The Relationship between C-reactive protein and Depression in Older Adults: Associations with Incidence of Type 2 Diabetes Mellitus

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DEDICATION

This work is dedicated to those who have been affected by depression and diabetes. And to my aunt, who passed away from diabetes complications before seeing me complete this thesis.
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<tr>
<td>AHA</td>
<td>American Heart Association</td>
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<tr>
<td>BBB</td>
<td>blood brain barrier</td>
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<td>BMI</td>
<td>body mass index</td>
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<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
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<td>CES-D</td>
<td>Center for Epidemiologic Studies Depression</td>
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<td>CI</td>
<td>confidence interval</td>
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<td>CRP</td>
<td>C-reactive protein</td>
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<td>CVD</td>
<td>cardiovascular disease</td>
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<td>DSM</td>
<td>Diagnostic and Statistical Manual of Mental Disorders</td>
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<td>ELSA</td>
<td>English Longitudinal Study of Ageing</td>
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<td>HDL</td>
<td>high density lipoprotein</td>
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<td>HR</td>
<td>hazard ratio</td>
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<tr>
<td>HPA</td>
<td>hypothalamic-pituitary-adrenal</td>
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<td>HSE</td>
<td>Health Survey for England</td>
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<td>ICD</td>
<td>International Statistical Classification of Diseases</td>
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<td>IDF</td>
<td>International Diabetes Federation</td>
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<td>IL-6</td>
<td>interleukin-6</td>
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<td>NOS</td>
<td>Newcastle-Ottawa Scale</td>
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<td>OR</td>
<td>odds ratio</td>
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<tr>
<td>RERI</td>
<td>relative excess risk due to interaction</td>
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<td>T2DM</td>
<td>type 2 diabetes mellitus</td>
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ABSTRACT

Background: Depression among older adults is a growing public health concern worldwide. However, the pathophysiological mechanisms remain elusive among older adults, where depression often occurs in co-morbidity with chronic medical conditions such as Type 2 diabetes mellitus (T2DM). Inflammation has been posited to be involved in depression and in diabetes. Evidence has shown that a higher concentration of C-reactive protein (CRP), a marker of inflammation, is associated with depressed mood and T2DM.

Objectives: The first manuscript is a systematic review and meta-analysis that seeks to review and summarize the evidence on the relationship between CRP and depression in a general population of older adults (50 years and older). The second manuscript is a longitudinal study that aims to examine the bidirectional association of high CRP levels with elevated symptoms of depression among older adults. The third manuscript is a longitudinal study designed to assess the joint association of high CRP and depressive symptomatology with diabetes incidence in a community sample of older adults.

Methods: The systematic review identified cross-sectional and longitudinal studies in scientific databases, and the meta-analysis was conducted using random-effects models. The two original research studies used data from the English Longitudinal Study of Ageing (ELSA), a prospective study of community-dwelling older adults. Logistic and Cox proportional hazard regressions were conducted while adjusting for socio-demographic, lifestyle, and clinical variables.

Results: From the meta-analysis of cross-sectional studies, we observed a weak but positive correlation between CRP and depressive symptoms that was attenuated in the most adjusted model. From the meta-analysis of prospective studies, there was a small positive correlation between CRP levels at baseline and depressive symptoms at follow-up. In the first ELSA study, a high CRP level at baseline was associated with elevated depressive symptomatology at follow-
up but was attenuated after adjusting for metabolic and co-morbidity factors. An association in the opposite direction was not observed. Lastly, older adults with both high CRP levels and elevated depressive symptoms were at risk for T2DM.

**Conclusion:** The associations between depressed mood, diabetes, and CRP are complex and multifaceted. Higher CRP levels are associated with depressive symptomatology in a general population of older adults, but this association involves interplay with metabolic and chronic health conditions. Further research is required to broaden our understanding of the inflammation, depression, and diabetes relationship, thereby allowing opportunities for clinicians and researchers to explore new avenues for research, prevention, and treatment of these conditions.
ABRÉGÉ

Contexte: La dépression parmi les personnes âgées est une préoccupation croissante de santé publique reconnue à l’échelle mondiale. Par contre, les mécanismes physiopathologiques parmi cette population qui expliquent la comorbidité entre la dépression et d’autres maladies chroniques, dont le diabète de type 2, ne sont toujours pas clairs pour les chercheurs. Il se pourrait que l’inflammation puisse expliquer cette association. Les données scientifiques indiquent qu’une concentration plus élevée de la protéine c-réactive (PCR), un marqueur de l’inflammation, est associée aux symptômes dépressifs et au diabète de type 2.

Objectifs: Le premier manuscrit de ce mémoire est une revue systématique et une méta-analyse qui résume la recherche portant sur la relation entre la PCR et la dépression parmi une population générale de personnes âgées (50 ans et plus). Le deuxième manuscrit est une étude longitudinale qui vise à examiner la relation bidirectionnelle entre la PCR et les symptômes dépressifs élevés. Le troisième manuscrit est une étude longitudinale qui évalue l’association entre la PCR élevée, les symptômes dépressifs, et l’incidence de diabète de type 2 dans un échantillon communautaire d’adultes âgés.


Résultats: La méta-analyse d’études transversales nous a permis d’observer une corrélation faible mais positive entre la PCR et les symptômes dépressifs. Cette corrélation fut atténuée lorsque le modèle fut ajusté pour tous les facteurs de confusion. La méta-analyse d’études
prospectives a démontré une corrélation positive entre la PCR de base et les symptômes dépressifs observés lors d'examens de suivi. Dans la première sous-étude ELSA, les hauts niveaux de base de PCR étaient associés aux symptômes élevés de dépression lors d'examens de suivi, même en contrôlant pour les symptômes dépressifs de base, et pour les facteurs sociodémographiques, cliniques, et de style de vie. Une relation dans le sens opposé n’a pas été observée. Les adultes âgés ayant un haut niveau de PCR et de symptômes dépressifs avaient un risque élevé de développer le diabète de type 2.

**Conclusions:** Les associations entre les symptômes dépressifs, le diabète, et la PCR sont complexes. De hauts niveaux de PCR sont associés avec les symptômes dépressifs, mais cette association interagie avec des facteurs métaboliques et des conditions chroniques de la santé. De la recherche supplémentaire est nécessaire afin d’approfondir nos connaissances sur la relation entre l’inflammation, la dépression et le diabète, ainsi que d’explorer de nouvelles avenues de recherche pour la prévention et le traitement de ces conditions.
ACKNOWLEDGEMENTS

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I would like to thank people who helped lead me to the Epidemiology degree at McGill University: Dr. Jean-Philippe Thivierge, for his great patience as my undergraduate thesis supervisor and writing my reference letter; and Dr. Vanessa Taler, for inspiring my interests in epidemiology and gerontology, and for writing my reference letter. I thank the Department of Epidemiology, Biostatistics, and Occupational Health as well as the Douglas Mental Health Institute for allowing me the opportunity to pursue my graduate studies at McGill University.

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I would like to thank my friends both old and new for being tremendously supportive during my graduate studies. Thank you Rien, Aemal, Jana, Wendy, Sylvie, Jenny, Ashley, Olha, Cindra, Eileen, and Nick. The memories and friendships we made along the way are ones that I will cherish for years to come.

Lastly, I would like to thank my parents and my family for their support and confidence in my abilities. Special thanks to my dad for his encouragement throughout my academic career; Andrew, for all the advice he has given me; Annie, for always being there to cheer me on; and my extraordinary grandmother - for everything listed above with a synergistic effect – I wouldn’t be who I am without your confidence and love.
PREFACE AND CONTRIBUTION OF AUTHORS

This thesis contains six chapters. Chapter one is the introduction outlining the background, rationale, and general objective of the thesis. Chapter two is the literature review on depression, C-reactive protein, and Type 2 Diabetes Mellitus, and summarizes the three primary objectives of the thesis. Chapter three investigates the first objective with Manuscript 1. Chapter four examines the second objective with Manuscript 2. Chapter five addresses the third objective with Manuscript 3. Chapter six contains a restatement of objectives, discussion of findings, thesis limitations, future directions, and concluding remarks. Tables and figures appear at the end of each corresponding manuscript. A full reference list appears at the end of the thesis. This thesis conforms to the guidelines and requirements of a manuscript-based thesis at McGill University.

As the first author of all three studies, I (Bonnie Au) have made significant contributions regarding the conceptualization of hypotheses, research design, statistical analysis, data interpretation, as well as drafting and revising of these manuscripts. Dr. Norbert Schmitz, as thesis supervisor, contributed significantly regarding the study design, interpretation of results, critical revisions of all manuscripts, attaining funding, and obtaining the English Longitudinal Study of Ageing dataset from the UK Data Archive. He is a co-author on all three of the manuscripts. Dr. Kimberley J. Smith, as thesis committee member, has provided substantial contributions regarding the research design, theoretical framework, interpretation of findings, and manuscript revision for each of these manuscripts. She was the secondary reviewer for the papers reviewed in Manuscript 1 of this thesis. She is a co-author on all three of the manuscripts. Geneviève Gariépy (PhD Candidate) provided significant input regarding data analysis, interpretation of results, and critical revisions of the second and third manuscripts in this thesis. She is a co-author on the second and third manuscripts.
CHAPTER 1 – INTRODUCTION

We are all aging — not just only as individuals or societies but as a world [1]. The world’s population continues aging at an unprecedented rate [2], and older adults (defined in this thesis as people aged 50 years and older) constitute the fastest growing population globally because of increasing life expectancy, declining birth rate, and a transition from infectious to chronic non-communicable diseases as leading causes of disability and death [3-5]. As a result, the global proportion of older adults is projected to increase from over 800 million people in 2014 to more than 2 billion in 2050 [1]. Europe is estimated to have the highest population of older adults into the mid-twenty-first century with more than one in four European older adults [6]. Specifically, over one-third of the population in England is comprised of older adults [7].

The growth of older populations places challenges on the health care systems [2]. Research and findings on the physical and mental health of older adults have substantial implications with regards to policy-making and support to manage this growing demographic group [2, 6]. As a result of the increased awareness of the aging population, many regions all over the world have longitudinal aging studies to study the changing biological, physical, psychosocial, lifestyle, and economic aspects of older people’s lives. For example, the English Longitudinal Study of Ageing (ELSA) in England is one of many prospective aging studies that examine a variety of topics related to the aging process.

Depression among older adults is a growing health concern because it is one of the most common psychiatric diseases among this population [2, 8]. Depression among older adults can increase the economic burden and health care utilization, reduce the quality of life, and increase the risks of disability, morbidity and mortality [9-12]. Broadly, the present thesis is seeking to better understand depression, its biological link, and its role in health conditions among a community-sample of older adults.
CHAPTER 2 – LITERATURE REVIEW

2.1 Depression

2.1.1 Definition of depression

Depression is a psychiatric disorder that is related to a feeling of sadness and loss of interest that affects how one feels, thinks, as well as behaves, and can lead to a variety of emotional and physical problems [13]. Symptoms of depression include depressed mood, loss of interest or pleasure, diminished concentration, feelings of guilt and unworthiness, disturbed sleep, changes in appetite, thoughts of suicide, and impairment of daily functioning [14, 15]. Two commonly used standards for assessing depression are the Diagnostic and Statistical Manual of Mental Disorders (DSM), published by the American Psychiatric Association [14] and the International Statistical Classification of Diseases (ICD), published by the World Health Organization [15]. The DSM criteria are predominately used in North America, whereas the ICD standards are used in Europe.

2.1.2 Measurement of depression

Within clinical and research settings, two frequent types of method to assess depression symptoms are structured clinical interviews and rating scales [16]. Structured clinical interviews are typically administered by trained interviewers and are generally derived from standard criteria based on the DSM or ICD [14, 15]. The ICD-10 criteria require that, for at least two weeks, the individual must have reported two or more of: 1) Abnormally depressed mood; 2) Marked loss of interest or pleasure; 3) Decreased energy. In addition, at least two further symptoms must be identified from a list that includes: 4) Poor attention and concentration; 5) Loss of self-esteem and confidence; 6) Ideas of guilt and unworthiness; 7) Bleak and pessimistic view of the future; 8) Thoughts of self-harm or suicide; 9) Sleep disturbance; 10) Diminished appetite [15]. The DSM-V criteria require that, for most of the day and nearly every day for at
least two weeks, the person must have reported five or more symptoms with at least one symptom of: 1) depressed mood or 2) loss of interest or pleasure. The additional symptoms include: 3) Significant weight loss when not dieting or gain or decrease or increase in appetite; 4) Insomnia or hypersomnia; 5) Psychomotor agitation or retardation; 6) Fatigue or loss of energy; 7) Feelings of worthlessness or inappropriate guilt; 8) Diminished ability to think or concentrate or indecisiveness; 9) Recurrent thoughts of death or suicidal ideation or attempt [14]. Although individual clinical interviews are commonly viewed as the “gold standard” for diagnosing depression [17], they are costly to conduct in terms of time and expense. Thus, most psychiatric epidemiology research utilises alternative forms of measurement. One of these alternatives is a symptom rating scale.

Rating scales, such as the Center for Epidemiologic Studies Depression Scale (CES-D) [18] and the Geriatric Depression Scale (GDS) [19], assess the presence and severity of depressive symptoms and can be completed by respondents themselves. Higher scores on such scales suggest greater severity of depressive symptoms [16]. The level of agreement between rating scales and clinical interviews may vary depending on the specific scale and population, but generally they have a moderate to excellent validity [16]. For example, rating scales such as the CES-D and GDS, which are frequently used among the elderly population, were found to have high sensitivity (92%-100%) and specificity (84%-87%) in identifying depression [20, 21]. In epidemiological studies, self-ratings are common because of the practical advantages of speed and cost since they do not require clinical observation [16]. The ELSA study uses the CES-D scale, which is one of the most widely used scale to assess late life depression in community-dwelling older adults [21, 22]. Overall, the coexistence of a variety of depression measurement scales indicates that depression is a complex syndrome rather than just a single condition. There
is not one single symptom that is diagnostic for depression and different populations will present different symptomatology.

2.1.3 Epidemiology of depression

Depression is a severe and potentially debilitating psychiatric disorder that is frequently undiagnosed and untreated [21], and it is predicted to become the second leading global cause of disease burden in the next decades [23]. Among older adults, depression has been reported as the most serious and prevalent psychological problem, with estimates of 1% to 12% of community-dwelling older adults affected by major depression [10, 24-26], while the prevalence of depressive symptoms are present in 10-39% of community-dwelling older adults [27-29]. Approximately 8-10% of older adults with depressive symptoms develop to become major depression each year [30]. Depression among older adults adversely affects the quality of life of older persons in terms of functioning and well-being [11]. Depression increases the risks of morbidity and mortality [9, 10]. In older adults, depression is a risk factor for cardiovascular diseases [31], cancer [32], and diabetes [33].

2.1.4 Biological aspects of depression

The complex pathophysiology of depression remains incomplete [34]. Numerous biological mechanisms have been proposed to be involved in depression [34, 35]. One of the most prominent biological focus in depression has been the “monoamine hypothesis of depression” [35]. The monoamine hypothesis of depression postulates that depression results from deficits in neurotransmitters including norepinephrine, serotonin, and dopamine [36]. Evidence in support of this hypothesis has mainly emerged from the success of pharmaceutical treatments which increase the concentrations of these neurotransmitters in the synaptic clefts throughout the brain [35]. Yet, despite the popularity of this hypothesis, many findings and observations conflict with this theory [35, 37]. For example, Ruhe and colleagues [37] found that
drugs that depleted these monoamines did not impair the mood among healthy people. Thus, other causes and mechanisms are likely involved in the pathophysiology of depression.

One burgeoning biological basis of depression is the “cytokine hypothesis of depression” [38]. This theory of depression posits that increased inflammation is involved in the pathophysiology of depression [39]. Evidence linking inflammation in depression comes from several findings: firstly, depression (even in the absence of medical illness) is associated with elevated inflammatory markers [34]; secondly, inflammatory medical conditions (e.g. arthritis) are associated with a higher rate of depression [40]; thirdly, people treated with cytokines are at increased risk of developing depressive symptoms [41]; fourthly, medications with an effect on the immune system (e.g. interferon) can have an effect on mood [42]; and lastly, depression share similar attributes with a syndrome known as “sickness behaviour” which is a response to increased pro-inflammatory markers associated with infection or inflammation [34, 35].

2.2 Inflammation

2.2.1 C-reactive protein as a marker of inflammation

C-reactive protein (CRP) is an acute-phase protein that plays a crucial role in the human immune system [43]. Produced and secreted by the liver, CRP is a non-specific marker of inflammation, infection, and tissue damage [44]. It is mainly regulated by the inflammatory cytokine interleukin-6 (IL-6) [45]. CRP is one of the most commonly assessed marker of inflammation due to its stability during long-term frozen blood storage and the availability of standardized assays that are precise and relatively inexpensive [46]. Elevated levels of CRP have consistently been found to be associated with an increased risk of cardiovascular diseases, diabetes, and other metabolic abnormalities [47]. CRP is present at trace levels (≤ 3 mg/L) among healthy individuals [48], but concentrations > 3 mg/L have been used to indicate high risk of cardiovascular diseases [46] and have also been defined as low-grade inflammation [49-51].
In addition to CRP’s role in many physical diseases, there has been increasing interest in assessing CRP’s relationship with mood disorders such as depression [44, 45, 52].

**2.2.2 Depression and C-reactive protein**

Higher concentrations of CRP have been found in patients with depressive disorders compared to controls [53-55]. However, many of the initial studies examining CRP with depression were limited to clinical samples, and it has only been a recent phenomenon that the association between CRP and depression were assessed among broader population-based samples [45]. The current evidence from epidemiological studies linking CRP and depression is unclear. Some studies have observed a relationship between CRP and depression [56-59]. For example, Penninx and colleagues [60] showed that among community-dwelling older adults, compared to the non-depressed participants, those who were depressed had higher median plasma levels of CRP (1.96 vs. 1.66 mg/L, p =0.03). After dichotomizing the scores of their depression scale, stratifying the CRP levels into quartiles, and controlling for potential confounders, there was an association between the highest quartile of CRP levels with depressed mood. However, there are also studies that have identified a weak or null association between CRP and depression [61-63]. For example, Tiemeier et al. [64] examined the associations between CRP with both depressive symptoms and depressive disorder but did not observe any associations between CRP and depressed mood. Thus, the association between high CRP levels and depressed mood among older adults is inconsistent. Part of the difficulty and inconsistency with clarifying the association between CRP and depression is the heterogeneity of study samples and adjustments for different potential confounders, especially given many possible factors that affect levels of inflammatory markers as well as mood, such as health conditions like cardiovascular diseases and diabetes.
2.3 Type 2 Diabetes Mellitus

2.3.1 Epidemiology of Type 2 Diabetes Mellitus

Type 2 Diabetes Mellitus (T2DM) is a highly prevalent chronic condition and a major global burden of disease as a result of population growth, ageing of population, increased rates of obesity, and decreased levels of physical activity [65]. According to the International Diabetes Federation (IDF), almost 400 million adults in the world have diabetes mellitus, a figure which is expected to increase to nearly 600 million people by 2035 [66]. T2DM affects how the body metabolizes sugar (glucose). This condition occurs when the pancreas produces inadequate amounts of insulin to maintain normal glucose levels or when the body becomes resistant to the effects of insulin, and several long term complications of diabetes include retinopathy, nephropathy, and neuropathy [67]. The risk of T2DM increases with age, particularly after 45 years [68], and the prevalence of T2DM is highest among older adults [69]. The microvascular and macrovascular complications associated with T2DM are especially more pronounced and detrimental among older adults by increasing the risks of cognitive decline, physical disability, falls, and fractures [70, 71]. Alongside the physical complications associated with diabetes, it has also been found that people with diabetes commonly suffer from depression [72].

