THE ROLE OF HYPERTENSION AND INFLAMMATION ON THE COGNITIVE SYMPTOMS OF ALZHEIMER’S DISEASE PATIENTS.

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Abstract

Background
This study aimed to assess the effect of blood pressure and inflammation on the cognitive symptoms of mild Alzheimer’s disease (AD) patients. Mid-life high blood pressure is known to be a risk factor for dementia, whereas low blood pressure in older age associates with poorer cognitive performance. Increased inflammation is also associated with the development of AD, and so this study assessed the impact of both blood pressure and inflammation on individual cognitive symptoms within AD patients. The possible synergistic effect of blood pressure, markers of vascular health asymmetric dimethylarginine (ADMA) and Fetuin-A, and markers of inflammation upon cognition was also assessed.

Measures
A history of hypertension was determined from patient records, and blood pressure was measured at time of cognitive testing. Inflammatory markers and markers of vascular health were collected from patient serum samples. Prospective memory, reaction time, emotional appraisal, visual selective attention and subjective memory awareness were the cognitive domains evaluated.

Results
There was no difference in cognitive presentation between AD patients with and without a history of hypertension. Current mean arterial pressure was positively associated with attentional performance, whereas sitting-to-standing systolic blood pressure decline was negatively associated with reaction time. Greater levels of pro-inflammatory markers and ADMA were associated with less report of memory failures. A synergistic effect of inflammation and ADMA was observed on retrospective memory awareness. This study adds to the growing consensus suggesting that inflammatory and vascular systems are involved with the aetiology of AD, and that differences in vascular risk factors and levels of inflammation may explain some of the variation in clinical and cognitive symptoms. Attentional tasks appear to have a different association with blood pressure than those of other cognitive domains in dementia patients and healthy older adults. The role of inflammation and vascular disease in memory awareness found in this study requires further elucidation.
## Contents

Abstract ........................................................................................................................................... 2  
List of Figures .................................................................................................................................. 4  
List of Tables.................................................................................................................................... 8  
List of common abbreviations........................................................................................................... 10  
Acknowledgements .......................................................................................................................... 12  
Declaration ....................................................................................................................................... 13  

1. Introduction .................................................................................................................................... 17  
2. Hypothesis ..................................................................................................................................... 56  
3. Methods ....................................................................................................................................... 57  

4. Blood pressure and cognitive performance ................................................................................. 69  
5. Inflammation and cognitive performance ................................................................................... 90  

6. Blood pressure, inflammation and cognitive performance ......................................................... 117  

7. General discussion ....................................................................................................................... 121  
8. Concluding remarks ................................................................................................................... 126  
9. Bibliography ................................................................................................................................. 127  

Appendix 1 ..................................................................................................................................... 160  
Appendix 2 ..................................................................................................................................... 165  
Appendix 3 ..................................................................................................................................... 167  
Appendix 4 ..................................................................................................................................... 168
List of Figures

Figure 1. Standard curves for pro-inflammatory markers IL1β, IL-2 and IL-6 (pg/ml). ................................................................. 63

Figure 2. Standard curves for pro-inflammatory markers CRP and TNF-α (pg/ml). 64

Figure 3. Standard curve of anti-inflammatory markers Fet-A and IL-10 (pg/ml)... 65

Figure 4. Standard curve of ADMA concentration (pg/ml).................................. 66

Figure 5 scatterplot showing relationship between age and blood pressure variables SBP decline and MAP..................................................... 72

Figure 6. Scatterplot showing relationship between age and subjective memory failure rating. .......................................................................................... 75

Figure 7. Scatterplot showing relationship between age and prospective memory rating. .......................................................................................... 76

Figure 8. Scatterplot showing relationship between age and retrospective memory rating. .......................................................................................... 76

Figure 9. Scatterplot showing there is no relationship between prospective memory score and subjective prospective memory rating in this study...................... 77

Figure 10. Scatterplot showing relationship between SBP standing decline and reaction time................................................................. 80

Figure 11. Scatterplot showing no relationship between accuracy on emotional recognition task and SBP standing decline........................................... 82
Figure 12. Scatterplot showing lack of relationship between SBP standing decline and reaction time on emotional recognition task. ................................................................. 83

Figure 13. Scatterplot showing lack of relationship between MAP and accuracy on emotional recognition task. .............................................................................. 83

Figure 14. Scatterplot showing lack of relationship between MAP and reaction time on emotional recognition task. .............................................................................. 84

Figure 15. Scatterplot showing lack of relationship between SBP decline when standing and attention score. .............................................................................. 86

Figure 16. Scatterplot showing relationship between MAP and attention score. ...... 86

Figure 17 Scatterplots showing relationship between age (years) and pro-inflammatory markers (pg/ml) in serum samples from AD patients. ........................................ 92

Figure 18. Scatterplots showing relationship between age and pro-inflammatory markers from serum samples from mild AD patients. Pearson’s R, Spearman’s Rho and $p$ values are displayed. ................................................................. 93

Figure 19. Scatterplots showing lack of relationship between pro-inflammatory markers and reaction time in AD patients. Pearson’s R, Spearman’s Rho and $p$ values are displayed. .............................................................................. 95

Figure 20. Scatterplot showing lack of relationship between pro-inflammatory cytokines and reaction time during prospective memory task in AD patients. Pearson’s R, Spearman’s Rho and $p$ values are displayed. ................................................................. 96
Figure 21. Scatterplots showing lack of relationship between prospective memory performance and pro-inflammatory markers in AD patients. Pearson’s R, Spearman’s Rho and $p$ values are displayed. ........................................................................................................ 97

Figure 22. Scatterplot showing relationship between IL-1β serum concentrations and subjective memory ratings in mild AD patients................................................................. 98

Figure 23. Scatterplot showing relationship between TNF-α serum concentration and subjective memory ratings in mild AD patients................................................................. 99

Figure 24. Scatterplots showing lack of relationship between reaction time on emotional recognition task and pro-inflammatory markers. Pearson’s R, Spearman’s Rho and $p$ values are displayed. ................................................................. 102

Figure 25. Scatterplots showing lack of relationship between reaction time on emotional recognition task and pro-inflammatory markers collected from the serum of mild AD patients. Pearson’s R, Spearman’s Rho and $p$ values are displayed. ......................... 103

