	0.42	0.01	.675	0.01	0.45	0.001	.979	0.001
	logCRP	0.59	0.41	0.06	.144					
	Waist-to-hip ratio (as %)	0.09	0.04	0.07	.026	0.09	0.05	0.07	.077	0.06
	Body mass index	0.14	0.08	0.07	.070					
	Steps per day (in thousands)	-0.04	0.08	-0.02	.631	-0.03	0.09	-0.01	.729	-0.01
	% energy from saturated fat	-0.04	0.08	-0.02	.660	-0.01	0.09	-0.01	.892	-0.01
	Smoke now (0 no, 1 yes)	0.77	1.18	0.02	.515	0.79	1.29	0.02	.541	0.02
	Alcohol use (reference: no use)									
	Safe use	-0.02	0.85	-0.002	.978	0.64	0.65	0.04	.327	0.03
	Hazardous use	-0.12	1.02	-0.006	.910	-0.10	0.89	-0.01	.907	0.004
	Use at unknown quantity	0.11	1.18	0.004	.924	0.36	1.11	0.01	.748	0.01

Lifestyle-adjusted multivariate Females: $F(9,539) = 2.57$, $p = .009$; $R^2 = .0458$; Males $F(8,542) = 0.72$, $p = .674$, $R^2 = .0068$.

*Excluding CRP and body mass index, as they are measuring similar latent variable as IL-6 and waist-to-hip ratio, respectively. For females, results were the same pattern when CRP ($p = .207$) was used instead of IL-6, although when body mass index was used, body mass index was not a significant predictor ($p = .577$), while energy from saturated fat remained significant ($p = .011$). For males, using CRP ($p = .370$) instead of IL-6, or body mass index ($p = .195$) instead of waist-to-hip ratio, did not change the pattern of results.

Table 5.4. Association of baseline inflamatory markers and baseline lifestyle factors, with dichtomous outcome of depression or no depression (Centre for Epidemiologic Studies Depression Scale [CES-D] scores ≥16 and <16, respectively), excluding people with CES-D≥16 at baseline.

| Gender | Predictor variable | Age adjusted | | | | Age and lifestyle adjusted* | | | |
		OR	95% CI		p	OR	95% CI		p
Female	logIL-6	**1.87**	**1.24**	**2.81**	**.003**	1.57	0.98	2.52	.062
	logCRP	0.96	0.69	1.32	.793				
	Waist-to-hip ratio (as %)	1.03	1.00	1.07	.075	1.02	0.98	1.06	.285
	Body mass index	1.03	0.98	1.09	.186				
	Steps per day (in thousands)	0.99	0.89	1.09	.798	1.03	0.92	1.15	.601
	% energy from saturated fat	1.07	0.98	1.16	.135	1.07	0.98	1.17	.132
	Smoke now (0 no, 1 yes)	**2.88**	**1.09**	**7.60**	**.033**	2.11	0.71	6.29	.179
	Alcohol use (reference: no use)								
	Safe use	0.70	0.37	1.32	.271	0.58	0.29	1.16	.125
	Hazardous use	1.03	0.21	5.09	.974	0.44	0.05	3.75	.452
	Use at unknown quantity	1.50	0.63	3.61	.362	1.10	0.39	3.11	.851
Male	logIL-6	0.82	0.48	1.38	.452	0.76	0.41	1.39	.376
	logCRP	1.38	0.94	2.03	.098				
	Waist-to-hip ratio (as %)	1.04	0.99	1.10	.106	1.06	0.99	1.13	.090
	Body mass index	1.05	0.98	1.13	.194				
	Steps per day (in thousands)	0.98	0.88	1.09	.732	0.99	0.89	1.12	.929
	% energy from saturated fat	0.98	0.89	1.08	.720	1.00	0.90	1.11	.952
	Smoke now (0 no, 1 yes)	2.29	0.74	7.05	.149	2.34	0.63	8.65	.201
	Alcohol use (reference: no use)								
	Safe use	0.92	0.40	2.10	.842	1.28	0.46	3.55	.631
	Hazardous use	0.88	0.30	2.62	.824	0.77	0.20	2.96	.709
	Use at unknown quantity	0.54	0.11	2.68	.449	0.84	0.15	4.72	.842

Lifestyle-adjusted multivariate Females: $\chi^2(10) = 13.96$, $p = .124$; pseudo $R^2 = .044$, area under ROC curve = .62; Males $\chi^2(10) = 8.18$, $p = .52$, pseudo $R^2 = .030$, area under ROC curve = .62.

*Excluding CRP and body mass index, as they are measuring similar latent variable as IL-6 and waist-to-hip ratio, respectively. For females, when CRP was used instead of IL-6, CRP was not a significant predictor ($p = .409$). When body mass index was used, body mass index was not a significant predictor ($p = .457$), and IL-6 was marginally associated with depression ($p = .087$). For males, the pattern of results did not change when using CRP ($p = .215$) instead of IL-6, or body mass index ($p = .146$) instead of waist-to-hip ratio.

depression residuals (b = 0.61, SE = 0.31, t = 1.98, p = .048) and not

significant for logCRP and depression residuals; and for males, non-

significant associations between logCRP/logIL-6 and depression residuals.

Inflammatory markers as mediators of the lifestyle to depression

relationship

We examined whether IL-6 acts as a mediator between several

lifestyle factors and residual depression scores (Table 5.5). For females, IL-6

acted as a significant mediator of the relationship between waist-to-hip ratio

and current smoking and residual CES-D score, with small but significant

indirect effects observed. For males, IL-6 was not a significant mediator of

relationships between lifestyle and residual score. However, the direct

effects were also not significant, indicating a poor model predicting

depression for males. There were no significant indirect effects of CRP as a

mediator between lifestyle and residual score for males and females.

5.5 Discussion

The current study sought to examine whether baseline levels of

inflammatory markers and aspects of unhealthy lifestyle predict later

depression outcomes. The strongest effects observed were that unhealthy

lifestyle factors drive depression both directly and indirectly via

inflammatory mediators. These effects were observed for females only,

indicating evidence of a gender difference in the relationship between

inflammatory markers, lifestyle and depression.

Table 5.5. Mediation results examining either interleukin (IL)-6 or C-reactive protein (CRP) as a mediator of the relationship between lifestyle factors and the residual score predicting follow-up depressive symptoms

Gender	Mediator	Predictor variable	Direct effect coefficient	Boostrapped 95% CI (bias corrected)		Indirect effect coefficient	Boostrapped 95% CI (bias corrected)		Ratio of effect (indirect: direct)
Females	IL-6	Waist-to-hip ratio	0.1163	0.0087	0.2185	0.0124	0.0007	0.0320	0.1066
		% energy from saturated fat	0.3193	0.1158	0.5488	0.0117	-0.0006	0.0450	0.0366
		Smoke now (0 no, 1 yes)	4.0399	0.3173	9.1437	0.2492	0.0014	0.7612	0.0617
	CRP	Waist-to-hip ratio	0.1374	0.0379	0.2465	-0.0087	-0.0281	0.0053	0.0633
		% energy from saturated fat	0.3334	0.1195	0.5471	-0.0024	-0.0261	0.0075	0.0072
		Smoke now (0 no, 1 yes)	4.3750	0.8317	9.2473	-0.0858	-0.5190	0.0608	0.0196
Males	IL-6	Waist-to-hip ratio	0.0940	0.0028	0.1789	0.0005	-0.0195	0.0181	0.0053
		% energy from saturated fat	-0.0369	-0.1977	0.1330	0.0000	-0.0081	0.0085	0.0003
		Smoke now (0 no, 1 yes)	0.7753	-1.5128	3.1275	-0.0064	-0.1914	0.0482	0.0083
	CRP	Waist-to-hip ratio	0.0813	-0.0027	0.1627	0.0131	-0.0072	0.0406	0.1611
		% energy from saturated fat	-0.0477	-0.2027	0.0993	0.0108	-0.0011	0.0440	0.2264
		Smoke now (0 no, 1 yes)	0.6951	-1.3758	3.3753	0.0739	-0.0476	0.4242	0.1063

This study replicates the clear cross-sectional inter-relationship between inflammatory markers, lifestyle and depression. We replicated previous observations of a cross-sectional logarithmic relationship between IL-6 (and CRP) and depressive symptoms in females, an effect which remained after controlling for aspects of unhealthy lifestyle. For males, the cross sectional relationship between IL-6/CRP and depressive symptoms were not significant after multivariate lifestyle adjustment. This may indicate that there are differences in contribution of inflammation to the experience of depression in males and females. Estrogen is generally anti-inflammatory, and thus, one possibility is that low levels of estrogen in these post-menopausal women may make them more prone to depression via inflammatory states (Straub, 2007). The sex differences observed in neuroendocrine stress responses may also contribute (Kudielka & Kirschbaum, 2005). There is other evidence of sexual dimorphism in relationships between inflammatory markers and health and lifestyle, for instance women show a greater increase in IL-6 after a high fat meal than men (Payette et al., 2009), women have a stronger association between adiposity and inflammatory markers (Thorand et al., 2006) and inflammatory markers have been related to self-rated health in women only (Lekander, Elofsson, Neve, Hansson, & Undén, 2004). Thus, gender seems to be an important effect modifier in the depression-inflammation relationship, particularly considering the contribution of lifestyle.

The current study also sought to explore several unanswered questions regarding prospective evidence for the inflammatory hypothesis of

depression: namely whether inflammatory markers have the potential to be useful biomarkers of later depression onset, and whether lifestyle factors may be important potential sources of elevated inflammatory markers. Regarding follow-up depressive symptoms, in females, but not males, IL-6 was a significant predictor of dichotomous high vs. low depressive symptoms and the residual variance in follow-up depressive symptom scores remaining after accounting for baseline scores and age. However, these effects were not significant after adjusting for lifestyle, which is consistent with previous studies (Duivis, de Jonge, Penninx, Ya Na, et al., 2011). As the association between IL-6 and depression outcomes were no longer significant after adjusting for lifestyle, it is unlikely that major drivers of inflammatory mediators were overlooked in the directed acyclic graph (Figure 5.1). It appears that unhealthy lifestyle factors are potentially confounders or mediators in the association between inflammatory markers and depression.

A noteworthy outcome in this study is that, for females, IL-6 mediates part of the relationship between several lifestyle factors and later depression. Previous prospective studies have made similar conclusions, including that CRP only partly explains the association between physical activity and risk of depression (Hamer, et al., 2009a) and that a healthy diet may minimise the effect of depression on inflammatory markers (Milaneschi, et al., 2009). The significant mediation effects observed in this study for measures of adiposity are particularly informative, given that waist-to-hip ratio endures as a significant predictor of residual CES-D scores after adjusting for other lifestyle factors. Inflammatory mediators

induced by hypertrophic, rather than lean, adipocytes (and excess nutrient intake) are already suggested to be involved in the pathogenesis of aspects of the metabolic syndrome, such as insulin resistance (Calay & Hotamisligil, 2013; Odegaard & Chawla, 2013). This concept of pathogenesis resulting from "cold inflammation", or subacute inflammation, may extend to depression.