2.3.2 Depression and Type 2 Diabetes Mellitus

Depression is a significant co-morbid condition with T2DM [73]. Among people with T2DM, the prevalence of having elevated depressive symptoms ranges between 26-33% [74]. The presence of co-morbid depression and diabetes is associated with worsening glycemic control [75], increased number and severity of diabetes complications [76], as well as increased mortality [77]. The bidirectional relationship between depression and diabetes has been well established in various systematic reviews and meta-analyses [33, 78-80]. Type 2 diabetes is associated with a 15-24% increased risk of developing depression [78, 80], while a larger
association is observed in the other direction in which depression is associated with a 37-60% increased risk of developing T2DM [33, 78, 79]. Although the directionality between depression and diabetes remains discussed, there is a general consensus that the mechanisms linking depression and T2DM are multifaceted and represent a complex interaction between behavioural and physiological factors.

Depression is related to impaired glucose intolerance [81] and obesity [82]. Furthermore, poor health behaviours such as smoking, physical inactivity, and caloric intake among depressed individuals could increase the risk of T2DM [83]. Depression is also associated with physiological abnormalities including activation of the hypothalamic-pituitary-adrenal (HPA) axis, over-activity of the sympathetic nervous system, and increased production of pro-inflammatory cytokines, which can induce insulin resistance and lead to the development of diabetes [33, 84]. A biological mechanism that is common within both depression and T2DM is systemic low-grade inflammation characterized by up-regulated levels of pro-inflammatory cytokines such as IL-6 and acute-phase reactants such as CRP [74, 85].

2.3.3 C-reactive protein and Type 2 Diabetes Mellitus

There is accumulating evidence to indicate that inflammation plays a role in the pathogenesis of diabetes [86]. Studies have demonstrated that a higher CRP level is associated with cardio-metabolic events [43, 87, 88], and there has been increasing interest in assessing the relationship between CRP and diabetes [87, 89, 90]. Numerous prospective studies have identified that an elevated level of CRP is associated with increased risk of developing T2DM [87, 89, 90]. However, the mechanisms between CRP and development of diabetes remain unclear [85, 91]. Since depression has been separately linked to inflammation and diabetes, it is possible that individuals who have both depression and inflammation might be particularly vulnerable to diabetes. Previous evidence has found that having both depressed mood and
inflammation increased the risk for abdominal obesity, metabolic syndrome, and coronary heart disease [92, 93]. Ladwig and colleagues [93] found a significant interaction between CRP and depressive symptoms in predicting cardiac-related events, in which levels of CRP predicted cardiac-related events to a larger extent among men with depression compared to those without depression suggesting that CRP and depressive symptoms have an additive effect on the development of CVD. Given the strong correlation between CVD and diabetes, it is probable that having both depressed mood and high CRP levels can also predict the development of T2DM. However, there is a paucity of studies that simultaneously examines inflammation, depression, and diabetes. In order to better understand the role of CRP in the link between T2DM and depression, it is essential to first conduct a comprehensive review on the relationship between CRP and depression.

2.4 Study Objectives

2.4.1 Investigate the relationship between CRP and depression in a general population of older adults

Since the emergence of interest in examining the link between inflammation and depression, there have been several reviews conducted on the relation between CRP and depression [44, 45, 94, 95]. However, the review by Kuo et al. [45] was limited to a small selection of cross-sectional studies using community-based samples with inconclusive results. The subsequent review conducted by De Berardis and colleagues [44] was a narrative review, which is methodologically less rigorous compared to a systematic review and is also more vulnerable to bias. The third review, a meta-analysis, by Howren et al. [94] demonstrated a positive association between CRP and depression, however, the collection of studies were restricted to cross-sectional designs and therefore not possible to address the directionality of the relationship between depression and CRP. Additionally, the effect of age was inconsistent,
possibly due to the heterogeneity of the study samples. A recent meta-analysis using only longitudinal studies found a small association between raised CRP levels with subsequent development of depressive symptoms [95]; however, it was based on a small number of studies and even fewer ones which assessed the association among older adults. Thus, there is a need to conduct a systematic review to summarize the relationship between CRP and depression in a general population of older adults using both cross-sectional and longitudinal studies and then to perform a meta-analysis to present pooled estimates of associations.

2.4.2 Investigate the longitudinal bidirectional associations between CRP and depression among older adults

A lack of longitudinal studies has made it challenging to elucidate the relationship between CRP levels and depression, and only one study has simultaneously assessed the bidirectional relationship among older adults [96]. However, the study conducted by Stewart and colleagues [96] was limited to a small sample size of healthy volunteers, where the range of depressive symptoms scores could have been potentially restricted. Furthermore, their study analyzed CRP and depressive symptomatology continuously, which may limit clinical interpretations in terms of systemic inflammation or depression [96]. Based on the research gap identified from the systematic review, we seek to examine the association between high CRP levels at baseline with elevated depressive symptoms at follow-up and vice versa. Additionally, we also aim to examine the role of important covariates such as socio-demographics, health behaviours, metabolic factors, and health conditions in the CRP-depression relationship among older adults using data from the English Longitudinal Study of Ageing (ELSA), a prospective study of community-dwelling older adults.
2.4.3 Investigate CRP, depressive symptoms and risk of Type 2 diabetes among older adults

High levels of CRP and elevated depressive symptoms have been separately linked to the development of T2DM [78, 79, 89, 90]. Given that inflammation and depressed mood are related to diabetes risk, then having one condition may stimulate or moderate the other condition (i.e., have an additive effect) on the risk of diabetes. The third objective of this thesis is to assess whether the joint association of raised CRP levels and elevated depressive symptoms is associated with incidence of diabetes in a general population of older adults from ELSA, beyond the risks associated with high CRP levels and elevated depressive symptoms alone.
The association between C-reactive protein and depression among older adults in the general population: a systematic review and meta-analysis

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3.1 Abstract

Objectives: There is a growing interest in examining the relationship between depression and inflammation. Evidence indicates that a higher level of the inflammatory marker, C-reactive protein (CRP), is associated with depressed mood. The aim of this systematic review and meta-analysis was to examine the link between CRP and depression in a general population of older adults and to present pooled estimates of associations.

Method: We performed a systematic search in five databases for both cross-sectional and longitudinal studies reporting an association between CRP and depression among older adults, aged 50 or older. Two independent reviewers screened articles, and publications that met eligibility criteria were analyzed in the meta-analysis using a random-effects model.

Results: We found 17 studies (11 cross-sectional, 4 longitudinal, and 2 assessing both) that met the selection criteria. There was a weak positive association between depression and CRP in cross-sectional analyses. The Pearson correlation ($r$) for depression and CRP was 0.051 (95% confidence interval [CI]: 0.025-0.076), $p < 0.0005$ and 0.018 (95% CI: 0-0.037), $p = 0.0605$ in the least adjusted and the most adjusted cross-sectional data, respectively. There was a small but significant correlation between CRP levels at baseline with subsequent depressive symptoms at follow-up in longitudinal analyses (most adjusted $r = 0.068$ [95% CI: 0.026-0.109], $p <0.005$).

Conclusion: There was a weak positive cross-sectional association between depression and CRP that was attenuated in the most adjusted model. Furthermore, CRP levels at baseline were associated with depression at follow-up; however the relationship from depression to CRP could not be inferred due to scarcity of data.
3.2 Introduction

Older adults constitute the fastest growing population globally because of longer life expectancy, decreasing fertility rate, and a transition from infectious to chronic non-communicable diseases as leading causes of disability and death [3-5]. Depression is one of the most common psychiatric disorders among older adults [8]. Approximately 10-19% of older persons within the general population have symptoms of depression [27-29], and 2-4% experience a major depressive episode each year [97, 98]. Depressive symptoms among older adults can adversely affect quality of life in terms of functioning and well-being [11] and also increase the risks for morbidity and mortality [9, 10, 99].

Depression among older adults differs from depression in other age groups with regards to etiology, symptomatology, risk and protective factors, as well as outcomes [10]. There is speculation that older adults are more biologically prone to depression [99], and one biological explanation proposed to be important for depression is increased inflammation [39].

There are several plausible mechanisms for a bidirectional relationship between depression and inflammation. Firstly, depression may lead to inflammation. Psychological stress induces pro-inflammatory cytokine expression in brain regions associated with emotional regulation, and negative mood enhances the production and over-activation of pro-inflammatory cytokines such as interleukin-6 (IL-6) [100, 101]. Depression could also affect inflammation through hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis by increasing the immune system's resistance to glucocorticoid hormones that are responsible for terminating the inflammatory response [102, 103]. Secondly, inflammation may lead to depression. Pro-inflammatory cytokines can cross the blood-brain barrier and act directly on emotion-regulating brain structures involved in depression such as the amygdala [34, 104]. These pro-inflammatory
cytokines have also been shown to induce sickness behaviour, a syndrome characterized by vegetative, somatic, and psychological symptoms that are similar to depression [39].

There has been increasing research to show that inflammation is associated with depression in the general population [94, 95]. However, there is a paucity of research to examine the association between inflammation and depression among older adults. Levels of inflammatory markers tend to increase with age [105], although the exact mechanisms remain incomplete [106]. Increased inflammation among older adults has been linked to medical co-morbidity, but there is also evidence of elevated inflammatory markers even in healthy individuals and in the absence of acute infections [106]. It is therefore important to examine the relationship between depressed mood and inflammation within this demographic group where these two conditions commonly occur. It is also relevant to assess the relationship between depression and inflammation in a population-based context to determine whether an association exists among community-dwellers perhaps before they are clinically diagnosed with depression or before they have to be institutionalized due to poor health outcomes.

One marker of inflammation that has been extensively studied is C-reactive protein (CRP). There have been two meta-analyses that have examined the associations between CRP and depression [94, 95]. The first review found a positive association between CRP and depression. However, the review was not focussed on older adults and was restricted to cross-sectional studies, thereby limiting inferences about the directionality of the relationship [94]. The second meta-analysis using only longitudinal studies found a small association between raised CRP levels with subsequent development of depressive symptoms [95]; however, it was based on a small number of studies and only three studies which assessed the association among older adults. The present meta-analysis includes an additional three community-based longitudinal studies, as well as cross-sectional studies, that examined the relationship between CRP and
depression among older persons. In this systematic review and meta-analysis, we aim to assess the relationship between CRP and depression in a general population of older adults.

3.3 Methods

3.3.1 Data Sources/Search Strategy

We conducted this meta-analysis according to the MOOSE (meta-analysis of observational studies in epidemiology) guidelines [107]. We performed a systematic literature search for studies describing the relationship between CRP and depression in five major databases: MEDLINE via PUBMED (United States National Library of Medicine, Bethesda, MD, USA), EMBASE (Elsevier, Amsterdam, Netherlands), PsycINFO (American Psychological Association, Washington, DC, USA), ISI Web of Knowledge (Thomson Reuters, New York, NY, USA), and ProQuest Dissertations and Theses Online (ProQuest, Ann Arbor, MI, USA). Two search themes, “C-reactive protein” and “depression” were combined using the Boolean operator “and.” Search themes were exploded with relevant synonyms and corresponding keywords also searched in titles and/or abstracts (see Appendix A for detailed search strategy for each database). We conducted the search with restriction to human studies. No language restriction was implemented, but only English or French papers were reviewed. In addition, we searched the reference lists of all identified relevant published primary studies and review articles. Conference proceedings for relevant abstracts were also examined and authors were contacted for potential published works.

3.3.2 Study Selection

Two authors (B.A. and K.J.S.) independently reviewed literature eligibility, and discrepancies were resolved by consensus, or, when necessary, by a third author (N.S.). Individual studies were considered for inclusion in the systematic review if 1) the authors reported data from an original, peer-reviewed study or from conference abstracts; 2) the authors
provided cross-sectional or longitudinal associations (or both) between CRP and depression; 3) the study population was a cross-sectional or prospective cohort of non-institutionalized older adults. Fifty years and above was used to define older adults, which is consistent with the cut-off age used for community-based ageing studies [108, 109] and in a previous meta-analysis [95]. Only community-based studies were included because the epidemiological inferences are more easily applicable to the general population compared to hospital- or clinical-based studies. Thus, specific populations (e.g. patients after gastric bypass surgery and those with cancer or respiratory disease) were excluded in order to decrease the potential effects of heterogeneity and confounding variables in the analysis. For publications using the same study cohort, the higher quality publication was included for final analysis. When both publications had the same quality rating, the publication that analyzed a smaller subset of participants was excluded. We assessed eligible articles by first screening titles and abstracts followed by full-text evaluation (Figure 3.1).

3.3.3 Data Extraction

Data extraction of eligible studies was performed by two reviewers (B.A. and K.J.S.) independently. Reviewers extracted the following information from each study using a predesigned extraction form: study characteristics (first author, publication year, study design, study name and country, number of participants, follow-up duration for prospective studies), participants’ characteristics at baseline (age range or mean age, proportion of males), method of assessment for depression, method of assessment for CRP, analysis strategy (statistical models and adjusted covariates), and brief results (least adjusted and fully adjusted effect measure were reported if available). Disparities were resolved through discussion.
3.3.4 Quality Assessment

We assessed the quality of studies using the modified Newcastle-Ottawa Scale (NOS) for observational studies. The NOS is a comprehensive instrument that has established content validity and inter-rater reliability [110, 111]. The original NOS is an eight-item checklist, and the interpretation of the scale is based on a “star” system wherein a study is assessed on three broad categories: 1) the selection of study groups; 2) the comparability of study groups; 3) the measurement of exposure/outcome.

Cross-Sectional Studies

The scale was modified for cross-sectional studies so that one star was presented to a study for each of the following items under the “selection” category that were met: representativeness of the exposed sample (e.g., depressed participants from general population), representativeness of the non-exposed sample (e.g., non-depressed participants drawn from the same population as the exposed cohort), and reliability of exposure assessment (e.g. validated depression measurement). A maximum of two stars were allocated to the “comparability” category that accounted for relevant confounders: one star was presented if the study adjusted for at least two confounders (socio-demographics, lifestyle behaviours, or clinical variables including medical co-morbidity) and one more star was given if the study accounted for very high CRP levels $\geq 10$ mg/L (considered indicative of having acute infection, chronic illness, or autoimmune disorder [112]). Additionally, one star was presented for describing the assay method used for the measurement of CRP. A cross-sectional study that satisfied all the items was given a maximum of six stars (see Appendix B for detailed quality assessment).

Prospective Studies

The methodological quality of the prospective studies was evaluated similarly to the cross-sectional studies. Stars were presented in the same way for each category, with the
exception of the possibility of awarding three additional stars. One extra star for prospective studies was given when a study demonstrated that the outcome of interest was not present at the start initially. An additional two stars were given in the “outcomes” category regarding the length and adequacy of follow-up: one star was presented for a follow-up period of one year or more and one more star was given if the study accounted for attrition (less than 20% of participants loss to follow-up or a description was of lost was provided). A prospective study that satisfied all the items was given a maximum of nine stars (see Appendix C for detailed quality assessment).

### 3.3.5 Statistical Analysis

We analyzed cross-sectional and prospective studies separately. We computed correlation coefficients ($r$) with their corresponding 95% confidence intervals (CI) and graphically presented these on Forest plots. The majority of the studies reported both unadjusted and adjusted findings. Therefore, we separated these results into two groups to make them more comparable: “least adjusted $r$” (unadjusted $r$ or $r$ adjusted only for basic covariates such as age and sex, when both were available we used the unadjusted value) and “most adjusted $r$” (the reported value from the fully adjusted model). We present data using random-effects models instead of fixed-effects models. Random-effects models provide more conservative estimates, which are appropriate for our analyses given the potential heterogeneity across studies and various methodologies used by these studies. We carried out subgroup analyses by type of depression assessment, method of CRP analysis, age group, and adjustments for clinical covariates including chronic conditions, BMI and/or obesity, and medication use (anti-depressants and anti-inflammatory drugs). The latter two factors are particularly relevant because there has been evidence showing a significant association between BMI and inflammation [94], while medications could affect inflammatory levels [113]. There were not enough studies to conduct a subgroup analysis by gender.
Heterogeneity was examined by calculating the Cochrane $Q$ statistic and the inconsistency index ($I^2$) statistic. The Cochrane $Q$ statistic is distributed as $\chi^2$ and tests whether the individual effects are farther away from the common effect beyond what is expected by chance (a p-value < 0.10 indicates significant heterogeneity). The $I^2$ statistic is the percentage of total variability in effect measure that is attributable to variation across studies (a value of 25%, 50%, and 75% indicates low, medium, and high heterogeneity respectively). Publication bias was assessed visually using Funnel plot and statistically with Egger’s regression intercept test (a significantly different intercept from zero suggests presence of publication bias). All statistical analyses were conducted using Comprehensive Meta-Analysis Software (version 2.2 Biostat, Englewood, NJ, USA).

3.4 Results

3.4.1 Study Selection

A total of 4528 studies were identified in our initial search (Figure 3.1). Studies were assessed using broad screening criteria and 4416 studies were rejected including duplicates. The major reasons for rejection were unsuitability of the study population, design, exposure, or outcome for our study objective. We found two additional studies through our hand search of references from published studies and relevant reviews, resulting in 114 potentially relevant publications for a more detailed assessment. Of these studies, 92 were further excluded on review of their full text. The primary reasons for rejection were non-representative sample, below age requirement, or no measure of association between CRP and depression. Subsequently, there were 23 potential studies put forward for quality assessment. Two studies were discarded because of study sample duplication (whichever publication that was of lower quality or that used a subset of participants were excluded), two studies were rejected because they adjusted for depression as a covariate, and one study was excluded because of low quality.
After quality assessment, there were 17 studies remaining for this systematic review and meta-analysis (two studies contained both cross-sectional and prospective analyses [114, 115], eleven studies provided only cross-sectional data [60, 64, 116-124], and four studies provided only longitudinal analyses [96, 125-127]). Inter-rater agreement for studying screening was good (kappa = 0.75, 95% CI: 0.68 – 0.83).