Figure 26. Scatterplots showing lack of relationship between pro-inflammatory markers from serum and selective attention score in mild AD patients. Pearson’s R, Spearman’s Rho and $p$ values are displayed. ................................................................. 104

Figure 27. Scatterplots showing lack of relationship between IL-10 and prospective memory performance in mild AD patients. Pearson’s R, Spearman’s Rho and $p$ values are displayed................................................................. 106

Figure 28. Scatterplots showing lack of relationship between Fet-A and prospective memory performance in mild AD patients. Pearson’s R, Spearman’s Rho and $p$ values are displayed................................................................. 110
Figure 29. Scatterplots showing lack of relationship between anti-inflammatory markers and selective attention task performance in mild AD patients. Pearson’s R, Spearman’s Rho and $p$ values are displayed. ................................................................. 107

Figure 30. Scatterplot showing lack of relationship between ADMA serum concentrations and performance on prospective memory task in mild AD patients. Pearson’s R, Spearman’s Rho and $p$ values are displayed........................................... 109

Figure 31. Scatterplots showing relationship between ADMA serum concentration and subjective memory rating in AD patients. Pearson’s R, Spearman’s Rho and $p$ values are displayed.................................................................................. 111

Figure 32. Scatterplots showing lack of relationship between ADMA serum concentrations and reaction time and accuracy on an emotional recognition task in AD patients. Pearson’s R, Spearman’s Rho and $p$ values are displayed. ...................... 112

Figure 33. Scatterplot showing lack of relationship between ADMA concentrations and selective attention score in mild AD patients. Pearson’s R, Spearman’s Rho and $p$ values are displayed. ................................................................. 113
List of Tables

Table 1. Sample size calculations for blood pressure and cognition using data from previous literature in AD patients. ................................................................. 68

Table 2. Demographic data of participants. Standard deviations are given in parentheses. ...................................................................................................... 68

Table 3. Blood pressure readings standing and sitting/mmHg in mild AD patients. 71

Table 4. No significant differences in current blood pressure between those with and without a history of hypertension.................................................. 73

Table 5. Average response by AD patients on prospective memory task; reaction time in milliseconds and accuracy. S.D in parentheses.......................... 74

Table 6 showing total average responses on subjective memory questionnaire Likert-Scale. .............................................................................................. 75

Table 7. Average response times and accuracy of AD patients for the different Ekman emotions in an emotional recognition task. .................................. 78

Table 8. No significant differences on cognitive test performance of AD patients with and without a history of hypertension......................................... 79

Table 9. Multiple hierarchical regression analysis of variance on the effect of blood pressure on subject memory rating when age is controlled for........ 82

Table 10. Multiple hierarchical regression analysis of variance in accuracy on happy trials of emotional recognition task when controlling for gender........ 85
Table 11. Multiple hierarchical regression analysis of variance on accuracy of surprise trials when gender is controlled for

Table 12. Concentrations of pro-inflammatory markers (pg/ml) in serum samples taken from mild AD patients

Table 13. T-tests showing no differences between average concentrations of pro-inflammatory markers between male and female mild AD patients

Table 14. Mann-Whitney U test showing no differences in pro-inflammatory markers between male and female AD patients

Table 15. Multiple hierarchical regression on the effect of TNF-α on total memory rating when controlling for age in mild AD patients

Table 16. Multiple hierarchical regression on the effect of TNF-α on prospective memory rating when controlling for age in mild AD patients

Table 17. Multiple hierarchical regression on the effect of TNF-α on retrospective memory rating when controlling for age in mild AD patients

Table 18. Average values of IL-10 (pg/ml)

Table 19. Average values of ADMA (μmol/l) and Fet-A (g/l) concentrations in total sample of mild AD patients, and in males and females. Mann-Whitney U test showed no significant difference between males and females

Table 20: Summary of key findings
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ</td>
<td>Amyloid-β</td>
</tr>
<tr>
<td>ACE</td>
<td>Addenbrooke’s Cognitive Exam</td>
</tr>
<tr>
<td>AChEIs</td>
<td>Acetylcholinesterase Inhibitor</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>ADMA</td>
<td>Asymmetric dimethylarginine</td>
</tr>
<tr>
<td>apoE</td>
<td>apolipoprotein E</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood brain barrier</td>
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<tr>
<td>CAA</td>
<td>Cerebral amyloid angiopathy</td>
</tr>
<tr>
<td>CBF</td>
<td>Cerebral blood flow</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebral spinal fluid</td>
</tr>
<tr>
<td>CVD</td>
<td>Cerebrovascular disease</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<tr>
<td>Fet-A</td>
<td>Fetuin-A</td>
</tr>
<tr>
<td>HTA</td>
<td>Human tissue authority</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>LTP</td>
<td>Long-term potentiation</td>
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<tr>
<td>MAP</td>
<td>Mean arterial pressure</td>
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<tr>
<td>MCI</td>
<td>Mild cognitive impairment</td>
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<tr>
<td>MMSE</td>
<td>Mini-mental state examination</td>
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<tr>
<td>Acronym</td>
<td>Full Form</td>
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<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>NART</td>
<td>National adult reading test</td>
</tr>
<tr>
<td>NFT</td>
<td>Neurofibrillary tangles</td>
</tr>
<tr>
<td>NINCDS-ADRDA</td>
<td>National Institute of Neurological and Communicative Disorders and Stroke, and the Alzheimer's Disease and Related Disorders Association</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>OH</td>
<td>Orthostatic hypotension</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>PIB</td>
<td>Pittsburgh-compound B</td>
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<tr>
<td>PM</td>
<td>Prospective memory</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RT</td>
<td>Reaction time</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic blood pressure</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SPRMQ</td>
<td>Subjective prospective and retrospective memory questionnaire</td>
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<tr>
<td>TNF-α</td>
<td>Tumour necrosis factor- α</td>
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<tr>
<td>VaD</td>
<td>Vascular dementia</td>
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<tr>
<td>WMH</td>
<td>White matter hyperintensities</td>
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</table>
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Declaration

I declare that the research contained in this thesis, unless otherwise formally indicated within the text, is the original work of the author. The thesis has not been previously submitted to this or any other university for a degree, and does not incorporate any material already submitted for a degree.