Nevertheless, the observed mediation effects observed in this study were small. Other social, psychological and biological effects are likely to be involved as mediators of the lifestyle-to-depression relationship, and also as sources of elevated inflammatory markers. For instance, psychosocial stress may be a key source of subacute inflammation (Raison & Miller, 2013), and social and emotional factors may also need to be taken into account.

The lack of association between physical activity and depression in this study is surprising, given that this relationship has been observed in previous studies (Azevedo Da Silva et al., 2012; Hamer, et al., 2009a; Song, Lee, Baek, & Miller, 2012). In the current study, physical activity was measured via pedometer. Although this is a good measure of walking compared with sedentary activity, which are critical and effective indicators of physical health (Ewald, McEvoy, & Attia, 2010), the pedometer does not capture the intensity of physical activity. It may be that intensity of physical activity is more closely related to depression.

This study must be viewed in light of several limitations. Depression was not defined through clinical interview, only through depressive symptom inventory, which may mean the effect sizes underestimate the

true effect, as suggested by meta-analysis of cross sectional studies comparing effect sizes for diagnosed depression and elevated depressive symptoms (Hiles, et al., 2012). Consequently, we also did not have the ability to examine subtypes of depression, although research indicates that melancholic vs. atypical depression are associated with differential inflammatory and immune profiles (Hickman, Khambaty, & Stewart, 2013; Kaestner et al., 2005; Rothermundt et al., 2001). There were also some missing data and participants lost to follow-up in this study. Blood was only collected from participants able to attend a clinic session within office hours, which may have led to a selective participant base of those who were not full-time employees or carers and were well enough to attend a clinic. This was a community-dwelling sample, and so does not represent institutionalised people, who have a high prevalence of mental and physical illness.

We must also acknowledge obesity is not a true lifestyle indicator in the same way as the other factors represent behaviour, although obesity is still an important indicator of health behaviour. Obesity is in part influenced by lifestyle, but also other factors such as genetics, endocrinology or other pathophysiology. It is possible that adjusting for obesity may have resulted in overadjustment; that is, controlling for this variable is unnecessary and therefore increases bias or decreases precision. In an attempt to minimise this risk, we completed a directed acyclic graph, where obesity created a unique path from exposure to outcome. This model indicated that obesity should be independently adjusted for. Ultimately, our

directed acyclic graph is a simplification and we suspect that in reality, there are likely to be bi-directional relationships between factors in our model. We selected the directions of relationships that had the strongest evidence. Based on the results of Chapter 4 (Hiles, Baker, Handley, de Malmanche, & Attia, under review), another potential factor to adjust for may be statin use. Preliminary evidence suggests that statins may have anti-inflammatory and antidepressant effects, however this literature has not been well established and the observed effects in the meta-analysis were small. If these results are robust after further study, it may be important to explore statin use in similar research questions in the future.

Our analyses have strengths over previous prospective studies on the depression-inflammatory marker relationship, particularly in the application of mediation analysis to identify central adiposity as one important potential source of inflammatory mediators. Furthermore, these results highlight that explorations of the inflammatory hypothesis of depression need to consider that gender may act as an effect modifier, and that the theory may need to account for gender differences.

The evidence from the current study that, in females, inflammatory markers precede depression and that adipose tissue may be one source of inflammation has consequences for the prevention and treatment of depression. For females in particular, it highlights unhealthy lifestyle as an important intervention target for the prevention and treatment for depression, through improvement of inflammatory pathways. Indeed, these results may extend to other aspects of psychopathology, as elevations in

inflammatory markers may be a feature of numerous psychopathologies, not simply depression, including anxiety (Copeland, Shanahan, Worthman, Angold, & Costello, 2012; Vogelzangs, Beekman, de Jonge, & Penninx, 2013), bipolar disorder (Modabbernia, Taslimi, Brietzke, & Ashrafi, 2013), psychosis (Miller, Buckley, Seabolt, Mellor, & Kirkpatrick, 2011), and suicidal ideation (O'Donovan et al., 2013). Future epidemiological studies which measure inflammatory markers, unhealthy lifestyle indicators and depression at multiple time-points are required to interpret the temporality and mediation between these factors.

5.6 References

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5.7 Supplementary tables

Supplementary table 5.7.1. Association between lifestyle factors and interleukin (IL)-6; adjusted for age only, and age and lifestyle.

Supplementary table 5.7.2. Association of lifestyle factors with C-reactive protein (CRP); adjusted for age only, and age and lifestyle.

Supplementary table 5.7.3. Association of baseline inflammatory markers and lifestyle factors with baseline depressive symptoms (Centre for Epidemiologic Studies Depression Scale; CES-D); adjusted for age only, and age and lifestyle.

Supplementary table 5.7.1. Association between lifestyle factors and interleukin (IL)-6; adjusted for age only, and age and lifestyle.

Gender	Predictor variable	Adjusted for age				Adjusted for age and lifestyle*				
		b	Robust SE	β	p	b	Robust SE	β	p	Semi-partial r
Female	CES-D	0.011	0.003	0.135	<.001					
	CRP	0.287	0.027	0.374	<.001					
	Waist-to-hip ratio (as %)	0.011	0.004	0.113	.003	0.006	0.004	0.061	.110	0.059
	Body mass index	0.033	0.004	0.271	.000					
	Steps per day (in thousands)	-0.049	0.008	-0.240	.000	-0.046	0.009	-0.222	.000	-0.205
	% energy from saturated fat	0.015	0.007	0.076	.040	0.014	0.008	0.072	.063	0.071
	Smoke now (0 no, 1 yes)	0.351	0.117	0.125	.003	0.285	0.120	0.104	.018	0.102
	Alcohol use (reference: no use)									
	Safe use	-0.118	0.060	-0.089	.049	-0.082	0.062	-0.061	.184	-0.051
	Hazardous use	-0.034	0.122	-0.010	.779	-0.026	0.127	-0.007	.838	-0.007
	Use at unknown quantity	-0.072	0.092	-0.034	.433	-0.065	0.099	-0.028	.515	-0.025
Male	CES-D	0.003	0.003	0.031	.393					
	CRP	0.293	0.027	0.369	.000					
	Waist-to-hip ratio (as %)	0.019	0.004	0.180	.000	0.018	0.004	0.164	<.001	0.160
	Body mass index	0.029	0.005	0.188	.000					
	Steps per day (in thousands)	-0.021	0.007	-0.113	.003	-0.013	0.008	-0.072	.079	-0.066
	% energy from saturated fat	-0.005	0.007	-0.024	.506	-0.004	0.008	-0.019	.612	-0.019
	Smoke now (0 no, 1 yes)	0.157	0.095	0.055	.098	0.036	0.097	0.013	.708	0.012
	Alcohol use (reference: no use)									
	Safe use	-0.023	0.064	-0.018	.726	-0.037	0.070	-0.029	.598	-0.019
	Hazardous use	0.071	0.080	0.043	.374	-0.039	0.084	-0.024	.643	-0.017
	Use at unknown quantity	0.025	0.100	0.011	.806	-0.052	0.107	-0.022	.628	-0.018

Lifestyle adjusted multivariate, females: $F_{(8,603)} = 9.67$, $p < .001$, $R^2 = .1253$; males: $F_{(8, 597)} = 13.27$, $p < .001$; $R^2 = .133$.

*Excluding CRP and body mass index, as they are measuring similar latent variables as IL-6 and waist-to-hip ratio, respectively. For both females and males, using body mass index instead of waist-to-hip ratio did not change the pattern of results, except that body mass index was a significant predictor ($p < .001$).

Supplementary table 5.7.2. Association of lifestyle factors with C-reactive protein (CRP); adjusted for age only, and age and lifestyle.

Gender	Predictor variable	Adjusted for age				Adjusted for age and lifestyle*				Semi-partial r
		b	Robust SE	β	p	b	Robust SE	β	p	
Female	CES-D	0.014	0.004	0.130	.001					
	IL-6	0.508	0.051	0.390	.000					
	Waist-to-hip ratio (as %)	0.018	0.006	0.147	.002	0.012	0.006	0.097	.044	0.096
	Body mass index	0.063	0.006	0.398	.000					
	Steps per day (in thousands)	-0.057	0.011	-0.211	.000	-0.051	0.012	-0.189	.000	-0.190
	% energy from saturated fat	0.020	0.010	0.079	.034	0.011	0.010	0.044	.249	0.047
	Smoke now (0 no, 1 yes)	0.242	0.137	0.066	.078	0.175	0.012	0.049	.219	
	Alcohol use (reference: no use)									
	Safe use	-0.192	0.076	-0.112	.012	-0.189	0.079	-0.108	.017	-0.089
	Hazardous use	0.016	0.157	0.003	.918	0.070	0.163	0.015	.666	0.014
	Use at unknown quantity	-0.254	0.112	-0.092	.024	-0.294	0.131	-0.110	.024	-0.096
Male	CES-D	0.009	0.004	0.085	.059					
	IL-6	0.534	0.054	0.424	.000					
	Waist-to-hip ratio (as %)	0.029	0.005	0.213	.000	0.026	0.005	0.192	.000	0.185
	Body mass index	0.065	0.007	0.334	.000					
	Steps per day (in thousands)	-0.028	0.010	-0.117	.006	-0.015	0.010	-0.064	.141	-0.060
	% energy from saturated fat	0.012	0.010	0.049	.208	0.010	0.010	0.049	.220	0.050
	Smoke now (0 no, 1 yes)	0.266	0.132	0.076	.045	0.215	0.145	0.058	.139	0.059
	Alcohol use (reference: no use)									
	Safe use	-0.173	0.085	-0.108	.042	-0.173	0.091	-0.107	.058	-0.073
	Hazardous use	-0.023	0.109	-0.011	.830	-0.122	0.117	-0.060	.295	-0.042
	Use at unknown quantity	0.053	0.129	0.681	.681	0.035	0.141	0.011	.805	0.010

Lifestyle adjusted multivariate females, $F_{(8,612)} = 5.81$, $p < .001$, $R^2 = .072$; males $F_{(8,597)} = 5.06$, $p < .001$, $R^2 = .063$.

*Excluding CRP and body mass index, as they are measuring similar latent variables as IL-6 and waist-to-hip ratio, respectively.

For females, using body mass index instead of waist-to-hip ratio lead to a different pattern of results, where only body mass index

($p < .001$) and alcohol use at an unknown quantity ($p = .023$) were significant predictors. For males, adding body mass index instead of waist-to-hip ratio, body mass index was significant ($p < .001$), with no other significant predictors.

Supplementary table 5.7.3. Association of baseline inflammatory markers and lifestyle factors with baseline depressive symptoms (Centre for Epidemiologic Studies Depression Scale; CES-D); adjusted for age only, and age and lifestyle.