Table 3.1 summarizes the characteristics and main findings of the thirteen cross-sectional analyses and Table 3.2 provides a summary of the six longitudinal analyses. Studies included in this review were published between 2002 and 2013. Most studies were from Europe (three from Netherlands, two from Italy, two from England, and one from Finland). Five studies were from North America (all from the USA), two studies from Asia (one in China and one in Singapore), and two studies from Australia. Sample size ranged from 192 to 5438 participants, with a total of 32,076 individuals from cross-sectional analyses and 10,562 individuals from prospective analyses included in this review. In prospective cohort studies, the observation period ranged from two to six years.

3.4.2 Study Review

Cross-Sectional Studies

Overall, 13 of the 17 studies conducted cross-sectional analyses on the association between CRP and depression. All the studies assessed depressive symptoms using rating scales. Three of these studies also assessed depression using diagnostic criteria [64, 114, 117]. Six studies assessed CRP as a continuous variable [64, 115, 119, 121, 122, 124], and seven studies analyzed CRP as a non-continuous variable by categorizing CRP levels based on cut-offs or quantiles [60, 114, 116-118, 120, 123].

All studies that measured depressive symptoms with rating scales and analyzed CRP as a continuous variable did not find a statistically significant association between increasing
depressive symptoms with higher CRP concentrations regardless of adjustment for confounders [64, 115, 119, 121, 122, 124].

Studies that measured depression through rating scales and analyzed CRP as a non-continuous variable produced various findings. There were several studies that did not yield a statistically significant association between elevated depressive symptoms and high CRP levels in the unadjusted and adjusted models controlling for basic covariates such as age and sex [64, 114, 117]. Adjustment for additional lifestyle and clinical covariates in these studies did not substantially change the effect size or overall conclusion. However, there were some studies that observed an association even after accounting for potential confounding. For example, Almeida et al.[116] studied 5438 Australian men, aged 70 and over, using the 15-item Geriatric Depression Scale (GDS) where a cut-off of ≥ 7 was used to indicate clinically significant depressive symptoms and high CRP was defined as > 3 mg/L. The authors [116] observed that men with CRP concentration > 3 mg/l had an increased odds of being depressed compared to men with CRP ≤ 3 mg/l after adjusting for variables including age, education, area of residence, smoking, obesity, treatment for depression, and cognitive impairment. However, this association became non-significant once they adjusted for physical co-morbidity (history of stroke and myocardial infarction). Similar studies produced point estimates which suggested that having elevated depressive symptoms was associated with increased odds of having high CRP levels when unadjusted and adjusted for basic covariates, but these associations were no longer statistically significant after adjustment for further confounding [116, 118, 120]. Conversely, there were also studies that found a significant statistical association between elevated depressive symptoms and high CRP levels even after accounting for socio-demographic, lifestyle and clinical covariates [60, 118]. Penninx and colleagues [60] analyzed data from 3024 older adults between 70-79 years old, using the 20-item CES-D scale where a cut-off of ≥ 16 was used to
indicate clinically significant depressive symptoms and CRP was dichotomized into above median levels (≥ 3.17 mg/L). They observed that the association between elevated depressive symptoms and high CRP levels remained after controlling for variables such as gender, age, total fat mass, diseases, use of anti-inflammatory drugs, use of anti-depressants, smoking, and alcohol use. Overall, various results were observed across studies that measured depression using rating scales and analyzed CRP as a non-continuous variable.

Of the three studies that assessed depression using diagnostic criteria, two of them analyzed CRP as a non-continuous variable [114, 117], and one of them assessed CRP as a continuous variable [64]. All three studies did not find a statistically significant association between having a diagnosis for major depression with high CRP concentrations regardless of adjustment for potential confounders.

**Prospective Studies**

There were six studies that conducted longitudinal analyses on the association between depression and CRP. All the studies assessed depressive symptoms using rating scales with one study that also measured major depression using diagnostic criteria [114]. All the studies examined CRP as the exposure and depressive symptomatology as the outcome.

There were only two studies that analyzed CRP as a non-continuous variable based on cut-offs or quantiles [114, 126]. Forti et al. [114] followed 4269 Italian older adults for four years and assessed depressive symptoms based on the 30-item GDS where a cut-off of ≥ 16 was used to indicate clinically significant depressive symptoms and CRP was categorized into quartiles. Using the bottom CRP quartile (< 2.0 mg/l) as the reference group, the authors did not find a significant association between the top quartile (> 5.0 mg/l) with having elevated depressive symptoms at follow-up. The second study conducted by Luukinen et al. [126] followed 404 Finnish older adults for 2.5 years and assessed depressive symptoms based on the
20-item Short Zung Self Rating Depression Scale where a score of $\geq 28$ was used to indicate clinically relevant depressive symptoms and CRP was grouped into standard cut-offs ($<1, 1 < 3$, and $\geq 3$mg/L). Using the lowest CRP group as the reference, the authors did not find a significant association between the highest CRP group and having elevated depressive symptoms at follow-up in the total population. However, they observed gender differences in the relationship between CRP and depressed mood where baseline high CRP levels were associated with depressed mood at follow-up only among males. Although Luukinen et al. [126] observed a gender interaction, the study was limited to a relatively small sample size and stratifying participants by sex and CRP group could have affected power. In sum, prospective studies analyzing depressed mood with CRP based on cut-offs is scarce and mixed. In particular, the method used for categorizing CRP is inconsistent.

There were four studies that assessed CRP as a continuous variable [96, 115, 125, 127]. Generally, most studies that assessed CRP as a continuous exposure found that having a higher CRP level at baseline was associated with increased depressive symptoms at follow-up even after accounting for potential confounders [96, 125, 127]. The study conducted by Hamer et al.[125] in 4323 English older adults, 50 years and older, using the 8-item CES-D scale showed that each standard deviation increase in baseline CRP was associated with a higher odds of depressive symptomatology at four years of follow-up after adjusting for age, gender, social occupational class, smoking, alcohol, baseline CES-D, and longstanding illness. However, there has also been evidence to indicate no association between baseline CRP and follow-up depressive symptomatology. For example, Baune et al. [115] analyzed data from 1037 Australian older adults, aged 70-79 years, using the 15-item GDS and found that CRP at baseline was not significantly associated with depressive symptoms at two years of follow-up after accounting for age, gender, education, medical disorders, body mass index, smoking status, and medication
usage. Overall, analogous to the cross-sectional findings, results from longitudinal analyses also demonstrate conflicting conclusions regarding the relationship between baseline CRP and depressive symptomatology at follow-up.

There was only one study that examined the association in the opposite direction (depression at baseline and CRP at follow-up) [96]. This study conducted by Stewart et al. [96] was the only study to analyze the bidirectional relationship among older adults. The authors followed 263 healthy volunteers, aged 50-70 years old, over a six year follow-up period and measured depressive symptoms using the Beck Depression Inventory-II (BDI-II). Using path analysis, Stewart et al. [96] observed evidence of a weak bidirectional relationship between BDI-II and CRP. However, neither of these longitudinal associations was statistically significant, possibly because the study was limited to a small number of healthy participating volunteers which could have underestimated the effect sizes of the associations.

3.4.3 Meta-analysis

**Cross-Sectional Studies**

Separate meta-analyses were performed using the least adjusted and most adjusted data that were available from the 13 cross-sectional analyses. Baune et al. [115] and Penninx et al. [60] did not report unadjusted findings, whereas Lu et al. [120] did not report adjusted results. The random effects summary correlation coefficient for 11 studies that reported least adjusted estimates showed a positive association between CRP levels and depressive symptoms (Figure 3.2). The effect was small yet highly significant, \( r = 0.051 \) (95% CI: 0.025-0.076), \( p < 0.0005 \). For the 12 studies that reported fully adjusted results (Figure 3), the association decreased and was no longer statistically significant, \( r = 0.018 \) (95% CI: 0.0-0.037), \( p = 0.0605 \). The \( I^2 \) was 14.85% and the \( Q \) statistic was 9.91 (\( p = 0.62 \)), suggesting a low and non-significant
heterogeneity. The funnel plot (Figure 3.4) was generally symmetric and Egger’s test produced a non-significant $P$-value (0.37), suggesting no publication bias.

**Prospective Studies**

Separate meta-analyses were also performed using the least adjusted and most adjusted data reported in the six longitudinal analyses. All studies reported adjusted results, whereas two studies conducted by Baune et al. [115] and Hamer et al. [125] did not report unadjusted findings. Luukinen et al. [126] presented separate results for men and women. The random effects summary correlation coefficient for four studies that reported least adjusted estimates showed a positive association between CRP at baseline and depressive symptoms at follow-up (Figure 3.5). The effect was small but significant, $r = 0.114$ (95% CI: 0.038-0.189), $p < 0.005$. For the six studies that reported fully adjusted results (Figure 3.6), the strength of the association reduced slightly but remained statistically significant, $r = 0.068$ (95% CI: 0.026-0.109), $p <0.005$. The $I^2$ was 26.55% and the $Q$ statistic was 8.17 ($p = 0.23$), indicating low to moderate and non-significant heterogeneity. The funnel plot (Figure 3.7) was fairly symmetric and Egger’s test produced a non-significant $P$-value (0.55), representing an absence of publication bias.

### 3.4.4 Subgroup Analyses

The subgroup results for the pooled most-adjusted Pearson’s correlations from cross-sectional and prospective analyses are presented in Table 3.3. In both cross-sectional and longitudinal findings, the pooled estimates for depression assessment based on rating scales were positive and significant, whereas assessments based on diagnostic criteria were not. The method of CRP analysis yielded contrasting findings in cross-sectional versus prospective analyses. Cross-sectional studies that analyzed CRP as a categorical variable showed a statistically significant and positive correlation between CRP and depression, whereas studies that analyzed CRP as a continuous measure did not. However, the opposite finding was observed with
prospective studies. The cut-off ages were also different between cross-sectional and longitudinal studies. Cross-sectional studies that consisted of adults 65 years or older were significant and positively correlated between CRP and depression, whereas studies comprised of adults 50 years or older were not. However, this finding was reverse with prospective studies.

Separate subgroup analyses were conducted for studies that adjusted for BMI or obesity, chronic conditions, use of anti-depressants, and use of anti-inflammatory medications. The association between depression and CRP in cross-sectional studies that adjusted for BMI or obesity as well as chronic conditions were not statistically significant. However, a significant association remained in prospective studies that controlled for either BMI or obesity and comorbidity. The association between depression and CRP in cross-sectional studies was not statistically significant regardless of controlling for the use of medications such as anti-depressants and anti-inflammatory drugs. There was a significant and positive correlation between CRP and depression for the prospective studies that did not account for medication usage, but no significant association was observed in the prospective studies that adjusted for anti-depressant and anti-inflammatory medication usage.

3.5 Discussion

The aim of this systematic review and meta-analysis was to examine the link between CRP and depression in a general population of older adults. To the best of our knowledge, this is the first study that includes both cross-sectional and longitudinal studies as well as presents pooled estimates of associations between CRP and depression among older persons. We identified a total of 17 observational studies (11 cross-sectional, 4 longitudinal, and 2 both) that examined the association between CRP and depression among older adults from community samples. Evidence from the least adjusted cross-sectional analyses indicated a weak but positive association between CRP and depressive symptoms. This association was no longer statistically
significant after accounting for potential confounding, although the point estimate remained indicative of a positive association.

Evidence from prospective analyses indicates that CRP levels at baseline have a small but significant association with subsequent depressive symptoms. However, given that all the longitudinal studies were assessing CRP levels at baseline as the exposure variable and depressive symptoms at follow-up as the outcome variable with only one study that assessed the relationship in the other direction, it was not possible to elucidate the bi-directionality between elevated CRP levels and depression.

Furthermore, there was some heterogeneity observed for both cross sectional and prospective analyses since many of the included studies differed by depression assessment, CRP analysis, and covariate adjustment.

3.5.1 Possible Mechanisms

It is unclear whether raised CRP levels are a causal or mediating risk factor for depressed mood. The small effect size between CRP and depressive symptoms may suggest that the involvement of CRP in depression as a condition is small. This is indicated by the statistically non-significant correlation in studies that used diagnostic criteria to assess for clinical depression. It is possible that the association between CRP and depression is more conservative because depression is a heterogenous condition and inflammation is related to certain symptoms or subtypes of depression. For example, pro-inflammatory cytokines have been shown to induce sickness behaviour, a syndrome characterized by vegetative, somatic, and psychological symptoms that are similar to depression [39]. Additionally, there is evidence indicating a differential role of inflammation in different depressive subtypes such as atypical versus melancholic depression [128]. Lamers and colleagues [128] observed that greater inflammation was associated with atypical depression (characterized by overeating, weight gain, and
hypersomnia) compared to melancholic depression (characterized by decreased appetite, weight loss, and insomnia).

Additional evidence to support that inflammation may be associated with atypical depression symptoms (i.e. weight gain) stems from findings that obesity is associated with CRP and depression [82, 129]. The adipose tissue is an important contributor of chronic low-grade inflammation in obesity, and weight gain is associated with increases in inflammatory factors [130]. In our results, the longitudinal association of baseline CRP with subsequent depressive symptoms was statistically significant in studies that adjusted for BMI or obesity, whereas the cross-sectional association in studies that adjusted for BMI or obesity was not significant. This contrasting finding could be due to the complexity of BMI and obesity in older adults. Although the prevalence of obesity in older adults is increasing, there is no correct definition of obesity in this demographic group [131]. The role of obesity in older adults is more complicated than it is in younger people [132]. This complexity is mainly due to sarcopenia, the age-related progressive loss of muscle and gain in fat, as well as due to the “obesity paradox” where obesity has an apparent protective effect and is associated with better health outcomes compared to normal weight [132]. The different cross-sectional and longitudinal findings could also be explained by the speculation that inflammatory markers correlate more with central adiposity in older adults than with BMI, which may not play a confounder role but rather a complementary or mediating role in the relationship [94, 130, 131].

Chronic diseases are more common among older adults than among younger adults [3]. People with chronic conditions such as cardiovascular diseases and diabetes have a higher prevalence of depression [78, 133, 134] as well as raised levels of inflammatory markers [46, 135, 136]. Thus, it was surprising to observe in the longitudinal subgroup analysis that CRP remained associated with depressive symptoms even after accounting for chronic co-morbidity.
There was no prospective study that did not adjust for chronic conditions, so we could not compare whether the association was substantially different in studies that adjusted versus studies that did not adjust for physical morbidity. However, the underlying mechanisms involving inflammation, depression, and chronic conditions are unclear. Multi-morbidity, the co-existence of two or more chronic conditions, is especially prevalent among older adults [137], and it is possible that the relationship between inflammation and depression varies among older adults with multiple conditions. Inflammatory conditions such as rheumatoid arthritis and periodontitis are common among older adults [138, 139], so it is also possible that the relation between inflammation and depression varies based on certain conditions that are associated with higher inflammatory levels.

Findings from this meta-analysis suggest that the method of analysis for CRP may play a role in the strength of the association between CRP and depressive symptoms. Distinguishing between whether CRP was analyzed as a categorical versus continuous variable has implications for clinical interpretation. Grouping CRP based on established cut-offs may indicate systemic inflammation, whereas assessing CRP continuously limits clinical applicability especially with regards to considering inflammation as a condition and risk factor. In cross-sectional studies, there was a stronger association between CRP and depressive symptoms when CRP was assessed as a categorical variable compared to studies that analyzed CRP continuously. In longitudinal studies, there was a statistically significant association between baseline CRP levels with subsequent depressive symptoms only when CRP was assessed as a continuous variable and not as a categorical variable. However, this statistically non-significant association could be the result of very few (only two) studies that analyzed CRP as a categorical variable.
3.5.2  **Strengths and Limitations**

One of the strengths of this review is the comprehensive search from several databases. By including cross-sectional as well as prospective analyses, we were able to examine not only cross-sectional associations between CRP and depressive symptoms but also able to assess causality via the longitudinal analyses. Furthermore, our study was conducted with a thorough evaluation of each included study and performed detailed analyses that took into account the least and most adjusted effect measures, methodology of exposure and outcome assessments, age groups, and adjustments for covariates allowing for additional comparability between findings among both cross-sectional and prospective studies. Lastly, by restricting our sample to older adults, potential heterogeneity across age groups could be reduced, which was observed from our findings of minimal heterogeneity with cross-sectional and longitudinal studies.

Despite these strengths, our review has several limitations. Firstly, our findings are based on only one inflammatory marker. The role of CRP in depression could be influenced by its primary inducer IL-6, a pro-inflammatory cytokine which could cross the blood-brain barrier and act directly on emotion-regulating brain structures involved in depression [34, 104, 140]. Secondly, our longitudinal analyses are based on a relatively small number of prospective studies. Additionally, there was only one study that examined depressive symptoms at baseline with subsequent CRP levels at follow-up, which did not allow this review to assess the bidirectionality between CRP and depressive symptomatology. In these studies, depression symptoms were assessed using several validated measures including a very few that used the *Diagnostic and Statistical Manual of Mental Disorders* (DSM) criteria, but many used rating scales. Although the rating scales have well-established psychometric properties and are commonly used in epidemiological studies, only the criteria based on the DSM that was used in a few of the included studies can offer a clinical diagnosis. Thus, results from this review should
be interpreted with caution since high scores on the rating scales are indicative of depressive symptoms and should not be inferred as a diagnosis for clinical depression. Although publication bias was not observed through statistical and visual analyses, such a bias is an inherent limitation in systematic reviews and meta-analyses. Similarly, language bias may also be an issue. Although we did not restrict our search by language, only English and French publications could be reviewed, which may account for the reason that the majority of the included studies were conducted in Western countries.

3.5.3 Future Directions

There are numerous research opportunities that can be explored regarding the relationship between inflammation and depression. Firstly, it is imperative to acknowledge the heterogeneity of inflammation and depression. There are many different types of inflammatory markers including pro-inflammatory cytokines such as IL-6 and acute-phase reactant proteins such as CRP. Thus, the association between inflammation and depression might vary across inflammatory markers. For example, the cross-sectional meta-analysis conducted by Howren et al. [94] found a larger effect size with IL-6 and depression compared to CRP and depression. Therefore, future studies should consider assessing multiple inflammatory markers simultaneously in order to better understand inflammation as a condition. Additionally, including a measure for clinical depression using diagnostic criteria rather than rating scales would increase the validity of the data and minimize overlap between symptoms of depression with symptoms of sickness behaviour. Subtypes of depression may also have different relationships with inflammation, but evidence for this theory is limited. Thus, a future step could group symptoms based on depression subtypes and assess whether inflammation is related to certain subtypes or cluster of symptoms.
Future research should also examine socio-demographics, lifestyle behaviours, and clinical variables including co-morbidities and metabolic characteristics to determine which factors may be mediators, moderators, or confounders in the relationship between depressed mood and inflammation among older adults. A subsequent important research would then be to examine similar questions among younger adults, allowing for comparability of findings in order to assess whether there is a difference between younger versus older adults and to better understand the possible mechanisms involved in the inflammation and depression relationship.