Signed

30/3/2016

Dated
Index
Abstract ......................................................................................................................... 2
List of Figures .............................................................................................................. 4
List of Tables ................................................................................................................ 8
List of common abbreviations .................................................................................. 10
Acknowledgements .................................................................................................... 12
Declaration .................................................................................................................. 13
1. Introduction ............................................................................................................ 17
  1.1 Alzheimer’s disease .............................................................................................. 17
  1.2 The cholinergic hypothesis .................................................................................. 19
    1.2.1 Current treatment for AD patients ................................................................. 19
    1.2.2 Effect of treatment and side-effects .............................................................. 20
  1.3 Cognition in AD ................................................................................................... 23
    1.3.1 Memory and attention .................................................................................. 23
    1.3.2 Prospective memory ....................................................................................... 25
    1.3.3 Emotional Processing ..................................................................................... 26
  1.4 AD neuronal pathology ......................................................................................... 28
    1.4.1 Amyloid-β and the amyloid hypothesis ......................................................... 28
    1.4.2 Cognition and amyloid .................................................................................. 30
    1.4.3 Amyloid and blood vessels .......................................................................... 31
    1.4.4 Tau ............................................................................................................... 33
    1.4.5 Cognition and Tau ........................................................................................ 34
  1.5 Ageing .................................................................................................................... 35
  1.6 APOE ..................................................................................................................... 36
  1.7 Vascular regulation and blood pressure .............................................................. 38
    1.7.1 Cerebrovasculature and pathology in AD .................................................... 41
    1.7.2 Blood pressure and AD risk .......................................................................... 44
  1.8 Inflammation ........................................................................................................ 45
    1.8.1 Inflammation in the brain ............................................................................. 46
    1.8.2 Inflammation and cognition ......................................................................... 47
    1.8.3 Inflammation in AD ....................................................................................... 48
5. Inflammation and cognitive performance ................................................. 90

5.1 Foreword ................................................................................................. 90
5.2 Methods .................................................................................................. 91
5.3 Results .................................................................................................... 92
  5.3.1 Pro-inflammatory cytokines .............................................................. 92
  5.3.2 Anti-inflammatory cytokine .............................................................. 105
  5.3.3 Fetuin-A and asymmetric dimethylarginine ..................................... 108
5.3 Discussion ............................................................................................... 114

6. Blood pressure, inflammation and cognitive performance ....................... 117

6.1 Foreword ................................................................................................. 117
6.2 Methods .................................................................................................. 118
6.3 Results .................................................................................................... 119
  6.3.1 Interactive effect of inflammation and blood pressure on cognitive performance .............................................................. 119
6.4 Discussion ............................................................................................... 120

7. General discussion .................................................................................... 121

7.2 Future Studies ......................................................................................... 125

8. Concluding remarks .................................................................................. 126

9. Bibliography .............................................................................................. 127

Appendix 1 ..................................................................................................... 160
Appendix 2 ..................................................................................................... 165
Appendix 3 ..................................................................................................... 167
Appendix 4 ..................................................................................................... 168
1. Introduction

1.1 Alzheimer’s disease

Alzheimer’s disease (AD) is a sporadic age-related clinical syndrome which causes a highly progressive dementia in the elderly. AD was first recognised as a distinct disorder by Alois Alzheimer in 1907, as described by Maurer et al., in (1997). The term ‘Alzheimer’s disease’ was first used by Kraepelin in the Handbook of Psychiatry published in 1910, which defined the occurrence of neurological pathologies plaques and tangles, as an integral part of this senile dementia.

Currently, AD affects over 520,000 people in the UK (Alzheimer's Society, 2013), and rates are expected to increase worldwide alongside an increasingly ageing population. Early symptoms usually present as problems with episodic memory and learning, and appear after 65 years of age. The presentation of AD typically progresses over time to include deficits in attention, executive function, and activities of daily living, as well as emotional and behavioural problems; however, there can be much variability between patients. The severity of AD rated as either mild, moderate or severe, is determined by scores achieved on standardised tests of global cognition such as the Mini Mental State Exam (MMSE), or Addenbrooke’s Cognitive Exam (ACE). A diagnosis is made using cognitive and neuropsychological tests according to guidelines such as those proposed by the National Institute of Neurological and Communicative Disorders and Stroke, and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al. 2011; McKhann et al. 1984). At diagnosis magnetic resonance imaging (MRI) or computerised tomography (CT) scanning may also be used to detect areas of neuronal atrophy associated with AD, and to rule out other possible causes of cognitive impairment. Much work has been done to determine a blood or cerebral spinal fluid (CSF) biomarker for AD, but this has not yet translated to clinical practice (O’Bryant et al. 2015). The diagnosis of AD is classed as ‘probable’ until a post-mortem investigation is available for a ‘definite’ diagnosis of AD. This post-mortem investigation studies the disposition of pathological hallmarks of AD; extra-cellular amyloid-β (Aβ) plaques and intra-cellular neurofibrillary tangles (NFT) of hyperphosphorylated tau which are present in brain tissue (McKhann et al. 2011) and
were first used to distinguish AD from other neurological conditions over 100 years ago (Maurer et al. 1997; Hodges 2006).

For a clinical diagnosis to be made under current guidelines, there must be a clear decline in functioning which affects everyday life and/or work, and individuals are often referred to a specialist memory service by their general practitioner after presenting with memory complaints (McKhann et al. 2011). If there is very mild memory complaint but no problems with other cognitive domains or activities of daily living, then the patient may be diagnosed with mild cognitive impairment (MCI). MCI is sometimes referred to as prodromal AD, as those with MCI have a higher risk of developing the dementia (Hodges 2006). AD has a slow onset, with neuropathological changes starting many years before the consequences are apparent, and there is much interest now in identifying pre-clinical AD.

The main risk factors identified for sporadic AD are age, presence of the ε4 allele of the APOE gene, and environmental factors such as low levels of education and cognitive stimulation. The importance of vascular and inflammatory factors are now becoming increasingly recognised from epidemiological research and population studies, which have suggested links between a history of high blood pressure, high inflammatory status, and the development of dementia and cognitive decline (Qiu et al. 2005; Aguero-Torres et al. 2006; van Exel et al. 2009; Misiak et al. 2012). Currently, the possible mechanisms behind these relationships are being uncovered with animal and molecular science investigations, as well as large population studies designed to identify biomarkers, prodromal phenotypes and genetic links to these systems and AD.