Gender	Predictor variable	Adjusted for age				Adjusted for age and lifestyle*				Semi-partial
		b	Robust SE	ß	p	b	Robust SE	ß	p	r
Female	logIL-6	1.712	0.480	0.143	.000	1.523	0.517	0.129	.003	0.120
	logCRP	1.199	0.396	0.130	.003					
	Waist-to-hip ratio (as %)	0.148	0.052	0.129	.005	0.119	0.058	0.105	.039	0.101
	Body mass index	0.193	0.071	0.135	.007					
	Steps per day (in thousands)	-0.084	0.114	-0.034	.463	0.025	0.118	0.010	.831	0.009
	% energy from saturated fat	0.231	0.113	0.097	.041	0.165	0.115	0.070	.152	0.069
	Smoke now (0 no, 1 yes)	3.280	1.500	0.101	.029	2.243	1.569	0.071	.153	0.069
	Alcohol use (reference: no use)									
	Safe use	-1.071	0.771	-0.068	.165	-0.496	0.787	-0.031	.529	-0.026
	Hazardous use	0.896	1.555	0.020	.565	0.566	1.552	0.013	.716	0.012
	Use at unknown quantity	-1.446	1.092	-0.056	.186	-0.760	1.361	-0.027	.577	-0.024
Male	logIL-6	0.448	0.525	0.036	.393	-0.052	0.528	-0.004	.922	-0.004
	logCRP	0.843	0.428	0.085	.049					
	Waist-to-hip ratio (as %)	0.182	0.057	0.134	.001	0.133	0.063	0.097	.034	0.092
	Body mass index	0.352	0.087	0.178	.000					
	Steps per day (in thousands)	-0.293	0.100	-0.123	.003	-0.212	0.099	-0.090	.033	-0.082
	% energy from saturated fat	0.208	0.095	0.085	.029	0.219	0.103	0.086	.033	0.085
	Smoke now (0 no, 1 yes)	1.294	1.357	0.036	.341	1.279	1.574	0.035	.417	0.034
	Alcohol use (reference: no use)									
	Safe use	-0.234	0.838	-0.014	.780	-0.072	0.889	-0.004	.935	0.003
	Hazardous use	0.728	1.091	0.035	.505	0.064	1.200	0.031	.594	0.021
	Use at unknown quantity	3.969	1.750	0.126	.024	3.242	1.800	0.102	.072	0.086

Lifestyle adjusted multivariate females: $F_{(9,558)} = 2.69$, $p = .005$, $R^2 = .049$; males: $F_{(9,574)} = 2.82$, $p = .002$, $R^2 = .044$.

*Excluding CRP and body mass index, as they are measuring similar latent variables as IL-6 and waist-to-hip ratio, respectively. For females,using body mass index instead of waist-to-hip ratio, body mass index was not significant ($p = .118$), but the pattern remained the same. Using CRP ($p = .030$) instead of IL-6 also did not change the pattern of results. For males, using body mass index instead of waist-to-hip ratio, body mass index ($p = .003$) and energy from saturated fat ($p = .043$) were significant, and steps per day ($p = .073$) was only marginally significant. Using CRP ($p = .584$) instead of IL-6 did not change the pattern of results.

6. Inflammatory markers as mediators of the association between depression and cardiovascular events

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6.1 Abstract

Objective: Depression is a risk factor for cardiovascular events, and inflammation may partly explain this relationship. The aim of this study was to examine whether inflammation may mediate the relationship between depression and incident cardiovascular hospitalisations.

Methods: Community-dwelling individuals aged 55-85 completed questionnaires, including depressive symptom assessment, and were assessed for physical health, including measurement of pro-inflammatory markers C-reactive protein (CRP) and interleukin-6 (IL-6) in the blood. Participants without self-reported cardiovascular events were followed prospectively for hospital admissions for angina, myocardial infarction and cerebral infarction for a median 937 days. Natural mediation effects were calculated using the mediators of CRP, IL-6, body mass index (BMI) and waist-to-hip ratio (WHR).

Results: Across 5140 person-years of risk ($N = 1692$), there were 47 incident cardiovascular hospitalisations (2.8%). Controlling for age and gender, depressive symptoms (HR = 1.03, 95%CI 1.00, 1.07; HR represents a one unit increase in depressive symptom score), CRP (HR = 1.47, 95%CI 1.02, 2.13), IL-6 (HR = 1.72, 95%CI 1.08, 2.72), BMI (HR = 1.06, 95%CI 1.00, 1.14) and WHR percentage (HR = 1.05, 95%CI 1.01, 1.10) were associated with future cardiovascular events. Mediation analysis showed that CRP accounted for 8.1% and IL-6 10.9% of the effect of depression on cardiovascular events. These were meaningful mediated effects; natural indirect effects through the inflammatory

277

markers were significant whereas the natural direct effects were no longer significant. BMI and WHR accounted for indirect effects of 7.7% and 10.4%, respectively.

Conclusions: Inflammatory markers partly mediate the association between depression and cardiovascular events, although other shared factors are also likely to contribute to the depression-cardiovascular disease relationship. Nevertheless, the management of inflammation may be one way to improve both depressive symptoms and cardiovascular event risk.

6.2 Introduction

Depression and cardiovascular disease have substantial disease burden globally (World Health Organization, 2008). Not only are they common separately (prevalence 0.8-9.6% for 12-month mood disorders, 5.4-26.4% for heart disease, and 1.8-6.6% for stroke worldwide), they frequently co-occur (Australian Institute of Health and Welfare, 2008; Laslett et al., 2012; The WHO World Mental Health Survey Consortium, 2004). People with depression are at higher risk of developing cardiovascular disease and *vice versa* (Aben et al., 2003; Gallagher et al., 2012; Thombs et al., 2006; Van der Kooy et al., 2007) and depression is a predictor of cardiovascular mortality (Surtees, Wainwright, Luben, et al., 2008). Individuals with both disorders concurrently have a substantially greater disease burden than either alone, with high rates of disability and complications (González & Tarraf, 2013; Rudisch & Nemeroff, 2003).

While there is much evidence that depression poses a risk for the development of cardiovascular diseases and events, the mechanism of this relationship remains unclear. Some suggested mechanisms are behavioural, for instance depression leading to reduced adherence to cardiovascular medications, or depression leading to increased unhealthy behaviours which increase fat mass and consequently the risk of cardiovascular disease (Nemeroff & Goldschmidt-Clermont, 2012). However, there is also a strong

argument for a shared underlying biological pathway for depression and cardiovascular disease in inflammation.

Inflammation is closely related to both depression and cardiovascular disease. Firstly, pro-inflammatory markers are elevated in people with depression and may predict the development of depressive disorders (Dowlati et al., 2010; Hiles, Baker, de Malmanche, & Attia, 2012b; Howren, Lamkin, & Suls, 2009; Matthews et al., 2010; Pasco, Nicholson, et al., 2010). Prolonged elevations of inflammatory mediators are associated with changes to some of the hallmark biological features of depression including neuroendocrine stress activity, neurotransmitter activity, neurodegeneration and oxidative stress (Miller, Maletic, & Raison, 2009). Secondly, pro-inflammatory markers are elevated in people with cardiovascular disease (Pearson et al., 2003) and predict risk of future cardiovascular disease (Danesh et al., 2008). Inflammation is implicated in the cause of atherosclerosis, involved in the formation and destabilisation of plaques (Hansson, 2005; Libby, Ridker, & Maseri, 2002). Furthermore, recent evidence indicates that acute immune challenge transiently diminishes mood and modulates risk factors for cardiovascular disease including increasing diastolic blood pressure and changing heart rate variability via changes to the central autonomic system (Harrison, Cooper, Voon, Miles, & Critchley, 2013). Many of the unhealthy behaviours observed in people with depression and cardiovascular events – such as increased body mass index (BMI) or high waist-to-hip ratio (WHR),

reduced physical activity, excessive alcohol intake, smoking and poor diet quality – also contribute to chronic inflammation (Bonnet et al., 2005; Hamer, Molloy, de Oliveira, & Demakakos, 2009; O'Connor et al., 2009). In particular, adipose tissue stimulates the release of inflammatory mediators (Miller, Freedland, Carney, Stetler, & Banks, 2003; Shelton & Miller, 2010).

C-reactive protein (CRP), a broad marker of inflammation, has been identified as a predictor of both depression and cardiovascular disease, including stroke (Kuo et al., 2005; Matthews, et al., 2010; Ridker, Rifai, Rose, Buring, & Cook, 2002). CRP is released by the liver and promotes a pro-inflammatory response (Black, Kushner, & Samols, 2004). Levels of CRP spike during acute inflammation and low-grade elevations are observed in several chronic diseases, including cardiovascular disease and depression (Black, et al., 2004). Chronic elevation of CRP has been suggested as a diagnostic predictor of cardiovascular risk, independent of previously identified factors such as high low-density lipoprotein cholesterol and high blood pressure (Ridker, 2007). Another pro-inflammatory marker particularly reliably associated with depression is interleukin (IL)-6 (Dowlati, et al., 2010; Hiles, et al., 2012b). The prospective studies and meta-analysis of Danesh et al. (2008) highlight that, like CRP, IL-6 is also associated with later development of coronary heart disease. CRP and IL-6 are relatively easily and inexpensively measured compared with other inflammatory markers, and make a good assessment of broad inflammatory status.

Although separate studies have identified CRP (Ridker, 2007), IL-6 (Danesh, et al., 2008), and depressive symptoms (Van der Kooy, et al., 2007) as prospective predictors of cardiovascular events, few have examined depression and inflammatory markers simultaneously and prospectively in general community samples. Doing so allows the investigation of whether inflammation accounts for the observed relationship between depression and cardiovascular disease. The previous studies in community samples free of existing cardiovascular disease and/or events generally conclude that inflammatory markers and depression independently predict cardiovascular events, including coronary events (Davidson et al., 2009; Empana et al., 2005), cerebrovascular events (Arbelaez, Ariyo, Crum, Fried, & Ford, 2007) and mixed cardiovascular outcomes (Hamer, Molloy, & Stamatakis, 2008; Surtees, Wainwright, Boekholdt, et al., 2008). However, some studies also indicate there is a synergy between inflammation and depression, for instance observing that CRP is only a key predictor of events in those with depressive symptoms compared to those without depressive symptoms (Ladwig, Marten-Mittag, Lowel, Doring, & Koenig, 2005), or observing the strongest association between depression and coronary heart disease for those participants in the lowest quartile of CRP (Surtees, Wainwright, Boekholdt, et al., 2008). Similar research conducted in samples with existing cardiovascular disease draw similar conclusions of an independence between inflammatory markers and cardiovascular events (Frazier, Vaughn, Willerson, Ballantyne, & Boerwinkle,

2009; Rallidis et al., 2011; Vaccarino et al., 2007; Whooley et al., 2008). Again, there is some evidence for an interaction between inflammatory markers and depression, with those with both low depressive symptoms and low CRP at low risk of further events, and either high CRP or depressive symptoms at similarly increased risk of events (Frasure-Smith et al., 2007). In these previous studies, the conclusion of independence is based on observations of only a small change in effect sizes after adjusting for inflammatory markers in the association between depression and a further cardiovascular event. Previous studies have not estimated the size and significance of the indirect effect of depression on cardiovascular disease through inflammatory markers using mediation analysis.

The current study investigates whether the observed reliable association between inflammation and depression explains the relationship between depression and cardiovascular hospitalisations. Specifically, the aim was to investigate how much of the effect of depression on incident cardiovascular hospitalisation is mediated through inflammation, as measured by the inflammatory markers CRP or IL-6. We then compared these effects to those obtained with BMI or WHR as a mediator of the relationship.