Finally, prospective data were scarce in this systematic review and their results were mixed. In particular, the majority of prospective data focused on CRP as the exposure at baseline and subsequent depressed mood as the outcome at follow-up. Thus, not only are high-quality longitudinal studies recommended to elucidate the temporal relationship between CRP and depression, but future longitudinal studies should simultaneously examine the bidirectional relationship between inflammatory markers and depression.

3.6 Conclusion

Overall, findings from this review suggest that there is a complex cross-sectional and prospective relationship between CRP levels and depressive symptoms. We found evidence to indicate a weak but positive correlation between CRP and depressive symptoms with cross-sectional analyses. However, the association was no longer statistically significant after accounting for additional confounders, thereby highlighting the need for future studies to assess the role of potential confounding variables in the CRP-depression relationship. Similar to cross-sectional analyses, prospective analyses indicated a small positive correlation between CRP levels at baseline and depressive symptoms at follow-up. The causal effect of depressive symptomatology on CRP levels could not be inferred based on the scarcity of available
longitudinal data, thereby highlighting the need for future studies to prospectively examine depression as the exposure at baseline and CRP as the outcome at follow-up.

**Acknowledgments:** The authors would like to thank Geneviève Gariépy for her statistical input.

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**Competing interests:** The authors have no competing interests to report.
Figure 3.1: Flowchart of study selection process
Table 3.1: Characteristics and main findings of included cross-sectional studies

<table>
<thead>
<tr>
<th>First author, year</th>
<th>Study name, Country</th>
<th>N</th>
<th>Sample Characteristics</th>
<th>Depression Measure</th>
<th>CRP Measure</th>
<th>Statistical Analysis</th>
<th>Confounders adjusted</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almeida et al., 2007 [116]</td>
<td>Health in Men Study (HIMS), Australia</td>
<td>5438</td>
<td>70-88 years</td>
<td>GDS-15 ≥ 7</td>
<td>CRP &gt; 3 mg/L vs. CRP ≤ 3 mg/L; Top Quartile (&gt; 3.82 mg/L) vs. Bottom Quartile (&lt; 1.01 mg/L)</td>
<td>Immuno-nephelometry</td>
<td>A) Spearman Correlation B) Logistic Regression</td>
<td>Age, non-English-speaking background, education, disadvantaged area of residence</td>
</tr>
<tr>
<td>Baune et al., 2012 [115]</td>
<td>Sydney Memory and Aging Study, Australia</td>
<td>1037</td>
<td>70-90 years</td>
<td>GDS-15 ≥ 6</td>
<td>Continuous</td>
<td>Turbidimetry</td>
<td>Linear Regression</td>
<td>Age, gender, years of education</td>
</tr>
</tbody>
</table>

Table 3.1 (Continued)
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<th>First author, year</th>
<th>Study name, Country</th>
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<th>Sample Characteristics</th>
<th>Depression Measure</th>
<th>CRP Measure</th>
<th>Statistical Analysis</th>
<th>Confounders adjusted</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bremmer et al., 2008 [117]</td>
<td>Longitudinal Aging Study Amsterdam, Netherlands</td>
<td>1285</td>
<td>65+ years</td>
<td>49, 14.8</td>
<td>1) CES-D ≥ 16 (threshold depression) 2) DSM-III Interview (major depression)</td>
<td>Above median (≥ 3.2 mg/L vs. Below median(&lt; 3.2 mg/L)</td>
<td>Immuno-assays</td>
<td>Multi-Nomial Logistic Regression</td>
</tr>
<tr>
<td>Forti et al., 2010 [114]</td>
<td>Conselice Study of Brain Ageing (CSBA), Italy</td>
<td>968</td>
<td>65+ years</td>
<td>44.9, 27.3% (Depressive symptoms) 9.7% (Major depression)</td>
<td>1) GDS-30 ≥ 10 2)retrospective DSM-IV diagnosis</td>
<td>Top Quartile (&gt; 5 mg/L) vs. Bottom Quartile (&lt; 2 mg/L)</td>
<td>Immuno-nephelometry</td>
<td>A) Spearman Correlation  B) Logistic Regression</td>
</tr>
<tr>
<td>Hamer et al., 2009 [118]</td>
<td>English Longitudinal Study of Ageing, England</td>
<td>5307</td>
<td>Mean Age (SD): 65.5 (10.1)</td>
<td>46% male</td>
<td>CES-D 8 ≥ 4</td>
<td>1)Very high: &gt;10 mg/L. 2)High: 3-10 mg/L. 3)Medium: 1 to &lt;3 mg/L vs. Low: &lt;1 mg/L (ref)</td>
<td>-</td>
<td>Logistic Regression</td>
</tr>
</tbody>
</table>

Table 3.1 (Continued)
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<th>Study name, Country</th>
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<th>Sample Characteristics</th>
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<th>CRP Measure</th>
<th>Statistical Analysis</th>
<th>Confounders adjusted</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kup et al., 2002 [119]</td>
<td>Cardiovascular Health Study, USA</td>
<td>4268</td>
<td>Mean Age (SD): 72.4 (5.5)</td>
<td>CES-D-10 ≥ 10</td>
<td>Continuous</td>
<td>Regression/ Mean</td>
<td>Age, gender, race</td>
<td>Smoking status, physical activity in kcal/week</td>
</tr>
<tr>
<td>Lu et al., 2013 [120]</td>
<td>Singapore Longitudinal Ageing Study, Singapore</td>
<td>2077</td>
<td>Mean Age (SD): 66.3 (7.8)</td>
<td>GDS -15 ≥ 5</td>
<td>Continuous</td>
<td>Logistic Regression</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>McDade et al, 2006 [121]</td>
<td>Chicago Health, Aging and Social Relations Study, USA</td>
<td>192</td>
<td>Mean Age (SD): 59.5 (4.3)</td>
<td>CES-D 20</td>
<td>Continuous</td>
<td>Multiple Linear Regression</td>
<td>Age, gender, ethnicity, education</td>
<td>Smoking symptoms in previous year, perceived stress</td>
</tr>
<tr>
<td>Milaneschi et al, 2009 [122]</td>
<td>Invecchiare in Chianti, aging in the Chianti area (InCHIANTI), Italy</td>
<td>991</td>
<td>Age: 65+</td>
<td>CES-D-20 ≥ 20</td>
<td>Continuous</td>
<td>Linear Regression</td>
<td>Age, gender, site, education</td>
<td>Alcohol use, smoking status, physical activity</td>
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</tbody>
</table>

Table 3.1 (Continued)
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<tr>
<th>First author, year, Country</th>
<th>Study name, Country</th>
<th>N</th>
<th>Sample Characteristics</th>
<th>Depression Measure</th>
<th>CRP Measure</th>
<th>Statistical Analysis</th>
<th>Confounders adjusted</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pan et al, 2008 [123]</td>
<td>Nutrition and Health of Ageing in China, China.</td>
<td>3289</td>
<td>50-70</td>
<td>CES-D-20 ≥ 16</td>
<td>Top Quartile (≥1.51 mg/L) vs. Below Quartile (&lt;1.51 mg/L)</td>
<td>Immuno-turbidimetric assay</td>
<td>B) Logistic Regression</td>
<td>Smoking, drinking, physical activity BMI, log-triglyceride, log-insulin, log-total cholesterol, use of anti-inflammatory medication, presence of comorbidity.</td>
</tr>
<tr>
<td>Penninx et al., 2003 [60]</td>
<td>Health, Aging and Body Composition Study, USA</td>
<td>3024</td>
<td>70-79 years</td>
<td>CES-D-20 ≥ 16</td>
<td>Above Median (≥3.17 mg/L) vs. Below Median (&lt;3.17 mg/L)</td>
<td>Immunosorbent Logistic Regression</td>
<td>Site, gender, age Smoking, alcohol use Total fat mass, diseases, use of anti-inflammatory drugs, and use of antidepressants</td>
<td>-</td>
</tr>
<tr>
<td>Stewart et al, 2008 [124]</td>
<td>Pittsburgh Healthy Heart Project, USA</td>
<td>316</td>
<td>Mean Age (SD): 60.6 (4.8)</td>
<td>BDI II continuous</td>
<td>Continuous</td>
<td>Immuno-nephelometric assay</td>
<td>Linear Regression</td>
<td>Age, sex, race, education, hostility Smoking, alcohol, physical activity Mean arterial pressure, HDL cholesterol.</td>
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</tbody>
</table>

Table 3.1 (Continued)
<table>
<thead>
<tr>
<th>First author, Year</th>
<th>Study name, Country</th>
<th>N</th>
<th>Sample Characteristics</th>
<th>Depression Measure</th>
<th>CRP Measure</th>
<th>Statistical Analysis</th>
<th>Confounders adjusted</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tiemeier et al., 2003 [64]</td>
<td>Rotterdam Study, Netherlands</td>
<td>3884</td>
<td>Age 60+ years</td>
<td>35.5, 7</td>
<td>1) CES-D-20 ≥ 16 2) Present State Examination (Depressive Disorder)</td>
<td>Continuous Nephelometric Logistic Regression Age, gender Smoking</td>
<td>History of stroke, functional disability, and atherosclerosis</td>
<td>1) Depressive Symptoms: OR = 1.06 (0.91-1.23); 2) Depressive Disorder: OR = 1.16 (0.96-1.39)</td>
</tr>
</tbody>
</table>

Abbreviations: ADL: activity of daily living; BDI: Beck Depression Index; BMI: body mass index; CES-D: Center for Epidemiologic Depression Scale; COPD: chronic obstructive pulmonary disease; CRP: C-reactive protein; CVD: cardiovascular disease; DSM: Diagnostic and Statistical Manual of Mental Disorder; GDS: Geriatric Depression Scale; IADL: instrumental activity of daily living; IDS: Inventory of Depressive Symptomatology; MMSE: mini–mental state examination; NSAID: Nonsteroidal anti-inflammatory drugs; OR: odds ratio; SDV: Socio-demographic variables; SD: standard deviation; SE: standard error; SEM: standard error of mean; SPPB: Short Physical Performance Battery.

Non-adjusted effect measures or effect measures adjusted exclusively for age and/or sex were considered “Least adjusted”; Fully-adjusted effect measures or effect measures adjusting for any additional demographic, lifestyle or clinical covariates were considered “Most adjusted.”
Table 3.2: Characteristics and main findings of included longitudinal studies

<table>
<thead>
<tr>
<th>First author, year</th>
<th>Study name, Country, Length of Follow-Up</th>
<th>Baseline Sample Characteristics</th>
<th>Depression Measure</th>
<th>CRP Measure</th>
<th>Statistical Analysis, Exposure, Outcome</th>
<th>Confounders adjusted</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baune et al., 2012 [115]</td>
<td>Sydney Memory and Aging Study, Australia 2 years</td>
<td>1037</td>
<td>70-90 years</td>
<td>45</td>
<td>GDS-15 ≥ 6</td>
<td>Continuous</td>
<td>Turbidimetry</td>
</tr>
<tr>
<td>Forti et al., 2010 [114]</td>
<td>Conselice Study of Brain Ageing (CSBA), Italy 4 years</td>
<td>4268</td>
<td>Mean Age (SD): 72.4 (5.5)</td>
<td>38.5</td>
<td>1) GDS-30 ≥ 10 2) retrospective DSM-IV diagnosis</td>
<td>Top Quartile (≥ 5 mg/L) vs. Bottom Quartile (&lt; 2 mg/L)</td>
<td>Immuno-nephelometry</td>
</tr>
</tbody>
</table>

Table 3.2 (Continued)
<table>
<thead>
<tr>
<th>First author, year</th>
<th>Study name, Country, Length of Follow-Up</th>
<th>Baseline Sample Characteristics</th>
<th>Depressions Measure</th>
<th>CRP Measure</th>
<th>Statistical Analysis, Exposure, Outcome</th>
<th>Confounders adjusted</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamer et al., 2009 [125]</td>
<td>English Longitudinal Study of Ageing, England 4 years</td>
<td>4323 Mean Age (SD): 63.3 (9.7)</td>
<td>CES-D ≥ 4</td>
<td>Continuous (looked at each SD)</td>
<td>Logistic Regression Exposure (CRP) Outcome (Dep)</td>
<td>Age, gender, social occupational class</td>
<td>Smoking, alcohol. Baseline CES-D, longstanding illness</td>
</tr>
<tr>
<td>Luukinen et al., 2010 [126]</td>
<td>Community, Finland 2.5 years</td>
<td>404 70+ years</td>
<td>SZSRDS ≥ 20 or use of an anti-depressant drug</td>
<td>Immuno-fluorometric assay</td>
<td>Logistic Regression Exposure (CRP) Outcome (Dep)</td>
<td>Gender, age, Current smoking</td>
<td>Medically treated diabetes mellitus BMI, MMSE, CVD event during the time between the SZRSRD examinations</td>
</tr>
<tr>
<td>Stewart et al, 2009 [96]</td>
<td>Pittsburgh Healthy Heart Project, USA. 6 years</td>
<td>263 Mean Age (SD): 61.0(4.8)</td>
<td>BDI II continuous</td>
<td>Continuous</td>
<td>Immuno-nephelometric assay</td>
<td>Path Analysis A) Exposure (CRP), Outcome (Dep) A) Exposure (Dep), Outcome (CRP)</td>
<td>Age, sex, race, education Smoking, alcohol, physical activity</td>
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</table>

Table 3.2 (Continued)
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<tr>
<th>First author, year</th>
<th>Study name, Country, Length of Follow-Up</th>
<th>Baseline Sample Characteristics</th>
<th>Depression Measure</th>
<th>CRP Measure</th>
<th>Statistical Analysis, Exposure, Outcome</th>
<th>Confounders adjusted</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>van den Biggelaar et al., 2007 [127]</td>
<td>Leiden 85-Plus Study, Netherlands 5 years</td>
<td>267</td>
<td>85+ years</td>
<td>37</td>
<td>GDS-15 &gt;2</td>
<td>Continuous</td>
<td>Linear Mixed Regression Exposure (CRP) Outcome (Dep)</td>
</tr>
</tbody>
</table>

Abbreviations: BAI: Beck Anxiety Index; BDI: Beck Depression Index; BMI: body mass index; CES-D: Center for Epidemiologic Depression Scale; CRP: C-reactive protein; CVD: cardiovascular disease; Dep: depression case; DSM: Diagnostic and Statistical Manual of Mental Disorder; GDS: Geriatric Depression Scale; MMSE: mini–mental state examination; NSAID: Nonsteroidal anti-inflammatory drugs; OR: odds ratio; SDV: Socio-demographic variables; SD: standard deviation; SE: standard error; SZSRDS: Short Zung Self Rating Depression Scale.

Non-adjusted effect measures or effect measures adjusted exclusively for age and/or sex were considered “Least adjusted”; Fully-adjusted effect measures or effect measures adjusting for any additional demographic, lifestyle or clinical covariates were considered “Most adjusted.”
<table>
<thead>
<tr>
<th>Study name</th>
<th>Correlation</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almeida et al., 2007</td>
<td>0.1266</td>
<td>-0.0503</td>
<td>0.2016</td>
<td>0.0012</td>
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<tr>
<td>Bremner et al., 2008</td>
<td>0.1302</td>
<td>-0.0504</td>
<td>0.2025</td>
<td>0.1672</td>
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<tr>
<td>Fodzi et al., 2010</td>
<td>-0.0754</td>
<td>-0.1928</td>
<td>0.0941</td>
<td>0.2156</td>
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<tr>
<td>Hamer et al., 2009</td>
<td>0.0981</td>
<td>0.0385</td>
<td>0.159</td>
<td>0.0018</td>
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<tr>
<td>Kop et al., 2002</td>
<td>0.0513</td>
<td>0.0214</td>
<td>0.0812</td>
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<tr>
<td>Lu et al., 2013</td>
<td>0.1083</td>
<td>0.003</td>
<td>0.2131</td>
<td>0.0430</td>
</tr>
<tr>
<td>McDade et al., 2006</td>
<td>0.0744</td>
<td>-0.0694</td>
<td>0.2153</td>
<td>0.3104</td>
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<tr>
<td>Milaneschi et al., 2009</td>
<td>0.0426</td>
<td>-0.0163</td>
<td>0.1033</td>
<td>0.17</td>
</tr>
<tr>
<td>Pan et al., 2008</td>
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<td>-0.0061</td>
<td>0.0822</td>
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<td>Stewart et al., 2009</td>
<td>0.0312</td>
<td>-0.0754</td>
<td>0.1411</td>
<td>0.6303</td>
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<td>Tiemeler et al., 2003</td>
<td>0.0161</td>
<td>-0.0025</td>
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</tr>
<tr>
<td></td>
<td>0.0504</td>
<td>0.0252</td>
<td>0.0755</td>
<td>1.054</td>
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</table>

**Figure 3.2**: Forest plot of included cross-sectional studies with least adjusted correlation coefficients and corresponding 95% confidence intervals.
**Figure 3.3:** Forest plot of included cross-sectional studies with most adjusted correlation coefficients and corresponding 95% confidence intervals.

<table>
<thead>
<tr>
<th>Study name</th>
<th>Correlation</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almeida et al., 2007</td>
<td>0.0547</td>
<td>-0.0414</td>
<td>0.1406</td>
<td>0.2643</td>
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<tr>
<td>Baune et al., 2012</td>
<td>0.0111</td>
<td>-0.0489</td>
<td>0.072</td>
<td>0.7201</td>
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<tr>
<td>Bremmer et al., 2009</td>
<td>0.0636</td>
<td>-0.143</td>
<td>0.2649</td>
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<td>Fodi et al., 2010</td>
<td>-0.0093</td>
<td>-0.2281</td>
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<tr>
<td>Hamer et al., 2009</td>
<td>0.0253</td>
<td>-0.0383</td>
<td>0.0005</td>
<td>0.4251</td>
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<tr>
<td>Kop et al., 2002</td>
<td>0.0177</td>
<td>-0.0124</td>
<td>0.0477</td>
<td>0.2489</td>
</tr>
<tr>
<td>McDade et al., 2006</td>
<td>0.0887</td>
<td>-0.0551</td>
<td>0.2239</td>
<td>0.2283</td>
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<tr>
<td>Milanesoli et al., 2009</td>
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<td>-0.1024</td>
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<td>0.016</td>
<td>-0.0E-4</td>
<td>0.0369</td>
<td>0.0605</td>
</tr>
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</table>
**Figure 3.4:** Funnel plot for publication bias of included cross-sectional studies.
Figure 3.5: Forest plot of included longitudinal studies with least adjusted correlation coefficients and corresponding 95% confidence intervals.
Figure 3.6: Forest plot of included longitudinal studies with most adjusted correlation coefficients and corresponding 95% confidence intervals.
Figure 3.7: Funnel plot for publication bias of included longitudinal studies.
Table 3.3: Results of most-adjusted subgroup analyses displayed as correlation coefficients with 95% confidence intervals

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>Cross-sectional</th>
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<td>No. of Studies</td>
<td>r (95% CI)</td>
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<td>0.020 (-0.014-0.055)</td>
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<td>0.018 (-0.039-0.074)</td>
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</tbody>
</table>

BMI = body mass index.