The current clinical diagnostic criteria for AD assume that it is independent from vascular processes. Therefore clinical signs of vascular dysfunction appear to rule out a diagnosis of AD and instead the patient may be diagnosed with vascular dementia (VaD). It is becoming apparent however, that post-mortem investigations showing pure forms of AD are relatively rare, and patients often have pathologies and symptoms of multiple conditions (Gorelick et al. 2011). Vascular lesions and white matter damage which historically have been associated with a diagnosis of VaD are common findings in AD brains (Kalaria et al. 2012), as well as cardiovascular comorbidities such as hypertension and atherosclerosis. Therefore, it is vital to further our understanding of the role of vascular disease and risk factors on the aetiology of AD to improve diagnosis.
and treatment. Although the aetiology of AD is still not fully resolved, there are several hypotheses.

1.2 The cholinergic hypothesis

One of the earliest theories as to the cause of AD was the cholinergic hypothesis. The cholinergic system is formed of three sub-systems of neuronal pathways; neurons from the forebrain to the cortex and hippocampus, neurons which innervate the thalamus and the dopaminergic system, and interneurons within the striatum. There are two types of receptor in the cholinergic system, muscarinic (mAChR) and nicotinic (nAChR), and both use acetylcholine (ACh) as a ligand. ACh is formed from choline and acetylCoA by the enzyme choline acetyltransferase (ChAT), and is broken down in the synaptic cleft by acetylcholine esterase (AChE). The role of cholinergic neurons in age-related memory loss was first suggested by Drachman and Leavitt in 1974 (discussed in Bartus et al. 1985). This research group used the muscarinic antagonist scopolamine in healthy young volunteers and found it caused impairments in learning and memory similar to those seen in elderly participants. Bowen et al., (1976) showed a positive correlation between a decrease in ChAT and a decline in cognitive functioning in AD patients. This discovery of a relationship between a measure of cognition and an enzyme was an important milestone in psychiatry and pharmacology, as it was the first to suggest a possible treatment for a degenerative cognitive disorder (Bartus et al. 1985).

1.2.1 Current treatment for AD patients

As the underlying cause of AD is not fully understood, the only drug treatment prescribed for patients is palliative symptomatic relief of cognitive symptoms. Acetylcholine esterase inhibitors (AChEIs) and N-methyl-D-aspartate (NMDA) receptor antagonists are prescribed to AD patients for cognitive aid.

AChEIs inhibit the degradation of acetylcholine by acetylcholine esterase. The two receptor types, mAChR and nAChR, facilitate both excitatory and inhibitory signals allowing the cholinergic system to exert a top-down control of attention and memory (Klinkenberg et al. 2011). MACr blocker scopolamine has been found to inhibit memory formation (Schon et al. 2005; Koller et al. 2003) and attentional processes
Markers of cholinergic activity such as ChAT, and mAChR and nAChR binding are decreased in AD brains, and this can be correlated with cognitive decline (Auld et al. 2002). The first AChEI to be licensed was tacrine in 1993 (Giacobini 1998). This was pivotal in being the first hypothesis-driven, proven treatment for a cognitive neurodegenerative condition such as AD. It is disappointing that this drug, and other AChEIs developed since, show only mild effects when effective, working to stabilise or slow rather than halt memory impairments, and there is wide inter-individual variability as to whether a patient will respond to AChEI treatment at all. Donepezil, galantamine and rivastigmine are AChEIs prescribed to AD patients in the UK for cognitive aid.

NMDA receptors are a type of glutamate receptor, situated on hippocampal postsynaptic neurons. Their activation is thought to be essential for memory encoding (Morris 2012) as their high plasticity allows for long term potentiation. The NMDA receptor has high permeability to Ca\(^{2+}\) ions, but to prevent calcium toxicity, this channel is voltage-dependently blocked by magnesium ions (Mg\(^{2+}\)). Although NMDA activation is necessary for cognitive function, over-activation has been implicated in neurological disorders such as AD, and so there must be a balance between agonist and antagonistic activity (Lemoine et al. 2012). Memantine is a NMDA receptor antagonist able to prevent pathological Ca\(^{2+}\) influx, while allowing sufficient influx of Ca\(^{2+}\) necessary for synaptic transmission. Due to its rapid unblocking kinetics, memantine block of the NMDA receptor channel is able to be released during strong pre-synaptic signalling which occurs during learning and memory processes (Danysz & Parsons 2012). It is used as a monotherapy in moderate-severe AD, or alongside continuation of AChEI treatment.

1.2.2 Effect of treatment and side-effects

A significant effect of AChEI treatment on cognitive performance in AD patients in the short term has been established. Double-blind placebo controlled studies with donepezil have found significant improvements in cognitive performance in mild-moderate AD patients with treatment of 5-10mg/d (Rogers & Friedhoff 1996; Rogers 1998), with a dose of 23mg/d showing some modest effect for moderate-severe patients (Sabbagh et al. 2013). Rivastigmine produces similar effects; 26 weeks of oral rivastigmine
treatment significantly reduced decline on Alzheimer’s Disease Assessment Scale (ADAS)-cog (a test of global cognition) compared to a placebo, with the strongest effect occurring in the memory domain (Farlow et al. 2010). The MMSE was used to evaluate the effect of oral rivastigmine treatment of 8.9mg/d over five years by Small et al (2005), who found a decline of 8.9 points in treated patients compared to nearly 14 points in a model of untreated patients. In this study however, 22.4% of treated patients dropped out of the study due to gastric distress. Rivastigmine prescribed as a transdermal patch reduces these side effects (Winblad et al. 2007) and is as clinically effective (Seibert et al. 2012). In one study, caregivers reported patch preference over oral medication, and so this may contribute to more successful treatment compliance (Articus et al. 2011). Galantamine is well tolerated at doses of 16-24mg/d and provides significant protection against cognitive decline when compared to placebo in mild to moderate patients (Wilkinson & Murray 2001). Little difference between the three AChEIs in their effectiveness was found in a Cochrane review by Birks (2006). Once the disease progresses beyond the moderate stages, however, these medications lose their effects, as even in the most responsive patients, AChEI treatment aims only to slow the natural progression of the disease (Gillette-Guyonnet et al. 2011). Indeed, some clinical trials with donepezil measured clinical effectiveness in only 30-40% of AD patients (Foster & Plosker 1999).