6.3 Method

Participants

Participants were drawn from the Hunter Community Study – a study of the health of older persons in the regional city of Newcastle, Australia (for details see McEvoy et al., 2010; Appendix 8.1). Briefly, between December 2004 and December 2007, community-dwelling individuals aged 55-85 years from the Newcastle area were randomly selected from the Australian electoral roll and invited to participate in the study. 3318 individuals agreed (45%). The gender and marital status of the participants were similar to national Australian profiles, although slightly younger.

Procedures

Participants completed self-report questionnaires and a face-to-face clinical assessment regarding health status, functioning, and health behaviours (see McEvoy, et al., 2010 for details on measures; Appendix 8.1). 2555 (78%) of participants provided a serum blood sample for routine blood testing and for storage for future use. 2762 participants (83%) also consented to provide linkage with state hospital admissions records via the Centre for Health Record Linkage, which provided prospective surveillance data to the end of 2009.

Measures

Inflammatory markers: 12 hours fasting blood was collected, 95% in the morning. Samples were centrifuged at 4°C and 3000g for 10mins, and serum

was stored at -80°C until analysis. High sensitivity CRP was analysed via CRP Flex System on Dimension Vista System immunonephelometry (Siemens Healthcare Diagnostics, Newark, DE, USA). The limit of detection was 0.16mg/L and coefficient of variation was 4.8%. High sensitivity IL-6 was analysed via Access IL-6 magnetic bead/chemiluminescent immunoassay (Beckman Coulter, Fullerton, CA, USA, ref A16369), performed on a Beckman DxI. The lower limit of detection was 0.5pg/mL and coefficient of variation was 12%.

Depressive symptoms: Depressive symptoms were measured via the 20-item self-report Centre for Epidemiologic Studies Depression Scale (CES-D; Radloff, 1977). It produces a continuous score (range 0-60) based on the frequency of depressive symptoms in the past week. The scale was designed for use in epidemiological studies and has been validated for use in older samples (Beekman et al., 1997). A cut-off score of 16 is used as a marker of at least mild and clinically relevant depressive symptomatology, and possible depression (100% sensitivity and 88% specificity for major depression) (Beekman, et al., 1997). Values were imputed if they were missing five or fewer items, based on the average of the items completed.

Cardiovascular hospitalisations: Hospital admission records were searched between 1st January 2004 and 31st December 2009 for separations where a relevant cardiovascular ICD-10 diagnosis appeared in the first three diagnosis coding positions. Relevant diagnoses included angina (I20), acute

myocardial infarction (I21), cerebral infarction (I63), or stroke not specified as infarction or haemorrhage (I64). The true positive rate for Australian hospital records of cardiovascular separations is high (Jamrozik et al., 2001). The primary outcome of hospitalisation was selected, excluding events resulting in out-of-hospital treatment or death, to reflect the contribution of depression and inflammation to severe events with ongoing morbidity. Thus, the current analyses bias toward the null and should be considered conservative.

Other variables: The baseline characteristics examined included self-reported values for age, gender, marital status, annual income before tax, employment, English as a first language, Short-Form 36 (SF-36) physical functioning subscale (Ware & Sherbourne, 1992), Kessler-10 (K-10) psychological distress (Kessler et al., 2002), smoking status, and self-reported disease history and medication use (some diseases prompted with specific questions including "angina", "heart attack", "stroke", others diseases free response; all medication free response). Alcohol consumption in the previous month was measured via a modified timeline follow-back method (Cumming & Mitchell, 1997; Skinner, 1982; Sobell et al., 1979) and was used to identify whether the participant consumed above contemporaneous Australian alcohol guidelines (>4 standard drinks per day for men, >2 standard drinks per day for women) (National Health and Medical Research Council, 2001). Participants also completed a previously validated semi-quantitative food frequency questionnaire (Smith, Mitchell, Reay, Webb, & Harvey, 1998), from which

nutrient intakes, including percentage of energy from saturated fat, were determined using a custom-made nutrient analysis programme based on the NUTTAB 2006 database (Food Standards Australia New Zealand, 2006). An objective measure of physical activity was used, namely average step count per day via a pedometer worn over a week. BMI was calculated from height and weight and WHR was calculated from waist and hip circumference measurements assessed by research staff. Blood concentrations of total cholesterol and systolic blood pressure were also measured and reported.

Data analysis

Analyses were conducted on a sample free of cardiovascular events at baseline, namely excluding people who self-reported "angina", "heart attack" or "stroke" during the baseline survey. Additionally, analyses were restricted to the participants who consented to linkage with hospitalisation records, were not using immunosuppressants, and had valid predictor data (CRP/ IL-6, CES-D, age, gender, BMI or WHR). We additionally excluded 134 individuals with CRP levels above 10mg/L as a conservative indicator of probable acute illness according to previously published guidelines (Clyne & Olshaker, 1999). Therefore, 1692 individuals remained (51% of original sample), with most excluded due to a self-reported history of cardiovascular events ($N = 231$) or the absence of a value for inflammatory markers ($N = 871$). We also completed a supplementary sensitivity analysis where instead of clinical criterion of "probable acute illness", the criterion for exclusion was statistical (values 3 standard deviations

above the geometric mean; > 37mg/L in this sample; $N = 8$). For the analyses involving IL-6, we excluded 23 participants with high values of IL-6 (>25pg/mL; 3 standard deviations above the geometric mean), and for analyses involving BMI, we excluded 4 participants with very high values (BMI>50). Compared to excluded participants, participants who were included in the analyses were younger, more frequently married, more frequently unsafe drinkers, more frequently currently working and, consequently, higher income earners. They also appeared somewhat healthier on some (although not all) measures, with lower CES-D and K-10, greater average steps per day, lower BMI and WHR, higher physical functioning scores, lower systolic blood pressure, fewer current and ex-smokers, less diabetes, less self-reported hypertension, and less use of cardiovascular medications, although more unsafe drinking.

Figure 6.1 shows the model used to guide these analyses. The outcome for the analyses was the time to first cardiovascular hospitalisation (angina, myocardial infarction or cerebral infarction) occurring between the baseline survey and the end of follow-up. The key mediators CRP and IL-6 were log-transformed for analysis to account for non-normality. The key exposure, CES-D score, also had some evidence of a skewed distribution which was not improved with transformation, so mediation analyses were undertaken using CES-D as a binary outcome with previously validated cut-off score of 16 (Beekman, et al., 1997) to indicate low vs. high depressive symptoms.
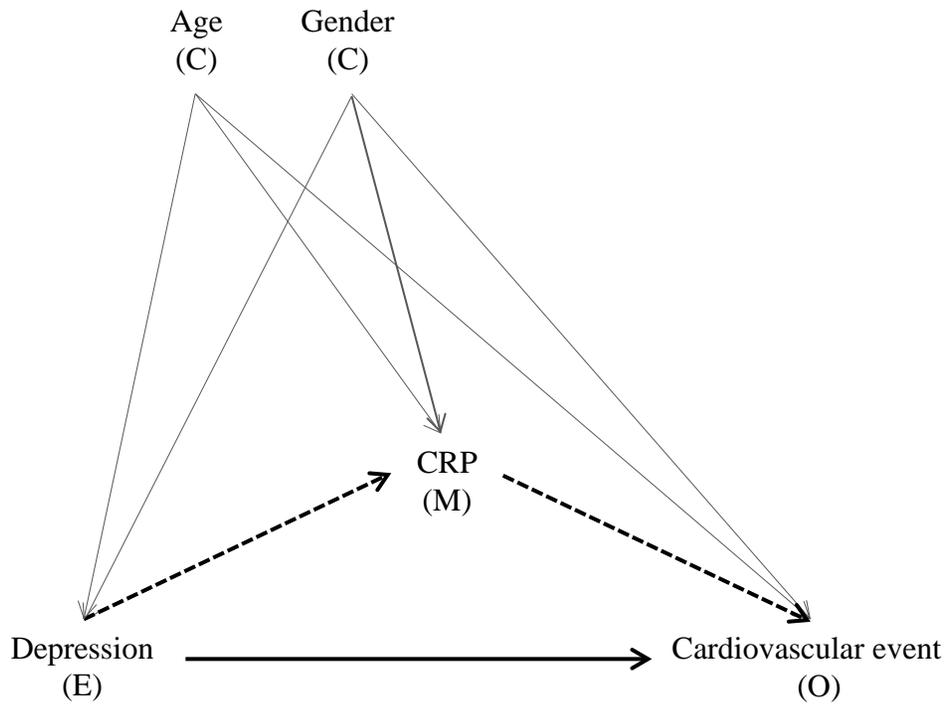
Figure 6.1. Simple causal diagram of exposure (E; depression), mediator (M; C-reactive protein [CRP]), confounders (C; age and gender), and outcome (O; incident cardiovascular events of hospitalisation for angina, myocardial infarction or stroke). The model demonstrates the direct effect (DE) from depression to cardiovascular effects, and the indirect effect (IE) mediated via CRP. Interleukin-6, body mass index and waist-to-hip ratio were also examined as alternative mediators.

Firstly, descriptive statistics regarding baseline demographic and lifestyle factors for those who experienced and did not experience a cardiovascular hospitalisation during follow-up were compared via t-test or chi-square analyses. Secondly, we conducted Cox proportional-hazard regression models using these baseline characteristics to predict time until first cardiovascular hospitalisation after baseline or censoring at the end of the follow-up period (or death if known), controlling for age and gender. Age and gender were considered the most established confounders identified via directed acyclic graph (Hernán, Hernández-Diaz, & Robins, 2004). Because hazard ratios are interpreted as an instantaneous ratio of risk of event for a one unit increase in the predictor variable, WHR was reported as a percentage rather than a ratio (waist circumference/hip circumference*100) to ease interpretation.

Finally, we conducted natural direct and indirect effects mediation analyses using the methods as described in Lange, Vansteelandt and Bekaert (2012) to model the natural direct relationship between depression and cardiovascular events and the natural indirect effect mediated via inflammation (as indicated by CRP or IL-6) or adiposity (BMI or WHR). The associations between the exposure and mediator were analysed with linear models to obtain weights for the marginal structural model. The direct and indirect associations between exposure and outcome were modelled through Cox marginal structural models. A Cox model was selected to represent results

as hazards, rather than parametric effect sizes, as proportional hazard assumptions tested via Schoenfeld residuals were met. Overall fit was evaluated by examining Harrell's C concordance statistic. To compare the effect size of mediators, the proportion of the mediated effect was calculated by dividing the indirect coefficient by the sum of the direct and indirect coefficients and converted to a percentage.

Descriptive analyses were conducted using Stata SE/11 (StataCorp LP, USA) and mediation analyses were conducted using the R statistical language version 2.15 (R Foundation for Statistical Computing, Austria).

6.3 Results

Descriptive statistics

Across approximately 5140 person-years of risk, there were 47 cardiovascular hospitalisations (2.8% of sample); an incidence rate of 9.1 cardiovascular events per 1000 person-years. This is within the range of rates observed in other similarly aged cohorts (myocardial infarction: 6-23 in men and 3-11 in women; stroke: 4-20 in men and 2-17 in women) (National Heart Lung and Blood Institute, 2006). Comparing the incidence by CES-D depression status, for those without depression, incidence was 8.6 events per 1000 person-years, whereas for those with probable depression it was 13.1 per 1000 person-years. Higher depressive symptoms at baseline were also

associated with a longer stay in hospital during admission, $\beta = 0.54$, SE $= 0.21$, $t(44) = 2.52$, $p = .016$.