*Numbers of studies do not add up to the overall number of studies due to studies using multiple depression measures.

n.a indicates that the subgroup analysis could not be conducted as there was no study.
BRIDGE: CONNECTING MANUSCRIPTS 1 AND 2

There has been emerging interest in the relationship between CRP and depression [52, 94, 95]. Manuscript 1 of this thesis was a systematic review and meta-analysis which assessed the cross-sectional and longitudinal associations between CRP and depressed mood in a general population of older adults. The review provided an important summary of the literature and highlighted several key findings. Firstly, there was a small but positive correlation between CRP and depressive symptoms that was attenuated after accounting for additional confounders. Secondly, the strength of the CRP-depressed mood association differed based on whether CRP was analyzed as a continuous or categorical variable. Thirdly, there is a paucity of prospective studies on the relationship between CRP and depression. All of the prospective studies examined CRP as the baseline exposure and depressed mood as the follow-up outcome, with only one study that simultaneously assessed the relationship in the reverse direction and analyzed CRP as a continuous variable although it was limited in terms of generalizability due to its small sample size of healthy volunteers [96]. Thus, Manuscript 1 identified research gaps for future studies.

In sum, there is a need for future studies to prospectively examine depression at baseline and CRP at follow-up. Studies should assess the role of potential confounders in the relationship. There has also not been a longitudinal bidirectional study analyzing CRP based on a cut-off to represent low-grade inflammation. Therefore, we address these research gaps in Manuscript 2.

The primary objective of the second manuscript is to assess the bidirectional associations between CRP and depression from the English Longitudinal Study of Ageing (ELSA). ELSA is one of the few prospective community studies of older adults with repeated assessments and detailed biological data. A secondary objective is to evaluate the role of important covariates such as socio-demographics, health behaviours, metabolic factors, and health conditions in the CRP-depression relationship.
The longitudinal associations between C-reactive protein and depressive symptoms: evidence from the English Longitudinal Study of Ageing (ELSA)

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This manuscript is currently under minor revision to be re-submitted for publication.

Keywords: Depression Symptoms; Inflammation; C-reactive protein; Older adults; Longitudinal
4.1 Abstract

Objectives: The inflammatory marker C-reactive protein (CRP) is associated with depression. We examined the bidirectional relationship between CRP and symptoms of depression among older adults.

Method: The sample consisted of 3397 participants from the English Longitudinal Study of Ageing, a prospective study of community-dwelling older adults. CRP and depressive symptoms were measured at baseline and follow-up. A high CRP level was dichotomized as > 3 mg/L. Elevated depressive symptomatology was defined as ≥ 4 using the 8-item Center for Epidemiologic Studies Depression Scale. Logistic regressions computed the association between high CRP levels at baseline with elevated depressive symptoms at follow-up, and vice versa.

Results: After adjusting for baseline depressive symptoms, baseline high CRP levels were associated with subsequent elevated symptoms of depression (OR = 1.49; 95% CI, 1.19 - 1.88). This relationship was no longer significant after simultaneous adjustments for metabolic and health variables. In the other direction, after adjusting for baseline CRP levels, baseline elevated depressive symptoms was not associated with subsequent high CRP levels (OR = 1.12; 95% CI, 0.88 - 1.42).

Conclusion: High CRP levels at baseline are related to elevated depressive symptomatology at follow-up due to clinical factors. No association was found in the opposite direction.
4.2 Introduction

The older population is the fastest growing age group in the world as a result of longer life expectancy, declining fertility rate, and a shift from infectious to chronic non-communicable diseases as prevailing causes of disability and death [3, 5]. Depression among older adults is a growing public health concern worldwide [141].

Over a third of the population in England is comprised of older adults aged 50 years or older [7], and the prevalence rate of depressive symptoms is estimated at more than 17% among community-dwelling English older adults [22]. Depressive symptoms adversely affect the quality of life of older persons in terms of functioning and well-being [11], as well as increase the risks of morbidity and mortality [9, 10]. Depression among older adults differs from depression earlier in the lifespan in terms of etiology, symptom presentation, risk and protective factors, and potential outcomes [10]. Despite extensive research, the complex pathophysiology of depression remains elusive among older adults.

Consideration of biological risk factors for depression is important in older age because depression often arises in co-morbidity with chronic medical conditions [98]. These chronic medical conditions are associated with changes in endocrine, cardiovascular, and inflammatory risk factors [10]. Given the disease-related processes involved in older ages, it is possible that dysregulation in these biological pathways could be involved in the etiology of depression [10, 98]. One biological theory of depression which incorporates disease-relevant pathways is the cytokine hypothesis of depression [38]. This theory of depression posits that increased inflammation is involved in the pathophysiology of depression [39]. Specific inflammatory markers that are suggested to be involved in the biological etiology of depression include interleukin-6 (IL-6) and its ensuing acute phase response C-reactive protein (CRP) [142]. CRP is
a well-established marker for inflammation and is gaining increasing attention for its role in the pathophysiology of depression [94, 104].

Findings from community-based aging studies that have examined whether CRP is associated with depression in older adults have been inconsistent. Some studies have detected an association between CRP with depressed mood in older adults [60, 143], whereas other studies have not [117, 122]. A meta-analysis conducted by Howren et al. [94] found a weak positive association between CRP and depression. However, the review was based on cross-sectional studies which limit inferences on the causal mechanisms between the two conditions.

Prospective data from aging cohorts are needed to ascertain whether inflammation is a cause or consequence of depression. There is a scarcity of studies that have examined the longitudinal relationship between CRP and depressive symptoms among older adults. Results from these studies have been mixed, with some studies finding a prospective association between CRP and incident depression [126, 127] and others finding no association [114, 115]. A recent review using only longitudinal studies found a small association between raised CRP levels with subsequent development of depressive symptoms [95]. However, the meta-analysis consisted of only three studies which assessed the association specifically among older adults, and all three studies analyzed CRP as the baseline exposure and depression as the follow-up outcome which limits inferences on the directionality of the CRP and depression relationship.

Previous bidirectional studies in other age groups have yielded mixed results. Copeland and colleagues [144] found that depression predicted later CRP levels, but CRP levels were not associated with later depression, among children and young adults. However, the reverse finding was observed in a study of middle-aged civil servants where CRP levels predicted symptoms of depression while baseline depression symptoms did not predict CRP levels [145]. In another study comprised of mid-life women, Matthews et al. [146] found a bidirectional association
between depressive symptoms and CRP levels. In sum, the bidirectional relationship is inconsistent, and findings from these younger cohorts cannot be generalized to an older population.

Only one study has examined the CRP-depression relationship in both directions among older adults [96] and evidence of a weak bidirectional relationship between depressive symptoms and CRP was observed, but neither of the associations were statistically significant. However, the study conducted by Stewart et al. [96] was limited to a small sample size of 263 healthy volunteers, which may not be representative of a general population of older adults and an underestimation of effects is possible. Furthermore, depressive symptoms and CRP levels were analyzed continuously, which may limit clinical interpretation with regards to defining clinically relevant depressive symptomatology or low-grade inflammation. Thus, we aim to add to this area of research by investigating the associations in a larger representative community-dwelling sample of older adults and analyzing depressive symptoms and CRP levels based on established cut-offs.

The main objective of this study was to assess the bidirectional relationship between CRP and depression from the English Longitudinal Study of Ageing (ELSA), a prospective study of community-dwelling English older adults. A secondary objective was to evaluate the role of important covariates such as socio-demographics, health behaviours, metabolic factors, and health conditions in the CRP-depression relationship.

4.3 Methods

4.3.1 Design/Setting and Participants

ELSA is an ongoing nationally representative cohort study of older English adults living in households. The ELSA cohort consists of men and women born on or before February 29 1952. The sample was derived from households that had participated in the Health Survey for
England (HSE) in 1998, 1999, and 2001. The HSE recruited participants using multistage stratified probability sampling with postcode sectors selected at the first stage and household addresses selected at the second stage. For the present analyses, data from wave 2 (2004-2005) were used as baseline since this was the first occasion when clinical assessments including blood samples were collected by a nurse. Wave 4 (2008-2009) was used as the follow-up phase since it contains data on both clinical measures (collected every four years) and depressive symptoms (collected every two years). Participants provided full informed written consent to participate in the study and ethical approval was granted by the London Multi-centre Research Ethics Committee.

4.3.2 Measures

Depressive Symptoms

Symptoms of depression were assessed using the 8-item Centre of Epidemiological Studies Depression (CES-D) scale. The 8-item CES-D is a well-validated instrument designed to measure depressive symptoms[18]; the brevity of the tool, relative ease of administration, and validity in older populations are reasons that the test is appropriate for large population-based studies such as ELSA [147, 148]. Using a binary response format (yes vs. no), participants were asked about depressive symptoms including depressed mood, restless sleep, decreased energy and enjoyment in life, as well as feelings of loneliness and sadness. Symptoms of depression were dichotomized into low (CES-D < 4) and elevated (CES-D ≥ 4) symptomology groups in line with recommendations [149] and previous studies [150, 151].

C-reactive Protein

Blood samples were taken by a study nurse and serum CRP was analyzed by The Department of Clinical Biochemistry at the Royal Victoria Infirmary (NewCastle-upon-Tyne,
UK) using the N Latex CRP mono Immunoassay on the Behring Nephelometer II Analyzer (Dade Behring, Milton Keynes, UK).

From a clinical point of view and for ease of prognostic interpretation, CRP was dichotomized as normal (≤ 3 mg/L) and high (> 3 mg/L) levels. This cut-off value for CRP was based on clinical guidelines from the joint scientific statement from the Centers for Disease Control and Prevention (CDC) and American Heart Association (AHA) that concentrations of CRP above 3 mg/L be used to indicate high risk of cardiovascular diseases [46]. Furthermore, CRP levels above this concentration have been defined as low-grade inflammation [49-51]. This classification has also been used in previous studies with depression [116, 143].

**Covariates**

**Socio-demographic Variables**

Demographic variables included age, sex, and marital status (married, single never married, divorced/separated, or widowed). Education was assessed by asking participants to report their highest attained educational qualification (university degree or equivalent, less than university degree, or no qualification). Additional socio-demographic variable included working status (employed, unemployed, or retired). Ethnicity was not included because almost the entire sample (99%) was Caucasian.

**Health Behaviours**

Health-related behaviour questions included baseline smoking status (smoker or non-smoker) and physical activity. High physical activity was a derived variable based on the combination of occupational and leisure activities that was classified using the Allied Dunbar Survey of Fitness [152].

**Metabolic Measures**

Nurses collected anthropometric data (height and weight) and blood samples at waves 2
and 4. Body mass index (BMI) was derived from body weight and height measures based on the standard formula (kg/m²).

Blood samples were also analyzed for high density lipoprotein (HDL) cholesterol and triglycerides. Detailed information on the technicalities of the blood analysis is available in the 2004 HSE technical report [153].

**Health Conditions**

Self-reported doctor’s diagnosis of health conditions up to and including wave 2 was recorded including cardiovascular disease (angina, myocardial infarction, congestive heart failure, arrhythmia, and stroke), hypertension, diabetes, asthma, and arthritis (including osteoarthritis and rheumatoid arthritis). Information about whether the participant had suffered from any respiratory infection in the last three weeks was also collected.

### 4.3.3 Statistical Analysis

Variables are presented as means and standard deviations (continuous) or proportions (categorical) for baseline characteristics of study participants stratified by CRP group and depressive symptomatology group. Significance of differences in mean values was performed with two-sided t-tests and significance of differences in proportions was conducted with χ² tests.

We used logistic regression to calculate the odds ratio (OR) and 95% confidence intervals (CI) to examine the direction of the association between CRP and depression. Firstly, the relationship between baseline CRP levels and the presence of having elevated depressive symptoms at follow-up were assessed, adjusting for baseline symptoms of depression. Similarly, the relationship between baseline depressive symptoms and the presence of having high CRP levels at follow-up were assessed, adjusting for baseline concentrations of CRP. Because the prevalence of depression across baseline CRP category and the prevalence of having high CRP
across baseline depression status were above 10%, odds ratio do not approximate risk ratios and should not be interpreted as such in this study.

The contribution of covariates to the longitudinal association between CRP and depression was examined in logistic regression models by including each set of factors as blocks in the following order: Block 1, socio-demographic variables; Block 2, health-related behaviours; Block 3, metabolic factors; Block 4, health conditions. In each subsequent block, all variables from the previous blocks were retained in the model. Thus, the final model consists of simultaneously adjusting for all of the covariates. All analyses were performed using STATA/SE version 12 (StataCorp LP, College Station, TX, USA).

4.4 Results

A total of 9432 participants attended the wave 2 core assessment. The present study reports only on participants who consented and were able to give blood (n = 7666) in the wave 2 clinical assessment. Missing biological data was mainly because participants did not consent to give blood or were ineligible (people with clotting and bleeding disorders or taking anti-coagulant medication). After excluding participants that did not attend the core and clinical assessments at wave 4 (n = 2506), participants that did not have valid baseline or follow-up CRP measurement (n = 1746) and participants with missing baseline or follow-up CES-D score (n = 17), the final analytic sample is comprised of 3397 individuals. In comparison with the baseline cohort, the sample used in the present analyses was slightly younger (64.6 vs. 65.8 years), more likely to be employed (35.8% vs. 24.4%) and involved in high levels of physical activity (23.0% vs. 18.8%). They also had a lower prevalence of chronic conditions including diabetes (6.2% vs. 30.2%), cardiovascular conditions (19.5% vs. 43.0%), arthritis (36.4% vs. 52.0%) and asthma (12.8% vs. 33.5%).
At baseline, approximately 32% of the total sample had high CRP concentrations and 13% had elevated depressive symptoms.

The baseline sample characteristics of the study participants according to CRP group are presented in Table 4.1. The high CRP group differed from the normal CRP in that they were more likely to have elevated depressive symptoms, higher BMI and triglyceride levels, but lower HDL-cholesterol. They were also more likely to be older, female, current smokers, have hypertension, arthritis, asthma, and a respiratory infection in the last three weeks. However, they were less likely to be married, have a university degree or equivalent, employed, and have high physical activity level compared to the normal CRP group.

The baseline sample characteristics of the study participants according to depressive symptomatology group are presented in Table 4.2. The elevated depressive symptoms group differed from the low depressive symptoms group in that they were more likely to have higher CRP and BMI. They were also more likely to be female, current smokers, have hypertension, arthritis, and asthma. However, they were less likely to be married, have a university degree or equivalent, employed, and have high levels of physical active compared to the low depressive symptoms group.

Table 4.3 presents the association between high CRP at baseline with elevated symptoms of depression at the fourth year of follow-up. In participants with normal CRP at baseline we identified 234 (10.0%) cases of elevated depressive symptoms at follow-up, and in participants with high CRP at baseline we identified 169 (15.7%) cases of elevated depressive symptoms at follow-up. In the prospective analysis adjusted for baseline depressive symptomatology, high CRP was associated with elevated symptoms of depression. After simultaneously controlling for socio-demographics and health-related behaviours, the relationship was attenuated but remained significant (OR = 1.30; 95% CI, 1.03 - 1.66). The association was no longer statistically
significant after further adjustment for metabolic factors (OR = 1.13; 95% CI, 0.87 - 1.47). Additional adjustment for health conditions in the fully adjusted model slightly attenuated the estimate but remained non-significant.

The relationship between depressive symptomatology at baseline with high CRP level at the fourth year of follow-up is displayed in Table 4.4. In participants with low depressive symptoms at baseline we identified 942 (31.7%) cases of having high levels of CRP at follow-up, and in participants with elevated depressive symptoms at baseline we identified 161 (38.2%) cases of having high levels of CRP at follow-up. In the prospective analysis adjusted for baseline CRP levels, the association of elevated baseline symptoms of depression with high levels of CRP at follow-up was not significant (OR = 1.12; 95% CI, 0.88 - 1.42). Adjustment for the four sets of covariates did not alter the non-significant relationship between depressive symptoms at baseline and high CRP levels at follow-up.

In order to examine if the results could be affected by acute infections, we excluded participants with CRP concentrations > 10 mg/L from the analyses, but this exclusion did not reveal any large differences in the associations between CRP levels and depressive symptomatology.

4.4.1 Sensitivity Analysis

We repeated the main analyses in a subcohort excluding 422 participants with existing elevated depressive symptoms (CES-D ≥ 4) at baseline. There were 225 incident cases of depression at follow-up. In the unadjusted association, high CRP levels at baseline was associated with incidence of depression (OR = 1.45; 95% CI, 1.10-1.92). The association was no longer statically significant after adjustment for socio-demographics (OR = 1.27, 95% CI, 0.95-1.70) and was further attenuated after additional adjustment for metabolic factors (OR = 1.13, 95% CI, 0.83-1.57).
Similarly, we excluded 1078 participants with existing high levels of CRP (> 3mg/L) at baseline. There were 417 incident cases of high CRP levels at follow-up. In the unadjusted association, elevated depressive symptoms at baseline was not associated with incidence of high CRP levels (OR = 1.15, 95% CI, 0.82-1.59).

4.5 Discussion

The aim of this study was to examine the bidirectional association between high levels of CRP and symptoms of depression over 4 years in a sample of older English adults. Our findings suggest that the direction of association is likely from high levels of CRP to symptoms of depression, whereas symptoms of depression do not appear to lead to high levels of CRP among older adults.

In this study a high level of CRP at baseline was associated with elevated depressive symptoms at follow-up, a finding which mirrors other studies in older adults [126, 127, 154]. This association was independent of baseline depressive symptomatology, socio-demographic characteristics and health-related behaviours, but was no longer significant after adjustment for metabolic factors. This is likely due to the relationship between metabolic factors such as obesity with CRP [129] and depression [155]. Our results contrast findings from Stewart et al. [96] with six years of follow-up and did not find a statistically significant association even in the basic model adjusted for demographics, thereby further adding to the notion that the relationship between CRP and depressed mood is complex and involves interplay with other behavioural or physiological processes. For example, circulating concentrations of CRP could be influenced by health conditions including cardiovascular diseases [156], infection [157], and obesity [129]. Previous studies have shown that the relationship between inflammation and depression is often attenuated by controlling for metabolic factors such as BMI [96, 143, 158].
4.5.1 Explanatory Mechanisms: CRP to Depression

Various hypotheses have been developed to explain how CRP levels may lead to depression since CRP cannot cross the blood-brain barrier (BBB) and directly affect emotion-regulating structures in the brain. It has been suggested that elevated CRP levels likely indicate elevated levels of pro-inflammatory cytokines (such as its primary inducer IL-6), which can cross the BBB and act directly on brain structures involved in depression such as the amygdala [34, 104, 140]. Furthermore, the cytokine theory of depression posits a direct role of inflammation in the pathogenesis of depression via interactions of inflammatory markers with biochemical pathways commonly activated in people with depression such as the hypothalamic-pituitary-adrenal axis [159] and serotonin system [160].