Unfortunately, AChEI treatment is not risk free resulting, for example, in clinically significant reductions in pulse rate (Masuda 2004). Cholinergic blockade in healthy volunteers leads to increases in arterial pressure and tachycardia, and so AChEIs may have a vasoactive effect (Hamner et al. 2012). Indeed, increased cerebral blood flow (CBF) has been observed in AD patients receiving AChEIs (Venneri et al. 2002).

Memantine is prescribed for moderate to severe AD, as it appears to have no benefit over placebo for those with mild AD (Schneider et al. 2014). It may be prescribed in combination with continuing AChEI treatment and is well tolerated in patients. Memantine treatment combined with AChEI treatment provided significant benefits in global cognition and psychiatric symptoms in moderate to severe patients compared to a placebo after six months (Tariot et al. 2004). However, this positive effect has not been replicated in all studies (Porsteinsson et al. 2008). Over a longer time frame, 30 months, memantine combination treatment has been shown to slow the decline in cognitive functioning and ADL greater than AChEI alone or no treatment (Atri et al. 2008). Also
importantly, combination treatment was found to significantly reduce the risk of nursing home admission (Lopez et al. 2009). Rive et al. (2013) carried out a comprehensive review and comparison of five meta-analyses of memantine treatment. This showed a significant effect on cognitive decline and ADL in moderate to severe AD patients compared to placebo. Also, there was some evidence for a decline in psychiatric symptoms, but findings were heterogeneous.

The cost-effectiveness of these medications in AD treatment were evaluated in a Health Technology Assessment on behalf of NICE by Bond et al. (2012). The AChEIs were found to have a small, dose-related, beneficial effect on global cognition; however the effect on behavioural and psychological AD symptoms is still unclear. A beneficial effect was also found for memantine, however the reviewer noted that this was based on the pooled results of only two trials, one of which could conclude no significant benefit from use of memantine (Bond et al. 2012). Therefore, evidence suggests that current treatment has at best a modest effect for some patients, and other treatment options must be investigated and evaluated.

Physiological differences in AD patients due to AChEI and NMDA antagonist treatment may provide useful biomarkers for disease progression and treatment response. A preservation of CBF, which is typically reduced in AD patients, was found after donepezil treatment compared to placebo (Nakano et al. 2001). Cholinergic innervation via mAChRs to cerebral microvessels induces vasodilation, and so protection of the cholinergic system may maintain this function (Van Beek & Claassen 2011). A reduction in hippocampal shrinkage has been observed in AD patients taking AChEIs compared to AD patients who are drug naive (Hashimoto et al. 2005; Krishnan et al. 2003), possibly due to improvements in regional blood flow. Treatment with AChEIs and memantine have also been observed to recover electroencephalogram (EEG) patterns in AD patients, returning the patterns to near those seen in healthy elderly (Babiloni et al. 2013). Also, a recent placebo controlled double blind study by Wang et al., (2013) found a protective effect of memantine treatment on glucose metabolism in the frontal and occipital cortex and hippocampal regions. It was appreciated by the authors however, that their patient sample, although in the moderate to severe stages of AD, was relatively young, and so further work is needed in larger groups with a range of ages to fully understand the neurological impact of these drugs. Treatment with memantine in an animal model has resulted in a reduction in AD-like
neuropathologies (Martinez-Coria et al. 2010) suggesting evidence of disease-modifying effects, but this has not yet been replicated in human studies (Wang et al. 2013).

The caveat with all of these medications is that they are only able to offer symptomatic relief, and in some cases offer no protection at all. So far the development of disease-modifying drugs targeting amyloid and tau aggregation has not been successful (Franco & Cedazo-Minguez 2014) but more clinical trials are underway. The mechanisms behind currently available AD medications and the effect they have on the brain are not fully understood. For instance the effect of AChEIs upon the cholinergic system is well established, however their influence on the other systems only recently becoming appreciated (Tabet 2006). Animal studies have shown increases in anti-oxidant levels with AChEI treatment in a dementia-mouse model (Saxena et al. 2008) and protection from Aβ-mediated toxicity in rat neurons (Kimura et al. 2005). Nicotinic pre-treatment reduced the release of pro-inflammatory molecules from immune cells in the brain via activation of nAChRs, suggesting an anti-inflammatory role for the cholinergic system (Shytle et al. 2004). However, human studies in AD patients have not yet supported these findings; AChEI treatment was found to have no effect upon blood anti-oxidant (Klugman et al. 2012) or pro-inflammatory biomarker levels (Richardson et al. 2013) in AD patients. An anti-inflammatory effect of memantine has been suggested by cell-culture studies; treatment of memantine has been observed to inhibit microglia activation (Rosi et al. 2009), and induce production of glia-cell derived neutrophic factors (Wu et al. 2009). However, this has not been clarified in patients.

1.3 Cognition in AD

1.3.1 Memory and attention

The diagnostic criteria suggested by McKhann et al., (2011) states that there should be an impaired ability to retain new information for a diagnosis to be made of AD. Elegantly designed cognitive tests exploring different areas of memory acquisition and retention have attempted to uncover whether this is due to a failure of memory formation or retrieval in AD. Experiments appear to have shown that failure to first retain information into the short term memory store is a characteristic symptom
(Germano & Kinsella 2005; Perry & Hodges 2000). For instance, Grober and Kawas (1997) retrospectively assessed the memory performance of elderly people who were later diagnosed with AD, and those who remained dementia free. Those who were found to have preclinical AD had an acquisition deficit when compared to the age-matched controls. Albert et al., (2001) also found that the best discriminator between probable early AD and normal cognitive decline associated with ageing was a measure of acquisition.