Table 6.1 shows the baseline demographics, medical history and health behaviour for participants with and without cardiovascular events during follow-up. Participants who had a cardiovascular event were significantly older, and were more likely to be males, low income earners, have a self-reported history of diabetes and hypertension and current use of cardiovascular medications (beta-blockers, warfarin or ACE inhibitors). They also had elevated levels of CRP and IL-6, had higher BMI and WHR, took fewer steps per day, had a lower total cholesterol, and lower SF-36 physical health rating.

Once age and gender were taken into account, higher CES-D scores were observed in those with a cardiovascular event ($M = 8.78$, $SD = 7.74$) compared to without ($M = 6.77$, $SD = 7.71$), and the Cox proportional-hazard ratio for time to cardiovascular event was significant at $\alpha = .05$ (Table 6.1). Furthermore, many of the variables that were significant in the unadjusted analysis were no longer significant in the age and gender adjusted Cox proportional-hazards analyses. Exceptions were CRP, IL-6, continuous CES-D score, BMI, WHR, presence of diabetes and current use of cardiovascular medications. Binary CES-D was a marginally significant predictor. Adding CRP or IL-6 to a model with age, gender and CES-D score significantly improved the model, CRP: $\chi^2(1) = 3.83$, $p = .05$; IL-6: $\chi^2(1) = 4.93$, $p = .03$ and the effectives size for CES-D remained stable. For the supplementary

Table 6.1. Self-reported baseline demographic, health behaviour and medical history characteristics for participants with and without a cardiovascular event during follow-up, yet free of cardiovascular events at baseline, and Cox proportional hazard ratios (HR) for cardiovascular event, adjusted for age and gender (mean observation time 1109 days, median 937 days, range 636–1875 days).

	Baseline characteristic	Missing	Cardiovascular event (*N*= 47)	No cardiovascular event (*N*= 1645)	*p*	HR (95% CI) (adj. for age and gender)		*p*
		N	Mean (SD)	Mean (SD)				
Demographic information	Age	0	68.6 (7.2)	65.1 (7.1)	<.01	1.06	(1.02, 1.10)	<.01
			N (%)	N (%)				
	Male	0	35 (74.5)	762 (46.3)	<.01	3.56	(1.85, 6.87)	<.01
	Married/de facto	34	35 (79.6)	1242 (77.0)	.69	1.08	(0.51, 2.31)	.84
	Employed (full/part-time)	30	10 (22.2)	498 (30.8)	.22	1.05	(0.47, 2.38)	.90
	Gross annual income above AU$40000	352	7 (16.7)	473 (36.4)	.01	0.45	(0.19, 1.05)	.07
	English first language spoken	85	39 (97.5)	1534 (97.9)	.86	0.79	(0.11, 5.76)	.81
			Mean (SD)	Mean (SD)				
Health information	CES-D	0	8.5 (7.4)	6.8 (7.7)	.14	1.03	(1.00, 1.07)	.05
	K-10	3	14.7 (5.3)	14.1 (4.9)	.40	1.03	(0.98, 1.08)	.29
	logCRP	0	0.9 (0.7)	0.7 (0.8)	.05	1.47	(1.02, 2.13)	.04
	logIL-6	189	0.9 (0.7)	0.6 (0.6)	<.01	1.72	(1.08, 2.72)	.02
	Average steps per day (in thousands)	158	6.0 (3.3)	7.1 (3.1)	.02	0.91	(0.82, 1.01)	.09

293

	BMI	5	29.6 (3.8)	28.4 (4.6)	.08	**1.06**	**(1.00, 1.14)**	.05
	WHR (as %)	5	**94.3 (6.7)**	**88.9 (8.5)**	**<.01**	**1.05**	**(1.01, 1.10)**	.02
	Total cholesterol	7	**4.9 (1.0)**	**5.2 (1.0)**	.03	0.86	(0.63, 1.17)	.35
	% saturated fat intake	75	11.4 (3.6)	11.6 (3.3)	.70	0.97	(0.88, 1.06)	.46
	SF-36 physical health subscale	13	**70.1 (26.3)**	**77.6 (21.9)**	.02	0.99	(0.98, 1.00)	.07
	Systolic blood pressure	3	137.5 (19.2)	138.1 (41.4)	.93	1.00	(0.99, 1.01)	.75
			N (%)	N (%)				
	CES-D ≥ 16	0	8 (17.0)	192 (11.7)	.26	**2.12**	**(0.97, 4.60)**	.06
	CES-D ≥ 16 or current antidepressant use	0	10 (21.3)	302 (18.4)	.61	1.51	(0.75, 3.06)	.25
	BMI ≥ 25	5	**44 (93.5)**	**1276 (77.8)**	.01	**3.55**	**(1.09, 11.57)**	.04
	Current smokers	39	4 (8.9)	105 (6.5)	.53	1.59	(0.56, 4.48)	.38
	Previous smokers	38	25 (56.8)	693 (43.0)	.07	1.54	(0.83, 2.85)	.17
	Alcohol consumption above guidelines*	188	9 (25.0)	282 (19.2)	.39	1.30	(0.60, 2.80)	.51
Self-reported health history	Depression/anxiety	59	4 (9.5)	322 (20.2)	.09	0.60	(0.21, 1.69)	.33
	Diabetes	59	**10 (23.8)**	**142 (8.9)**	**<.01**	**2.42**	**(1.19, 4.96)**	.02
	Hypertension	50	24 (54.6)	663 (41.5)	.08	1.60	(0.88, 2.92)	.12
	Current use of antidepressants	430	4 (11.1)	158 (12.9)	.75	0.98	(0.35, 2.78)	.97
	Current use of statins	430	13 (36.1)	383 (31.2)	.54	1.15	(0.58, 2.27)	.69
	Current use of beta-blockers, warfarin, or ACE inhibitors	430	**24 (66.7)**	**576 (47.0)**	.02	**2.07**	**(1.03, 4.17)**	.04

*Australian National Health and Medical Research Council alcohol use guidelines 2001 (contemporaneous with study): >4 standard drinks per day for men, >2 standard drinks per day for women.

Abbreviations:

ACE inhibitor: angiotensin-converting-enzyme inhibitor

BMI: Body mass index

CES-D: Centre for Epidemiologic Studies Depression Scale

CRP: C-reactive protein

IL-6: interleukin-6

K-10: Kessler-10

SF-36: short-form 36

WHR: waist-to-hip ratio.

sensitivity analysis excluding participants due to statistical criterion (CRP over 3 standard deviations from the geometric mean), rather than criterion of possible acute illness, the hazard ratio for logCRP remained statistically significant (HR = 1.34, 95% CI 1.01, 1.78, p = .04).

Mediation analysis

Controlling for age and gender, baseline CES-D category was significantly associated with logCRP (β = 0.06, p = .01) and logIL-6 (β = 0.05, p = .04). In both mediation analyses, the indirect effects from depression to cardiovascular hospitalisation via CRP or IL-6 were significant, while direct effects were not (Table 6.2). The proportion of effect mediated through CRP was 8.1% and for IL-6 was 10.9%. Concordance was equivalent for the models including CRP and IL-6, signifying that neither model outperformed the other in terms of fit (in each case, Harrell's C = .68; indicating correct identification of pairs of patients 68% of the time).

Given the results observed in the Cox proportional-hazards models, we also examined BMI and WHR as possible mediators between depression and cardiovascular disease. Controlling for age and gender, baseline CES-D category was significantly associated with BMI (β = 0.07, p < .01) and WHR (β = 0.05, p < .01). The mediation results were similar to the results for the inflammatory markers, with significant indirect but not direct effects, although the proportion explained through the indirect effect was slightly smaller for

Table 6.2. Mediation analysis for the natural direct effect from depression to later cardiovascular hospitalisations, and the natural indirect effect mediated via C-reactive protein (CRP), interleukin(IL)-6, body mass index (BMI) or waist-to-hip ratio (WHR) within strata of confounders age and gender.

	Direct effect HR (95% CI)		% of effect direct	Indirect effect HR (95% CI)			% of effect indirect
Depression (via CRP)	1.94	(0.86, 4.38)	91.9	1.06	(1.01, 1.11)		8.1
Depression (via IL-6)	1.61	(0.63, 4.09)	89.1	1.06	(1.00, 1.12)		10.9
Depression (via BMI)	2.02	(0.90, 4.55)	92.3	1.06	(1.00, 1.12)		7.7
Depression (via WHR)	1.91	(0.85, 4.31)	89.6	1.08	(1.01, 1.15)		10.4

BMI at 7.7%, and equivalent to that of IL-6 for WHR at 10.4% (Table 6.2; BMI Harrell's $C = .70$; WHR Harrell's $C = .68$).

6.5 Discussion

In a community-dwelling sample of older people without a self-reported history of cardiovascular events, depressive symptoms, CRP and IL-6 were positively associated with risk of cardiovascular hospitalisation over time, after taking into account age and gender. This is consistent with previous studies that conclude that depressive symptoms and inflammatory markers have a generally independent, although possibly overlapping, association with future coronary and cerebrovascular events (Hamer, et al., 2008; Surtees, Wainwright, Boekholdt, et al., 2008). However, extending on previous studies, we demonstrated that inflammatory markers at least partly mediate the association between depression and cardiovascular events. There was a significant indirect effect through inflammatory markers, while the direct effect from depression to cardiovascular events was rendered non-significant. Thus, the indirect effect via inflammatory markers is an important link in the depression-cardiovascular event association.

The mediation effect for CRP and IL-6 was not particularly large (8.1% and 10.9%, respectively). It may be that other inflammatory markers are more specific indicators of subacute inflammation in these disease processes. This may be the case, however the wealth of evidence to date supporting

associations between CRP, IL-6, depression and cardiovascular disease suggest that these would be the best two candidates. More likely is that there are other necessary mediators which help explain the association between depression and cardiovascular events. In this study, we explored the role of obesity and demonstrated that, like the inflammatory markers, BMI and WHR were significant mediators. The mediation effect for WHR was similar to that of the inflammatory markers, and was slightly smaller for BMI. Other suggested mechanisms that may be partly involved which were not captured in the current study include medication adherence, neuroendocrine deregulation, decreased heart rate variability or oxidative stress (Harrison, et al., 2013; Joynt, Whellan, & O'Connor, 2003; Lippi, Montagnana, Favaloro, & Franchini, 2009; Nemeroff & Goldschmidt-Clermont, 2012). Some of these factors are also associated with inflammation although they may also have independent physiological effects. Such factors should be explored systematically in future research. Existing studies suggest that even after adjusting for a range of factors including inflammation, lifestyle, and coagulation, there is still an association between depression and acute myocardial infarction (Janszky, Ahlbom, Hallqvist, & Ahnve, 2007). Perhaps there are as yet unknown mechanisms underlying the relationship.