4.5.2 Strengths and Limitations

The strengths of our study are the longitudinal design and controlling for various important confounding variables. Furthermore, this is the first study to take into consideration inflammatory conditions including arthritic disorders and asthma as important covariates. The nationally representative design of ELSA also offers value in the applicability of our results to the general community-dwelling population of older adults.

The limitations of the present study should be acknowledged. Although ELSA is designed to be nationally representative, the present study sample is likely healthier than the overall cohort due to restrictions implemented for eligibility of blood assessments. Additionally, the majority of the participants in the current study were white, which affects the generalizability to other ethnic groups. There is also possible measurement bias present in both CRP and depression. Measurement of CRP can be affected by acute infections, however our sensitivity analysis (removal of participants with possible acute infections; CRP > 10 mg/L [46]) did not substantially alter our results. There is also a possibility of measurement error for CRP. CRP is
an acute-phase reactant and guidelines suggest that it should be measured more than once and optimally two weeks apart to minimize fluctuations and provide a more stable estimate [46]. We were also unable to account for psychiatric or anti-inflammatory medications which could alter CRP levels [113].

Furthermore, depressive symptoms were assessed with a depression screening scale rather than a clinical interview. Inflammation has been shown to induce sickness behaviour, a syndrome characterized by vegetative, somatic, and psychological symptoms similar to depression [39]. Although we adjusted for common chronic conditions, it might be possible that the high depressive symptoms observed as a result of high CRP levels were due to sickness behaviours and scoring highly on somatic items such as increased fatigue and decreased sleep.

4.5.3 Future Directions

Additional research is necessary to ascertain the reliability of our findings and to elucidate the physiological mechanisms that underlie the relationship between inflammation and depression. Data on depression and CRP were collected simultaneously at waves 2 and 4 in ELSA, thus repeated data from more than two occasions as well as at varying time intervals are needed to determine their temporal association. For our present analyses, CRP was dichotomized based on a validated cut-off as a preliminary step in this research area to lay some groundwork for future studies in extending our findings by analyzing CRP in a different manner (i.e. as a continuous variable). Further research should also assess the bidirectional associations between other measures of inflammation (e.g. IL-6) and depressive symptoms. Future studies are also required to explore the causal pathways and determine whether interventions to decrease inflammation can reduce depression or whether interventions to reduce depression can lower inflammatory levels.
4.6 Conclusion

In conclusion, results from our analyses indicate that CRP is associated with elevated depressive symptoms at follow-up among older adults independent of socio-demographics and health behaviours. This suggests a possible inflammatory etiology of depression via clinical factors including metabolic and health conditions. Our results did not detect an association between depressive symptoms with high CRP levels at follow-up in our sample. Drawing attention to the role of inflammation in depression provides an opportunity to investigate the underlying mechanisms between inflammation and depression and to identify the metabolic pathways involved. Future research should focus on determining whether inflammatory markers can be of use for identifying people at risk for depression. Perhaps the action to prevent or treat inflammation might play a factor in averting depressive symptomatology. Thus, researchers and clinicians should recognize the complex relationship between inflammation and depression.

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Competing interests: The authors have no competing interests to report.

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<td>High physical activity level (%)</td>
<td>26.32</td>
<td>15.88</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>26.71 (3.98)</td>
<td>29.76 (5.28)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.69 (1.11)</td>
<td>1.99 (1.18)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.58 (0.39)</td>
<td>1.46 (0.37)</td>
</tr>
<tr>
<td>Hypertensive (%)</td>
<td>26.52</td>
<td>36.27</td>
</tr>
<tr>
<td>Diabetic (%)</td>
<td>5.78</td>
<td>7.14</td>
</tr>
<tr>
<td>CVD (%)</td>
<td>18.76</td>
<td>21.15</td>
</tr>
<tr>
<td>Arthritic (%)</td>
<td>32.38</td>
<td>44.99</td>
</tr>
<tr>
<td>Asthmatic (%)</td>
<td>11.21</td>
<td>16.23</td>
</tr>
<tr>
<td>Respiratory infection in last 3 weeks (%)</td>
<td>6.77</td>
<td>12.71</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; CES-D, Centre of Epidemiological Studies Depression; CRP, C-reactive protein; HDL, high-density lipoprotein.
Table 4.2: Baseline characteristics of study sample stratified by depressive symptomatology group (N = 3397)

<table>
<thead>
<tr>
<th></th>
<th>Low depressive symptoms (CES-D &lt; 4), n = 2975</th>
<th>Elevated depressive symptoms (CES-D ≥ 4), n = 422</th>
</tr>
</thead>
<tbody>
<tr>
<td>High CRP (%)</td>
<td>30.52</td>
<td>40.28</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64.54 (8.63)</td>
<td>64.75 (9.28)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>54.39</td>
<td>69.43</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married (%)</td>
<td>72.13</td>
<td>47.87</td>
</tr>
<tr>
<td>Single never married (%)</td>
<td>4.54</td>
<td>5.69</td>
</tr>
<tr>
<td>Divorced/separated (%)</td>
<td>9.61</td>
<td>21.09</td>
</tr>
<tr>
<td>Widowed (%)</td>
<td>13.71</td>
<td>25.36</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>University degree or equivalent (%)</td>
<td>15.53</td>
<td>9.52</td>
</tr>
<tr>
<td>Less than university (%)</td>
<td>55.13</td>
<td>50.00</td>
</tr>
<tr>
<td>No qualification (%)</td>
<td>29.34</td>
<td>40.48</td>
</tr>
<tr>
<td>Employment status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed (%)</td>
<td>37.10</td>
<td>26.30</td>
</tr>
<tr>
<td>Retired (%)</td>
<td>49.58</td>
<td>48.34</td>
</tr>
<tr>
<td>Unemployed (%)</td>
<td>13.32</td>
<td>25.36</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>12.07</td>
<td>21.67</td>
</tr>
<tr>
<td>High physical activity level (%)</td>
<td>24.21</td>
<td>14.49</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>27.56 (4.56)</td>
<td>28.41 (5.20)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.77 (1.13)</td>
<td>1.87 (1.23)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.54 (0.39)</td>
<td>1.56 (0.39)</td>
</tr>
<tr>
<td>Hypertensive (%)</td>
<td>27.56 (4.56)</td>
<td>28.41 (5.20)</td>
</tr>
<tr>
<td>Diabetic (%)</td>
<td>5.75</td>
<td>9.48</td>
</tr>
<tr>
<td>CVD (%)</td>
<td>18.69</td>
<td>25.36</td>
</tr>
<tr>
<td>Arthritic (%)</td>
<td>34.02</td>
<td>53.08</td>
</tr>
<tr>
<td>Asthmatic (%)</td>
<td>11.73</td>
<td>20.38</td>
</tr>
<tr>
<td>Respiratory infection in last 3 weeks (%)</td>
<td>8.20</td>
<td>11.85</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; CES-D, Centre of Epidemiological Studies Depression; CRP, C-reactive protein; HDL, high-density lipoprotein.
| Adjustments<sup>a</sup> | High CRP: > 3 mg/L  
<table>
<thead>
<tr>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude</td>
<td>1.49 (1.19 - 1.88)</td>
<td>Model 1</td>
<td>1.34 (1.06 - 1.70)</td>
<td>Model 2</td>
<td>1.30 (1.03 - 1.66)</td>
<td>Model 3</td>
<td>1.13 (0.87 - 1.47)</td>
<td>Model 4</td>
<td>1.08 (0.83 - 1.40)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Abbreviations: CRP, C-reactive protein; CI, confidence interval; OR, odds ratio.

<sup>a</sup>Additional adjustment for baseline depressive symptoms.

Model 1: adjustment for socio-demographics (age, sex, education, marital status, employment status).
Model 2: adjustment for Model 1 and health-related behaviours (smoking and physical activity level).
Model 3: adjustment for Model 2 and metabolic factors (body mass index, triglyceride, HDL cholesterol).
Model 4: adjustment for Model 3 and health conditions (hypertension, diabetes, cardiovascular disease, arthritis, asthma, recent respiratory infection).
Table 4.4: Association between elevated depressive symptomatology at baseline (2004-2005) and high CRP levels at follow-up (2008-2009)

<table>
<thead>
<tr>
<th>Adjustments\textsuperscript{a}</th>
<th>Elevated Depressive Symptoms: CES-D ≥ 4 OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>1.12 (0.88 - 1.42)</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.03 (0.81 - 1.31)</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.05 (0.78 - 1.29)</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.97 (0.75 - 1.25)</td>
</tr>
<tr>
<td>Model 4</td>
<td>0.95 (0.73 - 1.24)</td>
</tr>
</tbody>
</table>

Abbreviations: CES-D, Centre of Epidemiological Studies Depression; CRP, C-reactive protein; CI, confidence interval; OR, odds ratio.
\textsuperscript{a}Additional adjustment for baseline CRP levels.

Model 1: adjustment for socio-demographics (age, sex, education, marital status, employment status).
Model 2: adjustment for Model 1 and health-related behaviours (smoking and physical activity level).
Model 3: adjustment for Model 2 and metabolic factors (body mass index, triglyceride, HDL cholesterol).
Model 4: adjustment for Model 3 and health conditions (hypertension, diabetes, cardiovascular disease, arthritis, asthma, recent respiratory infection).
BRIDGE: CONNECTING MANUSCRIPTS 2 AND 3

Depression is a heterogeneous condition with a multi-faceted aetiology [94], and evidence has implicated higher levels of CRP in people with depressed mood [94, 95, 161]. However, the lack of longitudinal studies has made it challenging to elucidate the causal mechanisms linking CRP and depression [95]. Manuscript 2 of this thesis examined the longitudinal relationship in both directions between CRP and symptoms of depression among older adults. Results indicated that the direction of association is likely from high levels of CRP to symptoms of depression via clinical factors including metabolic and health conditions, whereas symptoms of depression did not appear to lead to high levels of CRP among older adults. These findings suggest a complex relationship between inflammation and depression involving multiple pathways and interactions with metabolic factors and physical morbidities.

CRP and depressive symptoms have been found to have an additive effect on the development of CVD [92, 93]. A subsequent research question is to examine whether having both depressed mood and high CRP levels can also predict the development of Type 2 diabetes mellitus (a prevalent condition among older adults), since each of these factors have been implicated with incidence of T2DM.

Although high levels of CRP and elevated depressive symptoms have been separately linked to the development of T2DM [78, 79, 89, 90], no study has simultaneously assessed these two conditions with risk of diabetes. Thus, the aim of Manuscript 3 of this thesis is to fill the gap in the inflammation, depression, and diabetes literature. The third manuscript assesses whether the joint association of raised CRP levels and elevated depressive symptoms is associated with incidence of diabetes in a general population of older adults from the ELSA study, beyond the risks associated with high CRP levels and elevated depressive symptoms alone.
C-reactive protein, depressive symptoms, and risk of diabetes: results from the English Longitudinal Study of Ageing (ELSA)

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Keywords: C-reactive protein; Depression; Diabetes; Longitudinal; Older adults
5.1 Abstract

Objectives: Raised levels of C-reactive protein (CRP), an inflammatory biomarker, and depressive symptoms are both independently linked to risk of diabetes. The purpose of this study was to assess the joint association of CRP and depressive symptomatology with diabetes incidence in a representative sample of English people ≥ 50 years old.

Method: Data were from the English Longitudinal Study of Ageing, a prospective study of community-dwelling older adults. The sample was comprised of 4955 participants without self-reported doctor-diagnosed diabetes at baseline. High CRP level was dichotomized as > 3 mg/L. Elevated depressive symptomatology was defined as ≥ 4 using the 8-item Center for Epidemiologic Studies Depression Scale. Incident diabetes was determined based on newly self-reported doctor-diagnosed diabetes. Cox proportional hazard regressions were used to examine the association between CRP and depressive symptoms with incidence of type 2 diabetes.

Results: During approximately 63.2 months of follow-up, 194 participants reported diabetes diagnosis. After adjustment for socio-demographics, lifestyle behaviours, clinical factors, and BMI, the hazard ratio for diabetes was 1.63 (95% CI 0.88-3.01) for people with elevated depressive symptoms only, 1.43 (95% CI 0.99-2.07) for people with high CRP only, and 2.03 (95% CI 1.14-3.61) for people with both high CRP and elevated depressive symptoms.

Conclusion: The presence of both high CRP levels and elevated depressive symptoms was associated with risk of diabetes. Further investigation into this relationship could aid in understanding the mechanisms underlying inflammation, depression, and diabetes.
5.2 Introduction

Diabetes mellitus is a major global burden of disease due to a combination of population growth, ageing of population, increased rates of obesity, and decreased levels of physical activity [65]. According to the International Diabetes Federation (IDF), almost 400 million adults in the world have diabetes mellitus, a figure which is expected to increase to nearly 600 million people by 2035 [66]. In England, over a third of the population is comprised of older adults, aged 50 years and older [7], with more than 15% of this population having diabetes mellitus [162].

Diabetes among older adults is associated with higher mortality, reduced functioning, and increased risk for institutionalization [163]. Furthermore, compared to their younger counterparts, older adults with diabetes are more prone to a number of microvascular and macrovascular complications, cognitive decline, physical disability, as well as falls and fractures [70, 71].

There is increasing interest among researchers and clinicians to explore the multifaceted link between mental health and risk of developing type 2 diabetes [164]. Various meta-analyses have shown that depression is associated with a 37-60% increased risk of developing type 2 diabetes [78, 79]. Behavioural and biological hypotheses have been proposed to explain the increased risk for type 2 diabetes among people with depressed mood. From the behavioural viewpoint, it is well-recognized that depressed individuals are less likely to be compliant with dietary and weight loss recommendations [165] and more likely to be physically inactive [166]. These behaviours are associated with the risk or worsening of obesity, which is an established major risk factor for developing type 2 diabetes [167]. From the biological aspect, depression stimulates the activity of the hypothalamic-pituitary-adrenocortical (HPA) axis and sympathetic nervous system, leading to raised levels of cortisol and catecholamines [166, 168].
neuroendocrine changes have been associated with abdominal adiposity and increased triglycerides and insulin, which are all recognized as predictors of type 2 diabetes [166].

Another posited biological explanation for the depression-diabetes link involves the inflammatory response. There is increasing evidence to support a link between inflammation and depression [94, 95]. Up-regulated levels of pro-inflammatory cytokines such as interleukin-6 (IL-6) and its ensuing acute-phase reactant C-reactive protein (CRP) have also been implicated in the pathophysiology of type 2 diabetes [74, 85]. Meta-analyses have found that an elevated level of CRP is associated with increased risk of type 2 diabetes [89, 90]. However, the mechanisms between CRP and development of diabetes remain unclear [91]. It is possible that CRP plays an indirect role in insulin resistance via obesity [91] and adipose tissue, which is a main source of pro-inflammatory cytokines [169]. CRP can also have direct harmful effects on vessel walls, which could impair endothelial permeability and lead to insulin resistance [170].

Individuals who have both depression and inflammation might be particularly vulnerable to diabetes. Previous evidence has found that having both depressed mood and inflammation increased the risk of abdominal obesity, metabolic syndrome, and coronary heart disease [92, 93]. However, there is a scarcity of studies that simultaneously examines inflammation, depression, and diabetes. A recent study by Doyle et al. [74] that investigated diabetes, depressive symptoms and inflammation among older adults found that higher levels of IL-6 and CRP are present in people with both type 2 diabetes and depression. However, the cross-sectional design of the study limits inferences on causality. No investigation has examined the association between CRP and depression with risk of diabetes, even though both depression and inflammation are associated with diabetes. The aim of this study was to examine whether the joint association of raised CRP levels and depression is associated with risk of diabetes in a
general population of older adults from the English Longitudinal Study of Ageing (ELSA), beyond the risks associated with high CRP levels and elevated depressive symptoms alone.

5.3 Method

5.3.1 Design/Setting and Participants

Data for this study came from ELSA, an ongoing national cohort of English adults living in households born on or before February 29 1952. The ELSA sample was drawn from households responding to the Health Survey for England (HSE) [108]. The HSE recruits participants using multistage stratified probability sampling with postcode sectors selected at the first stage and household addresses selected at the second stage. For the present analyses, data from wave 2 (2004-2005) were used as baseline since this was the first occasion when clinical assessments including blood samples were collected by a nurse. The present study used wave 3 (2006-2007), wave 4 (2008-2009), and wave 5 (2010-2011) as the follow-up phases for ascertaining newly diagnosed diabetes. Participants provided full informed written consent to participate in the study and ethical approval was granted by the London Multi-centre Research Ethics Committee.

5.3.2 Measurements

Assessment of depression

The 8-item Centre of Epidemiological Studies Depression (CES-D) scale was used to assess symptoms of depression. In the present sample, the CES-D demonstrated good internal reliability (Cronbach’s $\alpha = 0.79$). To determine depressive symptoms, each participant was asked the following questions with a binary option of ‘yes’ or ‘no’: Much of the time during the past week, 1) I felt depressed; 2) I felt everything I did was an effort; 3) My sleep was restless; 4) I was happy; 5) I felt lonely; 6) I enjoyed life; 7) I felt sad; 8) I could not “get going”. The total sum score was calculated, ranging from 0 to 8. We classified participants into low (CES-D < 4)
and elevated (CES-D ≥ 4) depressive symptom groups in line with recommendations [149] and what have been used in previous ELSA studies [118, 125, 150, 151].

**Assessment of inflammation**

Clinical assessments were conducted at wave 2 and included the collection of blood samples for the measurement of CRP. Serum CRP was analyzed by The Department of Clinical Biochemistry at the Royal Victoria Infirmary (NewCastle-upon-Tyne, UK) using the N Latex CRP mono Immunoassay on the Behring Nephelometer II Analyzer (Dade Behring, Milton Keynes, UK). Detailed information on the technicalities of the blood analysis is available in the 2004 HSE technical report [153].

Systemic inflammation was defined as CRP > 3 mg/L levels. This dichotomization was selected based on the clinical guidelines of the joint scientific statement from the Centers for Disease Control and Prevention (CDC) and American Heart Association (AHA) that CRP levels above 3 mg/L be used to indicate high risk of cardiovascular diseases [46]. This cut-off has been found to increase the risk of developing diabetes by four to six times compared to individuals below this level [171], and this categorization is frequently used in the research literature [172, 173].