This inability to hold on to new information may be due to a problem with the working memory system. This is a short term memory system, dependent upon attentional processes, responsible for co-ordinating storage and processing of information (Baddeley & Hitch 1974). Therefore a lack of attentional ability to sufficiently encode information into memory, may lead to memory deficits in early-stage AD. The episodic buffer, added to the working memory model by Baddeley (2000), is proposed to manipulate and integrate information from very short term passive memory stores into an episodic representation. The attentional resources of working memory are controlled by the central executive, which is thought to be dysfunctional in early AD; tasks which involve active manipulation of information are impaired in early AD, whereas passive storage of information is intact until the disease has begun to move towards the moderate stages (Perry & Hodges 2000; Germano & Kinsella 2005). Impairments in working memory attentional control may therefore contribute to deficits seen in acquisition of new information. Perry et al., (2000) found that very mild AD patients were significantly impaired on tests which involved control and switching of attentional resources compared to an age-matched group. Baddeley (2001) also found impairments in resisting interference from distraction and dividing attention in AD patients. Selectively attending to objects of interest and ignoring distractors appears to be impaired in early stage AD patients, particularly in the visuospatial domain (Parasuraman et al. 1992; Rizzo et al. 2000).

The observed deficiencies seen in AD populations relates to known dementia- and age-related changes within the brain. Age-related white matter degradation (which has also been suggested as a contributing factor to the aetiology of AD by Bartokis (2004) has been associated with working memory deficits in healthy ageing (Charlton et al. 2014; Charlton et al. 2010). As synchrony across neuronal networks is required for working memory, lack of neuronal specificity observed in aged participants could
represent a loss of neuronal co-ordination. Functional imaging work comparing older and younger participants has shown an increase in bilateral activation of the frontal cortex during verbal memory tasks in older participants (65≥ years) (Reuter-Lorenz et al. 2000; Park & Reuter-Lorenz 2009). Work by Gutchess et al., (2005) showed that additional frontal lobe activity in older adults improved memory performance, and suggested that this may be a compensatory mechanism for the age-related deterioration of the memory systems of the hippocampus and medial temporal lobe. In a longitudinal study by Persson et al., (2006) it was found that older adults whose memory performance declined over time had a decrease in hippocampal volume and an increase in bilateral frontal activation. Those whose memory decline was most severe also showed a decrease in fractional anisotropy in the white matter tracts, suggesting that loss of white matter integrity may contribute to impaired memory function. Functional MRI studies in AD patients have found reduced activity in medial temporal lobe areas during memory encoding tasks (Schwindt & Black 2009). Compensatory mechanisms in the frontal lobes may therefore accommodate for loss of neuronal integrity in hippocampal areas.

1.3.2 Prospective memory

Prospective memory (PM) is the encoding, storage and retrieval of future tasks; i.e. remembering to do something in the future. The retrieval of a PM is often triggered by external stimuli which provide a cue which has been previously associated with the PM.

There is current debate in the literature as to the role of working memory systems in the detection and processing of the cue which corresponds with a prospective memory task. Smith (2003) proposed that individuals enter into a retrieval mode at the onset of the intention to perform a task, and that without constant monitoring the PM intention could not be retrieved. However, this is very capacity-demanding, especially if the PM task is over a long period of time. Therefore Giles et al., (2005) argue that there is another process involved with PM which uses automatic associative-memory systems and so is less capacity-demanding.

Which of these processes contributes most to the retrieval process may be partly dependent upon whether the task cue is congruent to the ongoing task (a focal target event) or incongruent (a non-focal target event). The evidence that focal target events
rely upon monitoring, and non-focal target events rely on automatic associative-memory systems comes from studies using a duel-task paradigm with a prospective and working memory task. As monitoring is a capacity-demanding process, working memory for other tasks performed at the same time in a duel-task situation will be impaired. Studies by Smith (2003) showed that working memory task performance could be impaired by simultaneously performing a PM task. Also, those who scored higher on the PM task scored less on the working memory task, suggesting that the PM task was performed at a cost to the working memory task.

A meta-analysis suggests both focal and non-focal PM is impaired with increased age (Uttl 2011). PM has also been shown to be further impaired in those with AD (Duchek et al. 2006) perhaps in part due to the working memory problems seen in this population. Although retrospective memory impairment is a well-known characteristic of AD, PM is less studied in this population. In a recent study by Farina et al., (2013) healthy older adults performed significantly better in terms of reaction time and accuracy in an event-based non-focal PM task compared to AD patients. The neuronal circuits implicated in PM are also those affected in AD patients, such as the prefrontal and parietal cortex (Gonneaud et al. 2014). Therefore limited integrity of these areas may explain the observed deficits.

1.3.3 Emotional Processing

Within healthy populations there are age-related changes in emotional processing. Older adults report less negative response to stress (Neupert et al. 2007) and less time in highly negative emotional states than younger adults (Hay & Diehl 2011). It may be that older adults use more successful emotional filtering strategies; self-reported suppression of negative emotions increases with age (Nolen-Hoeksema et al 2011), and older adults have been found to selectively disengage attention away from negative stimuli in experimental paradigms (Mather & Carstensen 2003; Isaacowitz et al. 2006). Concordant with this, older adults are more successful at avoiding distraction from negative stimuli (Lamonica et al. 2010). Studies have also shown a response bias to positive emotional stimuli in older adults (Johnson & Whiting 2013), and a greater distraction by positive stimuli. This positive bias may be related to greater activity in the anterior cingulate cortex (Brassen et al. 2011). Interestingly, greater activation of
this area during the positive distraction in Brassen et al., (2011) was correlated with a measure of emotional stability. This may suggest that there is a protective tendency to preferentially engage with positive stimuli in ageing brains. This bias to positive stimuli and emotional regulation can be attenuated by concurrent task demands, suggesting that it is dependent upon attentional resources (Knight et al. 2007).

Alongside an increased attendance to positive stimuli, recognition of non-positive emotional face expressions is impaired in old-age. Suzuki and Akiyama (2013) propose that impairment in emotional recognition in older adults may be explained by age-related cognitive decline as determined by tests of global cognition. However, Lambrecht et al., (2012) found no association between age-related deficits in emotional recognition and age-related declines in working memory and verbal intelligence. As varying methods have been used, it is difficult to conclude whether age-related declines in emotional recognition can be explained by general declines in cognitive ability.