Finally, these data showed CRP and IL-6 to be similar in their mediating value, supporting previous literature which demonstrates these to be similar predictors of cardiovascular events (Danesh, et al., 2008; Kip et al., 2005). CRP

is a particularly appealing measure as studies have examined specific cut-off values as risk indicators for cardiovascular risk (Pearson, et al., 2003). The assay is relatively inexpensive and values are relatively stable within individuals. There was a slight advantage for IL-6 in terms of the proportion of variance explained in the mediation analysis. Furthermore, IL-6 may be more closely related to depression than CRP (Howren, et al., 2009). Therefore, IL-6 may be another suitable candidate for similar risk stratification procedures.

Surprisingly, there was no significant association between smoking and cardiovascular events in this study, despite these events being a known risk of smoking. This result may be related to sample size since there were few current smokers in the present Hunter Community Study sample (N = 109, 6.6%). The low number of smokers in the group with cardiovascular events compared to those without led to wide confidence intervals of the hazard ratio, which in turn failed to produce a reliable difference. Nevertheless, the relationship was in the expected direction. Furthermore, although more participants classified themselves as "previous smokers", neither time since smoking cessation or previous smoking intensity were measured and taken into account. Had the relationship between smoking and cardiovascular events been reliable, smoking would have been investigated as a mediator of the relationship between depression and cardiovascular events in light of the association between depression, smoking and inflammatory markers observed in Chapter 5 (Hiles et al., under review-a). However, with the data available,

this was not possible. This has been explored in previous studies (Hamer et al., 2008; Whooley et al., 2008) and is an important avenue for further research given the high prevalence of smoking in people with depression (Strine et al., 2008; Wilhelm et al., 2006).

The current results must be interpreted in light of several limitations which have generally resulted in a more conservative result. The outcome was limited to hospital recorded events, and not events that did not lead to hospital presentation (including death), although this biases toward the null so is conservative. Furthermore, history of cardiovascular events was only obtained through self-report. There were considerable missing data and so it is unclear whether the sample was representative of the broader older community. Some missing data was due to study design, for instance, provision of blood collection forms could only be provided to those people who could attend the physical clinic examination whereas the self-report measures were completed at the participant's convenience via mailed form. This may have led to underrepresentation of people who were in employment, with in-home carer duties and those too unwell to attend the clinic. Consequently, only a subset of the overall Hunter Community Study participants could be analysed in this study. Included participants were significantly younger, higher income earning and had better scores on some health measures including depressive symptoms (although not all health measures), and as such, the analyses of the current study may not represent a more depressed, physically unwell and potentially

socio-economically disadvantaged segment of older persons. Although this may mean the relationships may not be representative of the broader community, the relationships observed are still representative of healthier community-dwelling older persons. We underestimate the true effects that we may observe if more unwell people where included in the study. The study also does not address whether these mediators are overlapping or exclusive of each other in their effects; the current models of natural effects are unable to assess two or three mediators at once in order to see whether CRP and IL-6 for example are markers of the same mediation pathway.

Future studies may benefit from the measurement of inflammatory markers and depression at multiple time points to examine of the directionality of the relationships between these factors and cardiovascular disease. Existing prospective research indicates that depressive symptoms are predictive of white blood cell count in people with coronary heart disease, while the reverse direction was not supported (Duivis et al., 2013). Measurement at multiple time points would provide more convincing evidence as to whether depressive symptoms and inflammatory markers have additive effects on risk over time. Perhaps, as suggested in a recent editorial, rather than direction (or causality), it is more likely that there is interdependence and mutually reinforcing relationships between depression and cardiovascular disease (de Jonge & Roest, 2012). Furthermore, type II diabetes is an increasingly prevalent metabolic disorder and is highly comorbid with cardiovascular disease.

302

Diabetes was a substantial predictor of cardiovascular events in the current study. Diabetes is also highly comorbid with depression (Knol et al., 2006) and is associated with high levels of CRP (Lee et al., 2009). Given this, although beyond the scope of the objectives of the current study, it would be useful in future studies to examine whether there is an interaction between diabetes and depression in predicting cardiovascular disease and hospitalisations. Finally, although recent research suggests the inflammation-depression relationship may differ by gender (Hiles et al., under review), there were few females with cardiovascular hospitalisations in this cohort such that we were unable to assess effect modification by gender in this study. This is an important avenue for future research.

The present study demonstrates that inflammation may partly explain the association between depression and cardiovascular events. The mediating effect was small, indicating that although there is a theoretical association between depression and inflammation, this does not translate to completely dependent risks. Nevertheless, reducing inflammation may help prevent cardiovascular events and reduce depressive symptoms. Antidepressants may have anti-inflammatory effects, which could in turn reduce risk of cardiovascular events (Hiles, Baker, de Malmanche, & Attia, 2012a; Tynan et al., 2012). Furthermore, medications with anti-inflammatory effects such as statins and aspirin, which are often prescribed for primary or secondary prevention of cardiovascular events, are associated with reductions in

depression (Hiles, Baker, Handley, de Malmanche, & Attia, under review; Pasco, Jacka, et al., 2010). There is also a role for intervening on aspects of unhealthy lifestyle and improving adherence to lifestyle management programs in people at risk of cardiovascular events, as aspects of unhealthy lifestyle factors such as adiposity are pro-inflammatory (Miller, et al., 2003; Shelton & Miller, 2010). For instance, modifications including exercise have been shown to reduce inflammatory markers, and inactivity is often a large contributor to risk of cardiovascular event (Goldhammer et al., 2005; Hamer, et al., 2008; Whooley, et al., 2008; Woods, Vieira, & Keylock, 2006). These effects of physical inactivity may be mediated by BMI (Verdaet et al., 2004), so physical activity and diet with a view to improve adiposity may be particularly warranted. Furthermore, adhering to a lifestyle modification program may benefit the other psychological and physiological factors which may mediate the relationship between depression and cardiovascular diseases. Ultimately, this could provide a unified approach to managing mental and physical health.

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7. General discussion

7. General discussion

7.1 Findings, limitations and strengths of this thesis

Key findings

This thesis presented a series of studies designed to investigate the inflammation-depression relationship from an epidemiological perspective. The broad goal was to provide concurrent evidence to the growing body of experimental literature showing depression and depression-like outcomes following inflammation in animal and human models (Dantzer, 2001; Maes et al., 2009; Miller, Maletic, & Raison, 2009). The thesis explored several lines of cross-sectional and temporal evidence for the inflammatory hypothesis of depression. The findings highlight a close association between depression and markers of inflammation in the blood, the circumstances where we are most likely to observe this association, and the implications of this association for the inflammatory hypothesis of depression.

The first meta-analysis of cross sectional studies demonstrated consistent evidence that levels of interleukin (IL)-6 are significantly elevated in people with major depressive disorder or elevated depressive symptoms (Chapter 2; Hiles, Baker, de Malmanche, & Attia, 2012b). The in depth moderator analyses were a particular asset of this study. These analyses showed that the strength of the relationship between IL-6 and depression differed depending on particular factors such as depression diagnosis, body mass index, and comorbidity. IL-10, on the other hand, did

not significantly differ in people with and without depression, indicating that cytokines are not affected across the board.

The second meta-analysis of antidepressant treatment studies showed that levels of IL-6 and CRP significantly decline after treatment with any antidepressant (Chapter 3; Hiles, Baker, de Malmanche, & Attia, 2012a). This may indicate that pro-inflammatory markers normalise after depressive symptoms improve, or may also indicate the potential anti-inflammatory effects of antidepressants themselves (Janssen, Caniato, Verster, & Baune, 2010; Kenis & Maes, 2002; Tynan et al., 2012). Ultimately, antidepressant treatment evidence is difficult to interpret because it is unclear whether the biochemical activity of the antidepressant agent or the reduction in symptoms drives the change in subclinical inflammation. IL-10 did not show a significant change over treatment, although this may be due to the small number of included studies.

The final meta-analysis included in this thesis showed that statin treatment, compared with placebo, was associated with a greater decline in depressive symptoms and a reduced number of depression events (Chapter 4; Hiles, Baker, Handley, de Malmanche, & Attia, under review). Considering evidence that statins have anti-inflammatory effects (Craig et al., 2011; Farooqui, Ong, Horrocks, Chen, & Farooqui, 2007; Shen, 2005), this meta-analysis suggests that use of medications with observed anti-inflammatory effects may be associated with protection against depression, and therefore suggests that inflammation might be a possible cause of depression.

The first prospective study in this thesis demonstrated an inter-relationship between baseline levels of IL-6 and CRP, depressive symptoms, and aspects of unhealthy lifestyle (including measures of adiposity, steps per day, saturated fat intake, smoking and alcohol use) (Chapter 5; Hiles et al., under review-b). It also showed that for females, baseline levels of IL-6 were associated with follow-up depression as a dichotomised value and as a residual depression score (an outcome which represented the variation in follow-up depressive symptoms not explained by baseline depressive symptoms and age). This relationship was no longer significant after accounting for aspects of unhealthy lifestyle, indicating lifestyle is an important confounder of the depression-inflammation relationship. Aspects of unhealthy lifestyle were important predictors of later depression outcomes, and this association was partly mediated by IL-6. This implies that lifestyle may be an important source of the elevated inflammatory markers observed in people with depression. These relationships were not observed for males, indicating there may be a gender difference in the inflammation-depression relationship.

The second prospective study showed that baseline levels of CRP, IL-6 and depressive symptoms were significantly associated with later hospitalisations for myocardial infarction, angina and stroke (Chapter 6; Hiles et al., under review-a). In the relationship between baseline depressive symptoms and later cardiovascular hospitalisation, inflammatory markers had a mediating effect similar in effect size to more traditional risk factors such as body mass index and waist-to-hip ratio. The inflammatory

hypothesis purports to explain why depression has substantial comorbidity with physical illness. The aim of this study was to examine whether the inflammatory hypothesis of depression would have flow-on effects to other aspects of health; in this case whether inflammation could explain the observed comorbidity between depression and cardiovascular events. The effects observed were statistically reliable although small, indicating the involvement of other biological and psychological (emotional, behavioural and cognitive) mechanisms, not simply inflammatory mechanisms.

Taken together, the findings of this thesis provide some support for the inflammatory hypothesis of depression. There is clearly a substantial cross sectional relationship between depression and inflammatory markers, and some temporal relationships exist, including prospective cohort evidence that elevated inflammatory markers precede elevated depressive symptoms, and evidence of the "anti-inflammatory" effects of antidepressants and "anti-depressive" effects of statins. However, many of the effects observed were small, as is common in epidemiological studies. Inflammation may only partly explain the relationship between unhealthy lifestyle and depression, and between depression and comorbid conditions such as cardiovascular disease. Furthermore, aspects of comorbidity and unhealthy lifestyle seem to have a substantial impact on the strength of the relationship between depression and inflammatory markers. While the current findings do not discredit that inflammation may have a causative role in depression, it suggests that other factors such as psychosocial stress, physical and psychological comorbidity and unhealthy lifestyle may be

required for a cogent inflammatory hypothesis of depression. Factors such as psychosocial stress and physical illness are specified as potential sources of subacute inflammation in depression in recent explanations of inflammatory theories of depression (Maes, et al., 2009). Perhaps unhealthy lifestyle should also feature in such explanations.