**Assessment of diabetes incidence**

Incident diabetes was determined based on self-reported doctor-diagnosed diabetes at wave 3 (2006-2007), wave 4 (2008-2009), and wave 5 (2010-2011). For our study, participants who responded positively to having a doctor diagnosis of diabetes at or before baseline (2004-2005) were excluded from the analysis. Incident diabetes were epidemiologically classified as type 2 diabetes, which is in line with previous ELSA studies [174, 175].
Covariates

Socio-demographic, lifestyle, and clinical variables at baseline were used as covariates since they are established risk factors for diabetes [174]. Demographic variables included age, sex, and marital status (married, single never married, divorced/separated, or widowed). Ethnicity was not included because the majority of the sample (99%) was Caucasian. Socioeconomic status (SES) was assessed by highest education attainment (university degree or equivalent, less than university degree, or no qualification), working status (employed, unemployed, or retired) and quintiles of total net non-pension household wealth.

Lifestyle variables included smoking status (smoker or non-smoker), frequency of alcohol consumption during the past 12 months (none, occasional, or daily), and level of physical activity (sedentary, low, moderate, or high). Physical activity was a derived variable based on the combination of occupational and leisure activities that was classified using the Allied Dunbar Survey of Fitness [152].

Clinical variables included data on existence of any longstanding illness or disability (yes or no), self-reported doctor-diagnosis of cardiovascular conditions including hypertension, angina, congestive heart failure, abnormal heart rhythm, heart murmur, heart attack, and stroke (no comorbid cardiovascular condition or at least one comorbid cardiovascular condition) and non-cardiovascular morbidities including asthma, arthritis, chronic lung disease, cancer/malignant tumor excluding minor skin cancers, osteoporosis, Parkinson's disease, Alzheimer's disease, and dementia or other serious memory impairment (no comorbid non-cardiovascular disease or at least one comorbid non-cardiovascular disease).

Participant weight and height were collected by nurses, and body mass index (BMI) was derived from the standard formula (kg/m²).
5.3.3 Statistical Analysis

We classified participants according to their CRP (≤ 3 mg/L vs > 3 mg/L) and CES-D score (< 4 vs ≥ 4 depressive symptoms) status into the following four categories:

1) Low CRP and low depressive symptoms: CRP ≤ 3 mg/L, CES-D < 4;
2) Elevated depressive symptoms only: CRP ≤ 3 mg/L, CES-D ≥ 4;
3) High CRP only: CRP > 3 mg/L, CES-D < 4;
4) High CRP and elevated depressive symptoms: CRP > 3 mg/L, CES-D ≥ 4.

Variables are presented as means and standard deviations (continuous) or proportions (categorical) for baseline characteristics with respect to these categories. Cross-tabulations and analysis of variance with Scheffé post hoc tests were performed to examine baseline differences between these categories.

The prospective association between categories of baseline CRP levels and depressive symptom status with incidence of diabetes was assessed using Cox proportional hazard regression models, in a crude model, and after adjusting for a priori defined covariates in a stepwise approach: 1) socio-demographics (age, sex, marital status, educational attainment, total household wealth); 2) health behaviours (smoking status, physical activity level, alcohol consumption); 3) clinical variables (existing longstanding illness or disability, cardiovascular co-morbidity, non-cardiovascular co-morbidity); 4) and BMI. The group with low CRP and low depressive symptoms served as the reference group. Follow-up time was calculated as the time elapsed from the date of baseline interview to the date of reported diagnosis of diabetes. If the date of diabetes diagnosis was not reported, then the follow-up time was based on the average time between the baseline interview and most current interview dates. The estimates for the risk of developing diabetes in the Cox regression models are presented as hazard ratios (HR) and
95% confidence intervals (CI). Statistical tests (Schoenfeld residuals test) and graphical plots (Kaplan-Meier observed survival curves versus Cox predicted curves) were used to check the proportional hazards assumption.

In order to account for the possibility of undiagnosed diabetes at baseline, we conducted sensitivity analyses where participants were classified as having undiagnosed diabetes if they did not report being told by a doctor of having diabetes, but they had a fasting blood glucose ≥ 7.0 mmol/l after fasting for at least 8 hours. While not providing an exhaustive diagnosis of diabetes, this criterion has been employed by previous epidemiological studies as a proxy for undiagnosed diabetes [176, 177].

To provide further insight on the joint relationship of CRP and depressive symptoms with diabetes, we tested for the occurrence of biological interaction. An additive effect is when two conditions, acting independently of each other, are both needed to cause a disease outcome; that is, the risk of having a combination of two risk factors equals the sum of the risks from only one factor [178]. Biological interaction exists when the risk of disease having both conditions is in excess of what would be expected under the additive model [179]. In Cox proportional hazard regression models, biological interaction is different compared to statistical interaction because in Cox regression analyses, statistical interaction is exponential and is under the multiplicative model [180]. Biological interaction was assessed using the Relative Excess Risk due to Interaction (RERI) applying the methodology proposed by Andersson et al. [181]. From the hazard model, the RERI was calculated using the formula: $RERI = HR_{CD} - HR_{C} - HR_{D} + 1$, where $HR_{CD}$ is the HR for both high CRP and elevated depressive symptoms, $HR_{C}$ is the HR for high CRP levels only, and $HR_{D}$ is the HR for elevated depressive symptoms only. $RERI > 0$ implies a synergistic, additive interaction in which the two independent conditions are affecting
each other. We adjusted for the same covariates as those in the Cox regression models. All analyses were performed using STATA/SE version 13 (StataCorp LP, College Station, TX, USA).

5.4 Results

A total of 7666 people participated in the wave 2 clinical assessments. We included participants with valid baseline CRP measurement (n = 5899). Invalid CRP was primarily because there was insufficient blood for measurement as well as because a long period between blood collection and receipt by the laboratory rendered the blood sample to be invalid. Compared to those who provided a valid CRP measurement, those without a valid serum CRP sample were more likely to have elevated depressive symptoms (14% vs. 19%), be older (66 vs. 69), widowed (17% vs. 22%), have no qualifications (36% vs. 43%), retired (53% vs. 60%), in the lowest wealth quintile (17% vs. 24%), non-alcoholic drinkers (10% vs. 14%), sedentary (4% vs. 11%), have a long-standing illness (55% vs. 65%), cardiovascular morbidity (51% vs. 66%), as well as a non-cardiovascular condition (53% vs. 59%), and have a slightly higher BMI (27.8 kg/m² vs. 28.5 kg/m²). We included individuals with complete CES-D scores at baseline (n = 5897). People with baseline diabetes (n = 412) were excluded from the analysis. After excluding 530 individuals that did not return for any of the subsequent follow-ups, the present analysis sample is comprised of 4955 participants. Compared to those who attended subsequent waves, those who did not return for any of the follow-ups were more likely to be older (66 vs. 69), male (44% vs. 49%), have no qualifications (34% vs. 49%), retired (52% vs. 57%), in the lowest wealth quintile (16% vs. 21%), smokers (14% vs. 18%), non-alcoholic drinkers (9% vs. 13%), sedentary (3% vs. 10%), have a cardiovascular disease (48% vs. 53%), as well as non-cardiovascular condition (52% vs. 58%).
At baseline, 2901 (58.5%) participants had low CRP levels and low depressive symptoms, 392 (7.9%) participants had elevated depressive symptoms only, 1397 (28.2%) participants had high CRP levels only, and 265 (5.3%) participants had both high CRP levels and elevated depressive symptoms. Table 5.1 presents the baseline characteristics across these four categories. Having both high CRP levels and elevated depressive symptoms was related to being female, not being married, lower SES, being a current smoker, not drinking alcohol, not being highly physically active, higher prevalence of cardiovascular and non-cardiovascular morbidity, and higher BMI.

A total of 194 incident cases of doctor-diagnosed type 2 diabetes were reported over an average of approximately 63.2 months of follow-up (Table 5.2). Participants with low CRP and low depressive symptoms had the lowest incidence of diabetes (4.7 per 1,000 person-years), and participants with both high CRP levels and elevated depressive symptoms had the highest incidence of diabetes (18.0 per 1,000 person-years).

Table 5.3 shows the HRs for diabetes across the four groups. In comparison to individuals with low CRP and low depressive symptoms, the HR for diabetes was 1.84 (95% CI 1.07-3.16) for participants with elevated depressive symptoms only, 2.45 (95% CI 1.78-3.37) for people with high CRP levels only, and 4.31 (95% CI 2.70-6.86) for those with both high CRP levels and elevated depressive symptoms in the age- and sex-adjusted model (Table 5.3). Adjusting simultaneously for socio-demographics and health behaviours did not significantly alter the HRs for any of the groups, and they all remained to be associated with risk of diabetes. Further adjustment for co-morbidities rendered the group with elevated depressive symptoms only to be no longer associated with risk of diabetes. Additional adjustment for BMI attenuated the association with diabetes risk and was no longer statistically significant for the group with
high CRP levels only. For participants with both high CRP levels and elevated depressive symptoms, the association with incidence of diabetes decreased after adjustment for all covariates, but remained significant (HR = 2.03, 95% CI 1.14-3.61).

A sensitivity analysis that excluded those with undiagnosed diabetes at baseline (n = 40) produced results similar to those of the main analysis. The HR for diabetes was 1.69 (95% CI 0.89-3.21) for participants with elevated depressive symptoms only, 1.37 (95% CI 0.92- 2.03) for people with high CRP levels only, and 2.17 (95% CI 1.20-3.94) for those with both high CRP levels and elevated depressive symptoms in the fully adjusted model.

In the biological interaction analysis, the RERI for incidence of diabetes was 0.78 (95% CI -1.03 – 2.60) in the unadjusted analysis and 0.25 (95% CI -1.40 – 1.35) in the fully adjusted analysis. The point estimates are greater than 0 which could indicate that there is a positive interaction between high CRP levels and elevated depressive symptoms under the additive model. However, due to the confidence intervals containing the null value, the joint effect of high CRP levels and elevated depressive symptoms is not statistically significant in exceeding the sum of the individual effects of high CRP levels and elevated depressive symptoms.

5.5 Discussion

The aim of this study was to examine the association of CRP and depressive symptomatology in the development of diabetes over approximately 6 years of follow-up. To the best of our knowledge, this is the first prospective study that simultaneously assesses the joint relationship between CRP and depressive symptoms with risk of diabetes in a large population-based cohort. Our findings indicate that participants with both high CRP levels and elevated depressive symptoms were more likely to develop diabetes even after accounting for sociodemographics and potential confounding factors. However, this association was not beyond that expected for high CRP levels alone or elevated depressive symptoms alone as indicated by a
statistically non-significant biological interaction. We did not observe a statistically significant risk for diabetes in people with only high CRP levels or with only elevated depressive symptoms after adjusting for relevant confounders.

Previous work from ELSA has established that elevated depressive symptoms were associated with an increased risk of diabetes [174]. However, their study did not take into consideration the role of CRP, whereas our work expands on this research to account for CRP in the depression and diabetes relationship. Findings from our study indicate that elevated depressive symptoms are associated with higher risk of type 2 diabetes only when occurring together with high CRP levels after controlling for well-established diabetes risk factors. Despite the numerous prospective studies examining the relationship between depression and incident diabetes [78, 79], only a few have included CRP measurements [33, 182]. These studies found that adjusting for CRP levels as a covariate in their analyses did not explain the association between depression and diabetes. Unlike the previous studies that adjusted for CRP as a continuous variable in their statistical models, we opted to stratify CRP based on an established cut-off (> 3 mg/l) to explore the interaction effect of low-grade systemic inflammation in the relationship between depression and diabetes and for ease of clinical interpretation. Furthermore, there has not been a study on the relationship between CRP and risk of type 2 diabetes that accounted for depressed mood [89]. Thus, our study was the first to examine both CRP and symptoms of depression, and we found that people with both high CRP levels and elevated depressive symptoms have a higher risk for diabetes.

5.5.1 Possible Mechanisms

Both CRP and depression are independent risk factors for diabetes [183, 184]. However, results from our study indicate that these two risk factors in combination lead to an increased risk
of diabetes. The pathway linking depression and inflammation to diabetes incidence is likely complex and multifaceted due to the possibility of bidirectional relationships and interactions of overlapping diabetes risk factors associated with both predictors.

Both inflammation and depression have been shown to be associated with various risk factors for diabetes such as insulin resistance [81, 185], obesity [82, 129], smoking [186, 187], and a sedentary lifestyle [188, 189]. Furthermore, both CRP and depression are associated with the metabolic syndrome [190], which is recognized as a precursor to type 2 diabetes [191]. The metabolic syndrome is a cluster of cardiometabolic risk factors such as diabetes or elevated fasting glucose levels, hypertension, abdominal obesity, and dyslipidemia [85]. Previous evidence found that the inflammatory response, involving CRP and IL-6 levels, may represent a partial determinant for the link between depression and the metabolic syndrome [192]. Of all the metabolic factors, obesity and BMI have been speculated to be the major mediating factor between inflammation and diabetes [85] as well as between depression and diabetes [184, 193]. This could explain why the associations on diabetes risk with only high CRP levels and with only elevated depressive symptoms were substantially attenuated and no longer significant after adjustment for BMI.

5.5.2 Strengths and Limitations

One of the strengths of this prospective study is its large population-based design. The nationally representative nature of ELSA and its rigorous design offers value in the generalizability of our results to the community-dwelling population. Furthermore, the current study has an appropriately long follow-up period which made it possible to study the association between CRP and depressive symptoms with diabetes. An additional strength in our study is the detailed assessment of socio-demographic, lifestyle, clinical, and objectively measured BMI.
which all permitted sufficient adjustment for a variety of potential confounding variables.

The limitations of the current study should be recognized. One major limitation is that our study outcome, incidence of diabetes, was ascertained through self-report of doctor’s diagnosis and was not validated via clinical diagnosis or laboratorial tests. Thus, there is a possibility of reporting bias regarding diabetes diagnosis. Participants were epidemiologically classified as having type 2 diabetes, but we cannot omit the potential presence of type 1 diabetes in the sample. Thus, there is a possibility of misclassifying diabetes type in the present study. Although our sensitivity analysis showed that accounting for undiagnosed diabetes at baseline did not have a substantial effect on our results, we were limited to the measurement of fasting blood glucose as a proxy assessment for undiagnosed diabetes. A similar drawback of the current study is related to the assessment of depressive symptomatology using a self-report, based on the CES-D scale, and not a diagnostic interview for ascertaining depression. Thus, the CES-D scale can be considered as a screening tool rather than a diagnostic one [16]. The assessments of clinical depression (using criteria set by the *Diagnostic and Statistical Manual of Mental Disorders*[14] or by the *International Statistical Classification of Diseases*[15]) as well as depressive symptoms (using self-report rating scales) are typically based on a minimum of two week history of depressive symptomatology. Since the CES-D assesses depressive symptoms with respect to only the past week, its validity as a self-report screening measure is a concern as it may measure only transient distress which can consequently lead to false positives. Although ELSA is designed to be representative of the general population, the study sample used in the analyses is likely comprised of healthier older adults than the overall cohort due to restrictions placed upon eligibility for blood assessments. An intrinsic characteristic in aging studies is the possibility of survivor bias and examining a sample of relatively healthy participants could lead
to an underestimation of the relationship between CRP, depressed mood, and diabetes. Likewise, attrition is inherent in prospective national cohort studies such as ELSA. Thus, if participants with depression, high CRP or diabetes are more likely to leave the study, this could lead to a conservative association between CRP, depressive symptoms, and risk of developing diabetes. There were fewer incident cases of diabetes for the groups with elevated depressive symptoms and the effect sizes were relatively small, which could explain why the group with elevated depressive symptoms only was not statistically significant. Furthermore, the applicability to other ethnic groups may be limited since the majority of the participants in the present study were white. An additional limitation of the ELSA data is that there was no information regarding current psychiatric or anti-inflammatory medication usage. Thus, we were also unable to account for these variables which could possibly affect CRP levels, depressive symptoms, and metabolic factors.

5.5.3 Future Directions

Future epidemiological studies on chronic diseases should consider the measurement of both biomarkers including inflammatory markers and psychiatric assessments such as symptoms of depression in the study design in order to advance our understanding of the relationship between inflammation and mental health with chronic diseases such as diabetes. These studies are needed to ascertain the reliability of our findings and to elucidate the mechanisms that underlie the complex association between inflammation, depression, and diabetes. At the time of the present study, only 5 years of follow-up data was available. Thus, the use of more follow-ups will be essential to collect additional incident cases of diabetes. Further research should examine other measures of inflammation (e.g. IL-6) and depression (e.g. clinical interview). With regards to potential future statistical hypotheses, mediation analyses can be performed to test whether
inflammation is a mediator in the pathway from depression to diabetes or whether depression is a mediator from inflammation to diabetes among older adults.

5.6 Conclusion

Our findings suggest that having both high CRP levels and elevated depressive symptoms is related to higher incidence of type 2 diabetes among older adults even after accounting for socio-demographics, health behaviours, and clinical factors. However, this association was not beyond the expected effect on diabetes risk of high CRP levels and elevated depressive symptoms alone. Because of the high burden of diabetes, an understanding of the combination of depression and inflammation on the risk of diabetes among older adults could have important research, clinical and public health implications. Broadening our understanding of the inflammation, depression, and diabetes relationship offers an opportunity for clinicians and researchers to explore new avenues for research, prevention, and treatment of these conditions. Thus, action to prevent and treat inflammation and depression might play a factor in preventing diabetes.

Acknowledgments: We gratefully acknowledge the UK Data Archive for supplying the ELSA data. ELSA was developed by a team of researchers based at University College London, the Institute of Fiscal Studies and the National Centre for Social Research. The data creators or the funders of the data collections and the UK Data Archive do not bear any responsibility for the analyses or interpretations presented here.