Impairments in recognition of emotions has been observed in various neurological disorders; frontotemporal dementia (Oliver et al. 2014), Parkinson’s disease (Buxton et al. 2013) and Huntington’s disease (Labuschagne et al. 2013). A deficit in AD patients has been observed in some (Spoletini et al. 2008; Weiss et al. 2008) but not all (Burnham & Hogervorst 2004) studies, and so the impact of AD upon emotional appraisal requires further investigation. Visual recognition of emotional expression may be particularly important in AD patients, as there is a vocal processing deficit which may impair verbal communication (Hailstone et al. 2011). A longitudinal study by Lavenu and Pasquier (2005) showed increasing impairment after three years in AD patients, which the authors suggested may be due to greater degradation of emotional processing brain areas. The authors noted that this decline was despite use of AChEIs, however there was no treatment-naïve group available for comparison.

As reviewed by Klein-Koerkamp et al., (2012), studies have found a problem with emotional appraisal in AD patients, however these studies did not control for potential confounding variables, other than depression. It may be that emotional processing can also be affected by the cerebrovascular system. As will be discussed further, age-related deterioration of blood vessels, and vessel stiffness associated with hypertension, are common in AD patients. Arterial stiffness has been associated with cerebral small vessel disease and cognitive impairment (Singer et al. 2014), and mid-life hypertension is recognised as a risk factor for AD (Meng et al. 2014; Shah et al. 2012). As well as the
negative impact vascular risk factors may have upon cognition per se, essential hypertension has been associated with alexithymia – a neurological condition associated with emotional dampening (Grabe et al. 2010). Very recent work by Makovac et al., (2015) in healthy adults showed that stimulation of the carotid receptors was sufficient to reduce both amygdala neuronal response and intensity ratings of fearful faces. It will therefore be interesting to see if hypertension could have a further impact upon emotional recognition within a cognitively impaired AD population.

An impact of immune activation on depression incidence has been suggested (Charlton 2000), and high levels of inflammatory cytokines are associated with depressive symptoms (Young et al. 2014). Greater levels of pro-inflammatory interleukin (IL)-1, IL-6 and C-reactive protein (CRP) have been measured in plasma of depressive patients compared to healthy controls (Raison et al. 2006). However, the influence of inflammatory state on the ability to appraise the emotional state of others within an AD population has not yet been determined.

1.4 AD neuronal pathology

A collection of neuropathologies, NFT and Aβ, is used to distinguish AD from other dementias at post-mortem examination and to give an indicator of disease severity (McKhann et al. 2011). The exact relationship between these markers and cognitive symptoms however, is not fully understood.

1.4.1 Amyloid-β and the amyloid hypothesis

Various proteins associated with different diseases are able to form an amyloid fibril structure. In AD, the cleavage of amyloid precursor protein (APP) at the cell membrane by β- and γ- secretases forms Aβ peptide fragments of various lengths which go on to develop along the fibrillogenic pathway. Initial Aβ oligomer peptides which form in the brains of AD patients are small, spherical structures which go on to form proto-fibrils, and then fibrils which have a crossed β-sheet structure (Fändrich 2012). Mature fibrils are the end stage of the fibrillogenic pathway, which are able to aggregate together to form the macroscopic amyloid plaques first identified by Alois Alzheimer (Maurer et al. 1997). Variation in structure of the various amyloid peptides seen in AD and other disorders leads to their differing effects on their environment (Marshall et al. 2014).
Size, surface properties, and levels of solubility and stability influence Aβ’s interaction with its environment and therefore its potentially toxic effects (Marshall et al. 2014; Mucke & Selkoe 2012). The most common peptides formed in AD are Aβ_{40} and Aβ_{42}. Aβ_{42} is hydrophobic making it more likely to fibrillate and form a plaque structure, and is significantly increased in AD brains compared to non-demented controls (Chen & Glabe 2006).

The amyloid hypothesis of AD states that the development and build-up of Aβ is a main causal factor of the cognitive deterioration observed in AD patients (Hardy & Higgins 1992; Hardy & Selkoe 2002). This was supported by the finding that genetic mutations associated with familial AD increase propensity for the formation of Aβ_{42} (Hutton et al. 1998), and because Aβ depositions have been observed to develop before aberrant tau NFTs in AD (Stancu et al. 2014). However, it is now appreciated that Aβ plaque load often has poor correlation with clinical presentation (Marchant et al. 2013), and they can be found in older persons with no clinical dementia symptoms (Aizenstein et al. 2008; Katzman et al. 1988). Therefore, more recent work has focused upon soluble forms of Aβ in the pre-plaque stages. It must also be appreciated that oligomers of Aβ are often co-localised with Aβ plaques, and so their effects upon neurons may not be independent (Koffie et al. 2009).

Oligomeric and small peptide fragments of Aβ are able to interact with cell membranes; evidence suggests that Aβ_{42} may cause an influx of Ca^{+2} into the neuron by forming calcium ion channels (Williams & Serpell 2011). Aβ can also interact directly with membrane receptors. Oligomers are able to bind to GluN1 and GluN2B subunits of NMDA receptors at the hippocampal post-synaptic neuron, altering glutamatergic transmission (Patel & Jhamandas 2012). This binding also increases movement of Ca^{2+} into the cell to neurotoxic levels (Demuro et al. 2005; Kelly & Ferreira 2006). An increase in intracellular Ca^{2+} can lead to excitotoxicity in the cell by increasing after-hyperpolarisation (AHP). AHP is designed to limit the firing rate of action potentials and is mediated by flow of Ca^{2+} across the cell membrane. AHP has been observed to be increased in the hippocampus of aged animals, impairing memory and learning. This impairment can be rescued by calcium channel antagonists (Disterhoft & Oh 2006). Disruption of glutamatergic hippocampal neurons may lead to impaired memory formation by interfering with long term potentiation (LTP). This has been observed in rat hippocampal neurons by using Aβ treatment (Walsh et al. 2002). These interactions
may lead to synaptic loss and dysfunction before neuronal cell loss occurs. Indeed, post-mortem examination of AD patients has shown cognitive impairment to be associated with loss of synaptic density (Terry et al. 1991). Due to interaction with the cell membrane and its receptors, both soluble and fibrillar Aβ have been associated with reduced dendritic spine density and size on hippocampal neurons (Kirkwood et al. 2013; Wei et al. 2010). Reductions in dendritic complexity have been observed in the entorhinal cortex and hippocampus, as well as in cortical neurons after Aβ treatment (Cochran et al. 2014). Soluble forms of Aβ have also been shown in mature cultured hippocampal neurons to increase production of reactive oxygen species (ROS). This activity could be inhibited by blocking of the NMDA receptors, suggesting that Aβ is also able to increase oxidative stress through activation of these receptors (De Felice et al. 2007).