Strengths and limitations of the thesis studies

Before further discussion of the implication of these findings, the limitations of this thesis must be acknowledged. The first limitations are to do with the characterisation of the participant groups. There are differences in the experience and triggers of depression across the lifespan (Brenes et al., 2008; Leach, Christensen, Mackinnon, Windsor, & Butterworth, 2008; Nolen-Hoeksema & Ahrens, 2002). However, age groups differed in each study of this thesis, with a general adult range for the meta-analyses and a focus on older people in the primary prospective studies. This raises questions as to whether the implications are applicable for depression across the lifespan. Furthermore, this thesis has a heavier focus on depressive symptoms rather than depressive disorder. Although both are associated with similarly significant levels of dysfunction, there are some qualitative differences between depressive symptoms and depressive disorder (Gotlib, Lewinsohn, & Seeley, 1995; Lewinsohn, Solomon, Seeley, & Zeiss, 2000). These can translate into differences for the association between inflammatory markers and depression, with larger associations between IL-6 and depressive disorder than high depressive symptoms demonstrated in

Chapter 2 of this thesis (Hiles, et al., 2012b). It is often difficult to assess mental disorders via a clinical interview in large, epidemiological cohorts; particularly in cohorts such as the Hunter Community Study which focus on taking measurements of physical health and health behaviour.

The main difficulty with the epidemiological evidence presented both in this thesis and in the research body in general is the potential for unmeasured residual confounding to account for the relationship between depression and inflammation. One example is the rarely measured variable of sleep, which is affected during depression and is immunomodulatory (Kapsimalis et al., 2008; Lopresti, Hood, & Drummond, 2013; Motivala, Sarfatti, Olmos, & Irwin, 2005; Nolen-Hoeksema & Ahrens, 2002). One study in participants with coronary heart disease demonstrated an association between white blood cell counts and depression that persisted after adjusting for a range of behavioural and nascent disease covariates; additionally adjusting for sleep quality attenuated the relationship to non-significance (Duivis et al., 2013). Furthermore, in participants with major depressive disorder, sleep latency and frequency of rapid eye movement sleep were better predictors of IL-6 levels than depressive symptoms (Motivala, et al., 2005). This thesis also did not take into account current anxiety symptoms, as distinct from, or comorbid with, depression. In the prospective studies of this thesis, this was due to the Hunter Community Study focussing on physical health assessment at baseline. Although this focus allowed for a range of health and lifestyle behaviours to be accounted for as confounders in the analyses, it meant that mental health problems,

such as anxiety, were not measured adequately for differential diagnosis. Anxiety is associated with levels of CRP and other pro-inflammatory cytokines (Copeland, Shanahan, Worthman, Angold, & Costello, 2012; Duivis, Vogelzangs, Kupper, de Jonge, & Penninx, 2013; Liukkonen et al., 2011; O'Donovan et al., 2010; Vogelzangs, Beekman, de Jonge, & Penninx, 2013). There is substantial comorbidity between depression and anxiety, and people with depression often have subclinical anxiety (Fava et al., 2004; Kessler et al., 2003; Lenze et al., 2001; Rush et al., 2005; Teeson, Slade, & Mills, 2009). Thus, it is important to further examine the contribution of anxiety in this literature. An initial focus on whether it is particular clusters of anxious or somatic-vegetative (sleep, eating) behaviours that are associated with inflammatory mediators (as some previous studies have done) is recommended (Duivis, Vogelzangs, et al., 2013; Kupper, Widdershoven, & Pedersen, 2012).

The contribution that the evidence outlined in this thesis makes assessing the causal directionality of the inflammatory hypothesis of depression is limited. Firstly, although the cross sectional meta-analysis highlights the proof-of-concept that people with and without depression differ in inflammatory markers, the direction of the relationship is unknown. In terms of temporal evidence, both the treatment evidence and prospective evidence described in this thesis are problematic for determining directionality because change in inflammatory markers occurs concurrently with improvements in mood or overall functioning. In the prospective studies, we examined prospective validation and incremental

value of using inflammatory markers to predict later depressive events, and depressive symptoms and inflammatory markers to predict later cardiovascular hospitalisations. However, due to the limitation of providing a single blood sample for assay of inflammatory markers, we could not examine this question bi-directionally. Furthermore, for the epidemiological studies presented in Chapters 5 and 6, the exposure and mediator variables were measured concurrently. This means it is impossible to infer temporal or causal effects where one factor leads to the other. A better indication of the mediation effect would be measuring the exposure, followed by the mediator, then then outcome variable. Indeed, multiple measurements at various time points to compare change trajectories and interactions would have shown even stronger evidence of mediation effects. With the data available, such analysis was not possible. Further evidence is required to fully evaluate inflammatory markers as novel risk markers for depression. In particular, evidence regarding whether the inflammatory markers have clinical utility for predicting risk and improving clinical outcomes for depression in a cost-effective way is necessary (Hlatky et al., 2009; Karakas & Koenig, 2009), which could not be meaningfully evaluated in this thesis.

Despite these limitations, there are several strengths of this thesis. A strength is the complementary meta-analytic and prospective approaches employed to explore the relationship between depression and inflammatory markers. Systematic review and meta-analysis conveniently summarise published literature to determine the state of the field at a particular point, bringing a sense of coherence to the literature and allowing generalisation

from individual studies. Meta-analyses highlight gaps that new primary research can fill. The meta-analyses in this thesis demonstrated that cross-sectional research is extensive, convincingly demonstrating an association between depression and inflammatory markers. Conversely, further research is required to verify preliminary findings regarding the effects of antidepressant medication (and as an extension, psychological intervention for depression) on inflammatory markers, and regarding the effects of medication with anti-inflammatory effects, such as statins, on depressive symptoms. The primary prospective research complements the meta-analyses, allowing for more in depth evaluation of the moderators and mediators of the depression/inflammation relationship.

The studies also addressed key gaps in existing literature, some of which had not been previously addressed when the studies were conducted. The cross sectional meta-analysis built on that of Howren et al. (2009) to explore moderators of the depression-inflammation relationship in greater depth. At the time of conducting the studies, the antidepressant and statin treatment meta-analyses were the first to generate pooled estimates examining the temporal relationship between inflammation and depression (note that two similar studies were published while these meta-analyses were under review, Hannestad, DellaGioia, & Bloch, 2011; O'Neil et al., 2012). For the primary studies, the comprehensive health measures collected in the Hunter Community Study (McEvoy et al., 2010) meant that many potential covariates of the inflammatory-depression relationships were examined, particularly useful was diet derived from a validated food

frequency questionnaire (Smith, Mitchell, Reay, Webb, & Harvey, 1998).

The use of mediation analysis to explore whether inflammatory markers

may mediate the relationship between baseline unhealthy lifestyle and later

depressive symptoms is novel. Finally, this thesis goes beyond the

relationship between depression and inflammatory markers, and explores

the implications of this relationship for comorbid physical health problems,

namely cardiovascular disease. The inflammation hypothesis has the

potential to explain the significant comorbidity between depression and

physical illness, due to the proposed shared inflammatory pathway for

psychological and physical illness (Miller, et al., 2009). It is novel to use

mediation analysis to examine whether inflammatory markers mediate the

association between depressive symptoms and later cardiovascular

hospitalisation to address this question, with most previous research not

having addressed these risk factors simultaneously in a single study.

7.2 Limitations and implications of the inflammatory hypothesis of depression

Limitations of the inflammatory hypothesis of depression

Whether inflammation itself is the cause of depression is as yet an

unanswered question. It is clear that inflammation is at least associated

with depression (Chapter 2; Hiles, et al., 2012b). There is also evidence that

immune challenge in both animal models and humans is associated with

transient negative affect and sickness behaviours akin to the behaviours present in people with depression (Dunn, Swiergiel, & Beaurepaire, 2005; Raison et al., 2009; Reichenberg et al., 2001; Song & Wang, 2011). Whether there is conclusive evidence for inflammation as the cause of idiopathic depression is another question.

Although there is evidence for the inflammatory hypothesis of depression, as outlined throughout this thesis, the relationships are not large and the evidence for these broad types of inflammatory markers alone as a biomarker is not particularly strong. The cross-sectional meta-analysis in this thesis and others revealed a small-moderate effect size (Dowlati et al., 2010; Hiles, et al., 2012b; Howren, et al., 2009); arguably a good biomarker would be much higher. Furthermore, pro-inflammatory markers are elevated across many physical and mental disorders (e.g., Kuo et al., 2005; Miller, Buckley, Seabolt, Mellor, & Kirkpatrick, 2011; Modabbernia, Taslimi, Brietzke, & Ashrafi, 2013; Vogelzangs, et al., 2013), so the markers lack specificity. Although antidepressant treatment evidence was lacking, based on available evidence, the decline in IL-6 after treatment was small, albeit reliable (Hiles, et al., 2012a). The effect sizes for reduction in depression after statin treatment (presuming its anti-inflammatory nature) were also small, and although significant, perhaps only meaningful at a population level (Hiles, Baker, Handley, et al., under review). However, perhaps larger effects would have been observed if depression outcomes were primary rather than secondary outcomes, particularly in depressed populations. In our primary analyses, there was also no evidence in males

for an association between baseline inflammatory markers and follow-up depression outcomes (Hiles, Baker, et al., under review-b). For females, this relationship did not remain significant after adjusting for aspects of unhealthy lifestyle, and IL-6 delivers only a small mediation effect between baseline lifestyle and later depression (Hiles, Baker, et al., under review-b). Inflammatory markers only accounted for a small part of the relationship between depression and later cardiovascular events, which provides limited evidence to suggest the nature of depression as an "illness of inflammation", predicting another disease with possible inflammatory origins (Hiles, Baker, et al., under review-a). From a biomarker perspective, the signal provided by broad inflammatory markers studied to date may offer only limited improvement over other psychological and behaviour risk factors for identifying those at risk of depression and recovery from depression. Although, perhaps there are more specific inflammatory markers that could achieve this.

Alternatively, there may be other processes besides those indicated by broad inflammatory markers that are involved in the cause of depression and complement the inflammatory evidence. These may also act as better risk markers for early identification and indication of treatment response. One example could be markers of oxidative stress. The causative role of oxidative stress and resulting neurodegeneration in depression is becoming increasingly recognised as a complement to and extension of the cytokine hypothesis of depression (Lee et al., 2013; Maes, Galecki, Chang, & Berk, 2011; Maes, et al., 2009). Inflammation is associated with the activation of

oxidative and nitrosative stress pathways, which cause tissue damage under pathological conditions when they are not stabilised by antioxidants and other proteins, as occurs under normal conditions. The brain is particularly vulnerable to damage via oxidative stress. Thus, oxidative and nitrosative stress may cause the neurodegeneration observed in depression. Markers of oxidative stress are elevated and antioxidant markers are decreased in people with depression (e.g., Kodydková et al., 2009; Maes et al., 2010; Owen, Batterham, Probst, Grenyer, & Tapsell, 2004; Stefanescu & Ciobica, 2012). Both inflammatory and oxidative processes together may contribute to depression. Markers of oxidative stress in addition to inflammatory markers may provide the sensitive biomarkers required, and a specified combined inflammation-oxidative stress theory may provide a more complete account of depression.