Contributors: BA and KJS conceptualized the manuscript. BA and KJS contributed to the writing of the manuscript. BA was responsible for data analysis. KJS, GG, and NS contributed to the revision of the manuscript. All authors oversaw manuscript preparation and provided feedback for manuscript preparation.
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**Competing interests:** The authors have no competing interests to report.
Table 5.1: Baseline characteristics of the sample without prevalent diabetes according to the four CRP and CES-D groups (N = 4955)

<table>
<thead>
<tr>
<th></th>
<th>CRP ≤ 3 mg/L, CES-D &lt; 4</th>
<th>CRP ≤ 3 mg/L, CES-D ≥ 4</th>
<th>CRP &gt; 3 mg/L, CES-D &lt; 4</th>
<th>CRP &gt; 3 mg/L, CES-D ≥ 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>2,901</td>
<td>392</td>
<td>1,397</td>
<td>265</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64.77 (9.19)</td>
<td>66.82 (10.76)</td>
<td>66.77 (9.55)</td>
<td>66.20 (9.83)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>51.47</td>
<td>68.62</td>
<td>57.05</td>
<td>69.81</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>University degree or equivalent (%)</td>
<td>16.99</td>
<td>8.46</td>
<td>9.02</td>
<td>6.04</td>
</tr>
<tr>
<td>Less than university (%)</td>
<td>54.88</td>
<td>48.21</td>
<td>50.75</td>
<td>48.68</td>
</tr>
<tr>
<td>No qualification (%)</td>
<td>28.13</td>
<td>43.33</td>
<td>40.23</td>
<td>45.28</td>
</tr>
<tr>
<td>Employment Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed (%)</td>
<td>38.99</td>
<td>27.04</td>
<td>26.93</td>
<td>16.98</td>
</tr>
<tr>
<td>Retired (%)</td>
<td>12.71</td>
<td>22.19</td>
<td>15.40</td>
<td>26.79</td>
</tr>
<tr>
<td>Unemployed (%)</td>
<td>48.30</td>
<td>50.77</td>
<td>57.66</td>
<td>56.23</td>
</tr>
<tr>
<td>Total net non-pension household wealth(^a), median (interquartile range), £</td>
<td>230,800 (253021)</td>
<td>157,026 (231402)</td>
<td>181,750 (208700)</td>
<td>145,000 (203185)</td>
</tr>
<tr>
<td>Current Smoker (%)</td>
<td>10.93</td>
<td>17.22</td>
<td>17.18</td>
<td>26.04</td>
</tr>
<tr>
<td>Frequency of alcohol consumption</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None (%)</td>
<td>5.96</td>
<td>11.34</td>
<td>11.16</td>
<td>20.51</td>
</tr>
<tr>
<td>Occasional (%)</td>
<td>72.95</td>
<td>74.03</td>
<td>73.41</td>
<td>66.24</td>
</tr>
<tr>
<td>Daily (%)</td>
<td>21.10</td>
<td>14.63</td>
<td>15.43</td>
<td>13.25</td>
</tr>
<tr>
<td>Level of physical activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedentary (%)</td>
<td>1.55</td>
<td>9.44</td>
<td>3.44</td>
<td>7.58</td>
</tr>
<tr>
<td>Low (%)</td>
<td>15.93</td>
<td>27.55</td>
<td>27.36</td>
<td>43.94</td>
</tr>
<tr>
<td>Moderate (%)</td>
<td>55.95</td>
<td>50.51</td>
<td>52.36</td>
<td>40.15</td>
</tr>
<tr>
<td>High (%)</td>
<td>26.58</td>
<td>12.50</td>
<td>16.83</td>
<td>8.33</td>
</tr>
<tr>
<td>Longstanding illness or disability (%)</td>
<td>45.12</td>
<td>65.31</td>
<td>56.69</td>
<td>51.89</td>
</tr>
</tbody>
</table>
Results are presented as means (SD) or % unless otherwise indicated. \( ^a \)Total household wealth (excluding pension savings) minus household debt. \( ^b \)Cardiovascular comorbidities included hypertension, angina, congestive heart failure, abnormal heart rhythm, heart murmur, heart attack, and stroke. \( ^c \)Non-cardiovascular comorbidities included asthma, arthritis, chronic lung disease, cancer/malignant tumor excluding minor skin cancers, osteoporosis, Parkinson's disease, Alzheimer's disease, and dementia or other serious memory impairment. Less than 0.5% of sample had missing data for marital status, education, employment, smoking, physical activity, longstanding illness or disability, 1.4% for wealth, 8% for alcohol, and 4% for BMI.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular comorbidity(^b) (%)</td>
<td>43.98</td>
<td>53.83</td>
<td>53.54</td>
<td>61.13</td>
</tr>
<tr>
<td>Non-cardiovascular comorbidity(^c) (%)</td>
<td>45.43</td>
<td>64.80</td>
<td>57.55</td>
<td>75.85</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>26.01 (5.53)</td>
<td>26.16 (6.24)</td>
<td>28.27 (7.85)</td>
<td>27.83 (9.07)</td>
</tr>
</tbody>
</table>
Table 5.2: Incidence of type 2 diabetes according to the four CRP and CES-D groups ($N = 4955$)

<table>
<thead>
<tr>
<th></th>
<th>CRP $\leq$ 3 mg/L, CES-D $&lt; 4$</th>
<th>CRP $\leq$ 3 mg/L, CES-D $\geq 4$</th>
<th>CRP $&gt; 3$ mg/L, CES-D $&lt; 4$</th>
<th>CRP $&gt; 3$ mg/L, CES-D $\geq 4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>2,901</td>
<td>392</td>
<td>1,397</td>
<td>265</td>
</tr>
<tr>
<td>Incident cases ($n$)</td>
<td>74</td>
<td>16</td>
<td>80</td>
<td>24</td>
</tr>
<tr>
<td>Person-years of follow-up</td>
<td>15,589</td>
<td>2,032</td>
<td>7,148</td>
<td>1334</td>
</tr>
<tr>
<td>Incidence rate of type 2 diabetes (per 1,000 person-years)</td>
<td>4.7</td>
<td>7.9</td>
<td>11.2</td>
<td>18.0</td>
</tr>
</tbody>
</table>
Table 5.3: HRs (95% CI) of type 2 diabetes according to the four CRP and CES-D (N = 4955)

<table>
<thead>
<tr>
<th>Cox regression models*</th>
<th>CRP ≤ 3 mg/L, CES-D &lt; 4 (n = 2,901)</th>
<th>CRP ≤ 3 mg/L, CES-D ≥ 4 (n = 392)</th>
<th>CRP &gt; 3 mg/L, CES-D &lt; 4 (n = 1,397)</th>
<th>CRP &gt; 3 mg/L, CES-D ≥ 4 (n = 265)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>1.00</td>
<td>1.67 (0.97-2.86)</td>
<td>2.37 (1.73-3.25)</td>
<td>3.82 (2.41-6.05)</td>
</tr>
<tr>
<td>Model 1: age, sex</td>
<td>1.00</td>
<td>1.84 (1.07-3.16)</td>
<td>2.45 (1.78-3.37)</td>
<td>4.31 (2.70-6.86)</td>
</tr>
<tr>
<td>Model 2: model 1 + adjusted for marital status, educational attainment, employment status, and total household wealthb</td>
<td>1.00</td>
<td>1.55 (0.89-2.69)</td>
<td>2.07 (1.50-2.86)</td>
<td>3.10 (1.90-5.06)</td>
</tr>
<tr>
<td>Model 3: model 2 + adjusted for smoking status, alcohol consumption, and physical activity level</td>
<td>1.00</td>
<td>1.83 (1.03-3.26)</td>
<td>2.12 (1.49-3.01)</td>
<td>3.24 (1.88-5.57)</td>
</tr>
<tr>
<td>Model 4: model 3 + adjusted for reported longstanding illness or disability, cardiovascularb and noncardiovascularc comorbidities</td>
<td>1.00</td>
<td>1.76 (0.99-3.14)</td>
<td>2.03 (1.43-2.88)</td>
<td>2.88 (1.66-4.97)</td>
</tr>
<tr>
<td>Model 5: model 4 + adjusted for BMI</td>
<td>1.00</td>
<td>1.63 (0.88-3.01)</td>
<td>1.43 (0.99-2.07)</td>
<td>2.03 (1.14-3.61)</td>
</tr>
</tbody>
</table>

Data are presented as Hazard Ratios (95% CI). aTotal household wealth (excluding pension savings) minus household debt. bCardiovascular comorbidities included hypertension, angina, congestive heart failure, abnormal heart rhythm, heart murmur, heart attack, and stroke. cNon-cardiovascular comorbidities included asthma, arthritis, chronic lung disease, cancer/malignant tumor excluding minor skin cancers, osteoporosis, Parkinson's disease, Alzheimer's disease, and dementia or other serious memory impairment. Model 1: n = 4955, Model 2: n = 4868, Model 3: n = 4482, Model 4: n = 4480, Model 5: n = 4319.
CHAPTER 6 – DISCUSSION

6.1 Restatement of Objectives

The world’s population is aging rapidly, and the global proportion of older adults is estimated to increase from over 800 million people in 2014 to more than 2 billion in 2050 [1]. Depression is one of the most common psychiatric disorders among older adults [8]. Depressed mood can negatively affect the quality of life of older persons with regards to functioning and well-being [11], as well as increase the risks of morbidity and mortality [9, 10]. Depression among older adults differs from depression in other age groups in terms of etiology, symptomatology, risk and protective factors, as well as potential outcomes [10]. One major characteristic of depression among older adults is that it often co-exists with chronic conditions such as Type 2 diabetes mellitus (T2DM) [98, 194]. A biological response that has received attention for possibly being the underlying mechanism between depression with chronic medical conditions such as T2DM is the inflammatory process [85, 93]. This speculation arises from evidence in support of the “cytokine hypothesis of depression” (theory postulating that increased inflammation is involved in the pathophysiology of depression) [38, 39], and from hypotheses suggesting that T2DM is a disease of the innate immune system [87, 183]. Thus, the present thesis aimed to elucidate the relationship between depression, inflammation, and T2DM.

In the systematic review and meta-analysis (Manuscript 1), we first conducted a comprehensive literature review to examine the relationship between CRP and depression among a general population of older adults utilizing both cross-sectional and longitudinal studies.

From Manuscript 1, we identified a research gap in the literature concerning the need to assess the longitudinal associations of CRP and depression among older adults (Manuscript 2). We then conducted longitudinal analyses of 3397 participants from the English Longitudinal Study of Ageing (ELSA) to examine the bidirectional association of CRP with symptoms of
depression among older adults.

Based on findings from Manuscript 2, we recognized the complex relationship between CRP and depression especially when accounting for metabolic and chronic conditions that are related to both CRP and to depression. Our last study (Manuscript 3) incorporates Type 2 diabetes, a prevalent chronic condition among older adults, into the CRP and depression relationship. High levels of CRP and elevated depressive symptoms have been separately linked to the development of diabetes [78, 79, 89, 90]. Thus, we assessed the association of both CRP and depressive symptomatology with incidence of diabetes over six years of follow-up in 4955 participants from ELSA.

6.2 Principal Findings

In Manuscript 1, we observed a small but positive correlation between CRP and depressive symptoms in a general population of community-dwelling older adults; however this association is often reduced after adjustment for additional potential confounders. Furthermore, we identified that the strength of the CRP and depression association differed based on whether CRP was analyzed as a continuous versus categorical variable particularly among cross-sectional studies, but this difference was less prominent among longitudinal studies due to the scarcity of available prospective studies. Overall, we identified an important need for more longitudinal studies to assess the CRP and depression relationship. In particular, it is necessary to simultaneously assess both variables as the exposure and outcome in order to be able to draw inferences on the direction of causality.

In Manuscript 2, we found that CRP is associated with elevated depressive symptoms at follow-up among older adults independent of socio-demographics and health behaviours. The direction of association is likely from high levels of CRP to symptoms of depression via metabolic pathways and co-morbidity with chronic conditions. We did not observe an
association in the reverse direction from elevated symptoms of depression to high levels of CRP among older adults in the unadjusted nor adjusted analyses.

From Manuscript 3, our findings suggested that having both high CRP levels and elevated depressive symptoms is related to the incidence of Type 2 diabetes among older adults even after adjustments for socio-demographics, health behaviours, and clinical factors. However, this association was not beyond the expected effect on diabetes risk of high CRP levels and elevated depressive symptoms alone.

6.3 Implication of Findings

The findings from this thesis are particularly relevant for mental health professionals, clinicians, and researchers. Findings from this thesis suggest that the relationship between inflammation, depression, and diabetes is a complex phenomenon, and our results add to the burgeoning fields of biological psychiatry and psychosomatic research. One major implication from Manuscripts 1 and 2 recognized the fact that inflammation and depression are both heterogeneous conditions and drew attention to the potential role of inflammation in depression, thereby providing an opportunity to investigate the underlying mechanisms between these conditions and to identify the possible behavioural or physiological processes involved.

At the time this thesis was written, no study had simultaneously assessed CRP, depression, and diabetes in a prospective analysis. Our study in Manuscript 3 was the first to observe that older adults with both high CRP levels and elevated depressive symptoms were more likely to develop diabetes even after accounting for socio-demographics and additional potential confounding variables. A key implication from Manuscript 3 was expanding our understanding of the inflammation, depression, and diabetes relationship and providing suggestions for clinicians and researchers to consider in terms of opportunities for research,
prevention, and treatment of these conditions. For example, perhaps the action to prevent and treat inflammation and depression might play a factor in preventing diabetes.

6.4 Limitations

There are several major limitations that need to be considered for the studies presented in this thesis. Firstly, we only examined one inflammatory marker. The role of CRP in vivo remains incomplete and the inflammatory response may involve a cascade of other inflammatory markers including pro-inflammatory cytokines as well as other acute-phase reactants that may have a stronger association with depression or diabetes. Secondly, the majority of the studies included in the review (Manuscript 1) assessed depressed mood using rating scales, and the CES-D scale was used in the ELSA studies (Manuscripts 2 and 3). Therefore, depressive symptomatology was based on self-report and not based on a structured interview for ascertaining a clinical diagnosis of depression. Furthermore, the CES-D scale is a swift assessment of depressive symptoms that is based on the past one-week history, and ELSA did not have information regarding previous history of depression or antidepressant use. Thirdly, by focussing on a general population of older adults where there is a possibility for survivor bias since relatively healthy older individuals are able to participate in these studies, there is a potential for a conservative estimation of effect sizes on the relationship between CRP with depressed mood, as well as on the relationship between CRP and depressive symptoms with diabetes. Consequently, issues with generalizability should be also recognized. Findings from this thesis, particularly from the ELSA studies, would not be representative of clinical samples. Even among community-dwelling samples, findings from the ELSA studies may not be applicable to other age groups, ethnicities, or regions with different health care systems.
6.5 Future Directions

There are many directions that future research could potentially undertake to further elucidate the relationship between inflammation, depression, and diabetes. Additional research is necessary to establish the reliability of our findings and to elucidate the mechanisms that underlie the relationship between these conditions. However, it is important to acknowledge the multifaceted nature of inflammation and depression since there are numerous types of inflammatory markers (e.g. pro-inflammatory cytokines such as IL-6 and acute-phase reactant proteins such as CRP) as well as subtypes of depression (e.g. melancholic and atypical depression). Therefore, there are numerous opportunities for future research to examine various inflammatory markers with depression as a whole condition or with specific depression subtypes among clinical or community samples. Furthermore, future studies should consider assessing multiple inflammatory markers simultaneously instead of just one marker in order to better understand inflammation as a condition.

Undoubtedly, future epidemiological studies on chronic diseases should consider collecting information on biomarkers (e.g. inflammatory markers) and detailed psychiatric assessments (e.g. depression symptoms, symptom severity, depression history, antidepressant use) to enhance our understanding of the relationship between inflammation and mental health with chronic diseases such as diabetes. Similarly, more prospective studies are certainly needed to infer the causal mechanisms involved in the inflammation, depression, and diabetes relationship. Additionally, repeated assessments from more than two occasions as well as at varying time intervals are needed to determine the temporal associations between these conditions, thereby allowing future studies to potentially assess inflammation as a mediator from depression to diabetes or depression as a mediator from inflammation to diabetes.
6.6 Conclusion

In conclusion, the associations between CRP, depressed mood, and diabetes are complex and multifaceted. Higher CRP levels are associated with depressive symptomatology in a general population of older adults, but this association involves interplay with metabolic and chronic health conditions. When Type 2 diabetes was incorporated as an outcome into the CRP-depression relationship, having both high CRP levels and elevated depressive symptoms is related to higher risk of diabetes among older adults even after adjustments for socio-demographics, health behaviours, and clinical factors. However, the association was not beyond the expected effect of high CRP levels alone nor elevated depressive symptoms alone. Thus, further research is warranted to unravel the underlying processes involved in the relationship between these conditions. Given the increasing global burden of mental illness such as depression and its somatic co-morbidities such as diabetes among the older population, it is essential to ascertain the reliability of these findings and for this research area to be further investigated upon in order to potentially contribute to prevention or treatment strategies for these conditions.
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## APPENDIX

### Appendix A: Search Strategies

**PubMed (until July 8, 2013)**
1. C-reactive protein [MeSH]
2. CRP [Title/Abstract]
3. C reactive protein [Title/Abstract]
4. 1 or 2 or 3
5. Depression [MeSH Terms]
6. Depressive disorder [MeSH Terms]
7. Depressive symptom [Title/Abstract]
8. Depressive symptoms [Title/Abstract]
9. Depress* [Title/Abstract]
10. 5 or 6 or 7 or 8 or 9
11. 4 and 10
12. Limit 11 to humans

**EMBASE (until July 8, 2013)**
1. C-reactive protein.mp
2. CRP.mp
3. C reactive protein.mp
4. 1 or 2 or 3
5. Depression.mp
6. Depressive disorder.mp
7. Depressive symptom.ti.ab
8. Depressive symptoms.ti.ab
9. Depress*.ti.ab
10. 5 or 6 or 7 or 8 or 9
11. 4 and 10
12. Limit 11 to humans

**PsychINFO (until July 8, 2013)**
1. C-reactive protein.af
2. CRP.af
3. C reactive protein.af
4. 1 or 2 or 3
5. Depression.mp
6. Depressive disorder.mp
7. Depressive symptom.ti.ab
8. Depressive symptoms.ti.ab
9. Depress*.ti.ab
10. 5 or 6 or 7 or 8 or 9
11. 4 and 10
12. Limit 11 to humans

**ISI Web of Science (until July 8, 2013)**
1. C-reactive protein [topic]
2. CRP [topic]
3. C reactive protein [topic]
4. 1 or 2 or 3
5. Depression [topic]
6. Depressive disorder [topic]
7. Depressive symptom [topic]
8. Depressive symptoms [topic]
9. Depress* [topic]
10. 5 or 6 or 7 or 8 or 9
11. 4 and 10
12. Limit 11 to articles only

**ProQuest Dissertations and Theses Online (until July 8, 2013)**
1. C-reactive protein.ab
2. CRP.ab
3. C reactive protein.ab
4. 1 or 2 or 3
5. Depression.ab
6. Depressive disorder.ab
7. Depressive symptom.ab
8. Depressive symptoms.ab
9. Depress*.ab
10. 5 or 6 or 7 or 8 or 9
11. 4 and 10
## Appendix B: Quality assessment of cross-sectional studies

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<th>Information bias</th>
<th>Comparability</th>
<th>Quality*</th>
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<td>Baune et al., 2012</td>
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<td>Bremmer et al., 2008</td>
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<td>Lu et al, 2013</td>
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*Based on the total number of stars, overall quality was evaluated as follows: five or more stars for high-quality studies, three or four stars for moderate-quality studies, and two or fewer stars for low-quality studies.
## Appendix C: Quality assessment of longitudinal studies

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<tr>
<td></td>
<td>Representative sampling procedure of participants in community base</td>
<td>People with depression and reference group from same community</td>
<td>Demonstration that outcome of interest was not present at start of study</td>
<td>Depression measured using clinical interview or validated scale</td>
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* Based on the total number of stars, overall quality was evaluated as follows: seven or more stars for high-quality studies, four to six stars for moderate-quality studies, and three or fewer stars for low-quality studies.