Practical implications of the inflammatory hypothesis of depression

Limitations from a biomarker perspective do not preclude subacute inflammation as a potential cause of depression. A causal relationship between inflammation and depression is biologically plausible, and this thesis outlines some epidemiological evidence that supports an association. Consequently, the theory suggests that inflammatory mediators may be targets for treatment. The two practical treatment and prevention implications of a causal inflammatory hypothesis of depression are pharmacological and lifestyle interventions.

Firstly, the most obvious implication is that anti-inflammatory medications may be an innovative treatment for individuals with depression, or prophylactic for individuals at very high risk of depression. Evidence that directly using anti-inflammatory medications improves clinical depression in humans is lacking (Raison et al., 2013, as the exception). Anti-inflammatories have been used successfully as adjunct treatments to traditional antidepressants, suggesting superior response to a combined treatment with cyclooxygenase-2 inhibitor and antidepressant (selective serotonin reuptake inhibitor or norepinephrine reuptake inhibitor), compared to antidepressant alone (Akhondzadeh et al., 2009; Muller et al., 2006). There is also evidence in non-clinical human populations such as the evidence already outlined in this thesis that statin medications, with their anti-inflammatory properties, are associated with reductions in depression and depressive symptoms in randomised controlled trials (Hiles, Baker, Handley, et al., under review). This is supported in cohort studies, with statins and aspirin each associated with reduced incidence of *de novo* major depressive disorder (Pasco et al., 2010). Another avenue of research may be central anti-inflammatory medications such as minocycline. There is evidence in animal models that minocycline is associated with reduced depressive-like behaviour (Arakawa et al., 2012; Henry et al., 2008; Hinwood, Morandini, Day, & Walker, 2012; Pae, Marks, Han, & Patkar, 2008).

Secondly, the inflammatory hypothesis supports the promotion of interventions addressing pro-inflammatory lifestyle factors such as high

fat/low fibre diet, sedentary activity, alcohol and tobacco use. These lifestyle factors are often observed in people with depression and other chronic diseases such as cardiovascular disease, and are also associated with elevations in pro-inflammatory markers (Bonnet et al., 2005; Lopresti, et al., 2013; O'Connor et al., 2009; Spring, Moller, & Coons, 2012; van Gool et al., 2007; Verdaet et al., 2004). Emerging evidence demonstrates that interventions focussed on improving exercise and diet and tobacco cessation are associated with improvements in inflammatory markers (Esposito et al., 2003; Hastie, Haw, & Pell, 2008; Kasapis & Thompson, 2005; Wannamethee et al., 2005; Watzl, Kulling, Möseneder, Barth, & Bub, 2005). Lifestyle intervention is appropriate as a selected intervention for individuals with depression and at the public health level to reduce population levels of risk. Innovative research suggests that targeting these unhealthy lifestyle behaviours together leads to positive outcomes across multiple domains in both general community populations (Spring et al., 2012) and populations with mental illness (Baker et al., 2009; Brown & Chan, 2006; Tsoh, Chi, Mertens, & Weisner, 2011).

Besides treatment implications, another practical implication of the inflammatory hypothesis may be toward informing diagnostic criteria for depression, particularly relating to depression subtypes. Depression diagnosis using subtypes based on symptom clusters (and perhaps eventually, subtypes based on cause) may better account for the observed heterogeneity in people with depression. The idea is controversial in terms of its utility in clinical settings and whether the subtypes generated are

genuine categories or better exist on a continuum of severity of the broad

disorder "depression". Nevertheless, currently the two most prevalent

subtypes of depression are the melancholic type (often regarded as a severe

and "biological" type depression featuring anhedonia and lack of mood

reactivity) and atypical depression (highly related to psychosocial stress,

characterised by reactivity of mood), each identified in the DSM-IV-TR

(American Psychiatric Association, 2000). Differential inflammatory

cytokine and immune cell profiles have been observed in melancholic and

atypical depression, for instance, greater numbers of leukocytes and greater

production of IL-1β in atypical patients (Kaestner et al., 2005; Rothermundt

et al., 2001). Most recently, observational research indicates that it is those

with atypical, rather than non-atypical, depression who demonstrate

elevated CRP, compared to controls without depression (Hickman,

Khambaty, & Stewart, 2013). People with atypical depression also show

significantly elevated CRP, IL-6 and tumor necrosis factor compared to

people with melancholic depression and controls (Lamers et al., 2013).

Furthermore, people with atypical depression also show differences in their

metabolic profile, with higher odds of overweight, diabetes and metabolic

syndrome, even after adjusting for demographic characteristics, medication

and comorbidity (Glaus et al., 2013). A recent review concluded that people

with atypical depression may demonstrate an altered inflammatory and

metabolic profile whereas people with melancholic depression may show

altered cortisol profiles (Penninx et al., 2013). This has important

implications for research in terms of further examining the mechanisms and

extent of these effects. It also encourages investigating options to increase the efficiency of antidepressant medications, providing adjuncts to traditional medications or investigating whether certain treatments may have superior benefit for atypical compared with melancholic depression. This may ultimately have consequences for the treatment and diagnosis of depression, including the possible need for greater integration of lifestyle management into standard mood intervention for those with atypical depression who may be at greater risk of metabolic disorder.

To take this further, if we use the inflammatory hypothesis of depression to inform depression subtypes, we may see something like (1) an "inflammation/immune type" depression, with an inflammatory cause treated primarily via anti-inflammatory pharmacology and behavioural means, vs. (2) a psychogenic type depression where inflammation is neither necessary nor sufficient to cause depression, but may be a by-product of other processes (e.g., inflammation due to the relationship between the immune system and the hypothalamic-pituitary-adrenal axis/autonomic nervous system involved in the reaction to a psychosocial stressor; pro-inflammatory effects caused by a decline in physical activity or changes in sleep habits).

(1) Trigger → Inflammation → depressive episode

(2) Trigger/Stressor → depressive episode (→ inflammation?)

There is already some preliminary evidence of this in the recently published randomised controlled trial of the tumor necrosis factor antagonist, infliximab (Raison, et al., 2013). Those with improved depression after anti-inflammatory treatment were those with elevations of CRP at baseline, whereas those with lower CRP had somewhat greater improvements on placebo. Furthermore, there is evidence that people who do not respond to traditional serotonergic antidepressant treatment have higher baseline inflammatory makers than responders (Yoshimura et al., 2009). If not a subtype of depression, perhaps the depression in some may be sensitive to first-line treatment with anti-inflammatory medications.

7.3 Recommendations and final conclusions

Key recommendations and future research

There are several useful research directions to add validity and enhance the explanatory status of the inflammatory hypothesis of depression. The next step is to further examine the impact of established anti-inflammatory medications on major depressive disorder. Beyond one initial randomised controlled trial of infliximab (tumor necrosis factor antagonist) as a primary treatment for people with treatment-resistant major depression (Raison, et al., 2013), little has been done to examine whether anti-inflammatory medications on their own resolve clinical depression. Furthermore, randomised controlled trials in behavioural interventions are clearly indicated, comparing the efficacy of exercise and

dietary interventions and anti-inflammatory pharmacotherapy in people with depressive disorders on both depression and inflammatory marker outcomes. It would also be useful to examine whether existing antidepressant trials should simultaneously address pro-inflammatory unhealthy behaviour for enhanced outcomes. Another useful treatment design would be to compare depression and inflammatory marker outcomes in different activities used in behavioural activation or activity scheduling. Specifically, to examine whether outcomes are improved by using activities that improve inflammatory functioning, such as exercise, rather than sedentary activities that would not have the same degree of impact on inflammation (beyond indirect effects such as improvements in stress), such as doing crosswords or puzzles, or learning touch typing.

Further explanation of confounding and the role of other lifestyle factors are also required to examine the circumstances under which an inflammation-depression relationship is observed. This approach of examining lifestyle as the potential trigger for depression-causing subclinical inflammation could also be complemented by genetic research. Altered polymorphisms of genes including IL-6 (Bull et al., 2008), CRP (Almeida et al., 2009) and IL-10 (Jun et al., 2002; Misener et al., 2008; Traks et al., 2008) indicate that a genetic vulnerability to inflammatory parameters is present in people who develop depression following treatment with interferon-α but also in community samples. Exploring the interaction between genes and environment would be a useful next step.

Final conclusions

The inflammatory hypothesis of depression is plausible from psychological, biological and evolutionary perspectives (Raison & Miller, 2013). It has the potential to tie together the diagnostic symptoms of depression, many of the neurobiological correlates of depression, and the evident high frequency of co-occurring diseases and unhealthy behaviours in people with depression. The epidemiological evidence presented in this thesis provides both cross-sectional and temporal support for the inflammatory hypothesis of depression. A particular strength is the proof-of-concept treatment evidence suggesting that antidepressants may affect inflammatory functioning, and that treatments with anti-inflammatory effects may influence depression outcomes. It also highlights the importance of unhealthy lifestyle for the strength of the relationship between depression and inflammation. The inflammatory hypothesis of depression provides a clear therapeutic target, the opportunity to explore inflammatory biomarkers and re-evaluate diagnostic criteria in depression. Continued research into the inflammation hypothesis of depression has the potential to provide novel avenues for the prevention and treatment for depression. Improving aspects of unhealthy lifestyle may benefit depression, inflammatory mediators, and other comorbid diseases such as cardiovascular disease.

7.4 References

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8. Appendices

8.1 Hunter Community Study Cohort Profile (McEvoy et al. 2010)

Reference: McEvoy, M., Smith, W., D'Este, C., Duke, J., Peel, R., Schofield,

P., et al. (2010). Cohort profile: The Hunter Community Study.

International Journal of Epidemiology, 39(6), 1452-1463. Available

at: http://ije.oxfordjournals.org/content/39/6/1452.extract

8.2 PRISMA 2009 checklist for Chapter 2: Cross sectional meta-analysis

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	75
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	76-77
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	78-80
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	80
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	81
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	81-82
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	81-82
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	82-83
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	82

8.2 PRISMA 2009 checklist for Chapter 2: Cross sectional meta-analysis

Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	82, 84, 85
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	82, N/A
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	84
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	91
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	92, 94, 95
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	85

RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	83
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	N/A
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	N/A
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	87, 96
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	87, 96
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	92
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	89-95, 97

DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	97-104
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	102-103
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	97-104
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	No role of funder

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8.3 PRISMA 2009 checklist for Chapter 3: Antidepressant meta-analysis

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	134
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	135-136
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	137-138
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	138
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	128-139
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	139
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	139
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	139-140
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	140

8.3 PRISMA 2009 checklist for Chapter 3: Antidepressant meta-analysis

Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	140-141
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	N/A
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	140
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	141-142
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	141-142
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	142

RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	143
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	144-154
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	144-154
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	155-164
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	159-164
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	159-160
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	165-166

DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	167-174
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	170-171
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	167-174
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	No role of funder

8.4 PRISMA 2009 checklist for Chapter 4: Statin meta-analysis

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	184
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	185
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	186-189
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	189
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	189-190
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	190-191
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	190-191
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	191
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	191

8.4 PRISMA 2009 checklist for Chapter 4: Statin meta-analysis

Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	191
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	N/A
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	193
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	193
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	191, 194
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	194

RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	192
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	195-199
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	195-199
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	200, 204
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	194, 200, 204
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	202-203
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	205

DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	205-211
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	209-211
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	205-211
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	No role of funder

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