1.4.2 Cognition and amyloid

Researchers have investigated the relationship between cognitive phenotype and amyloid deposition in the brain using Pittsburgh compound B (PIB). PIB is a radiopharmaceutical used in positron emission tomography (PET) scans to trace fibrillar Aβ (Rowe & Villemagne 2011). PIB positivity has been associated with greater atrophy over time in healthy elderly (Chételat et al. 2012), as well as in MCI and AD patients (Jack et al. 2009). Other studies have found correlations between Aβ plaque load as measured by PIB and cognitive symptoms, however the correlations are often weak in AD patients (Villemagne et al. 2011), and this relationship is lost in older patients (Prohovnik et al. 2006). Greater associations have been found in post-mortem studies which have assessed the relationship between cognitive status and smaller Aβ peptides in the brain. For instance Ikonomovic et al., (2009) found a correlation between soluble Aβ levels in the frontal cortex of AD patients and MMSE score taken less than twelve months before death. Naslund et al., (2000) found that levels of both Aβ40 and Aβ42 in cortical regions, correlated with Clinical Dementia Rating (CDR) score within the last six months of life. It appears that Aβ load as determined by PIB reactivity may be useful as a biomarker in preclinical AD, but not once clinical presentation of AD symptoms has begun.
Due to the common co-morbidity of both AD and vascular disease, Marchant et al., (2013) measured the relative impact of both PIB positivity and presence of cerebrovascular disease (CVD) on cognitive performance. In a sample of elderly participants it was found that presence of CVD was associated with greater impairments in executive function tasks, but that PIB status did not independently effect cognition. Further work by this group (Marchant et al. 2013) found that infarcts were particularly important in determining cognitive function in cognitively impaired elderly participants. This recent finding supports earlier work by Snowden et al., (1997) in the infamous Nun study. Here, post-mortem examination suggested that presence of infarcts lowered the Aβ threshold required in the brain for cognitive symptoms to be apparent.

Many studies have used a measure of total CVD and AD pathology load or burden, however it may be that specific regional damage may tell us more about the aetiology of AD. AD pathology has a pattern of deposition through brain regions (Braak & Braak 1991; Thal et al. 2014), and CVD presentation is dependent upon the specific pattern of cerebrovasculature. Brickman et al., (2012) showed that regional white matter lesion volume, as indicated by white matter hyperintensites (WMH) on MRI, in the parietal lobe predicted time to incident AD in an elderly population, but not WMH in other areas. This raises the possibility that WMH may only be associated with cognitive decline and AD in specific brain regions, rather than overall load of WMH. Meguro et al., (2012) investigated the importance of different areas of CVD in AD and VaD, and found that lesions in the caudate head and thalamus were particularly associated with dementia; even a small lesion in the left thalamus was sufficient to cause enough impairment to be clinically diagnosed with VaD. It was also found in this study that AD with CVD was the most common cause of dementia, and that those with more severe dementia rating had increased numbers of CVD. Therefore due to the pattern of cerebrovasculature and AD pathology, certain brain regions relating to different types of cognition may be selectively vulnerable.

1.4.3 Amyloid and blood vessels

Brain imaging for detection of amyloid as a biomarker for AD has limitations; small soluble forms cannot be detected, and imaging procedures may be intolerable for some patients. However, amyloid can also be detected in the peripheral blood stream. Decreased plasma levels of Aβ40 and Aβ42 along with a greater Aβ42/40 ratio, has been
associated with increased risk of developing AD when measured fifteen years before diagnosis (Shah et al. 2012). High levels of cerebral amyloid angiopathy (CAA) was associated with lower Aβ levels in the blood in this study, suggesting a problem with amyloid clearance from the brain. A meta-analysis by Song et al., (2011), suggested that at an older age, and approximately three to five years before diagnosis, higher levels of Aβ₄₀ and Aβ₄₂ predicted conversion from cognitively typical to AD, but that the difference between levels in AD populations and healthy controls is not significant. Work by Rembach et al., (2014a) found a decrease of Aβ₄₂ to be predictive of AD over eighteen months. These results show the dynamism of Aβ levels in the blood over time. In persons diagnosed with AD, Rembach et al., (2014b) showed an increase over time in Aβ plasma levels. A change in plasma Aβ however has not been associated with measures of cognitive decline in clinical trials of AD patients, so this may have limited clinical significance (Donohue et al. 2014). A review by Toledo et al., (2013) highlighted the inconsistencies in the literature surrounding Aβ plasma levels in AD. Although some studies show that a decrease over three to five years in plasma Aβ₄₀ and Aβ₄₂ (e.g Schupf et al. 2008) is associated with AD incidence, others show that it is high Aβ₄₀ (van Oijen et al. 2007; Hansson et al. 2012) and Aβ₄₂ (Blasko et al. 2010), and others find no effect (Lopez et al. 2008; Toledo et al. 2013). Therefore, it seems plasma Aβ levels are not useful as a biomarker until they are further understood.

CAA is the deposition of amyloid peptides in the cerebral blood vessel wall, thought to occur due to their impaired removal from the brain. Protein deposits are removed from the brain alongside the vasculature via periventricular drainage. As perivascular drainage is produced by the pulsating force of the blood vessels, it is impaired with age-related vessel stiffening (Vasilevko et al. 2010). This will decrease the effectiveness of drainage of Aβ peptides and other protein debris from brain tissue. CAA and hypertension are leading causes of intracerebral haemorrhage (O’Donnell et al. 2000) and are associated with microinfarcts (Okamoto et al. 2012) which may contribute to cognitive impairment. CAA is also associated with white matter damage and stroke, and significant levels of complement cascade proteins have been measured in CAA deposits (Vasilevko et al. 2010), suggesting an inflammatory component, which is likely to lead to further tissue damage.
1.4.4 Tau

Intra-neuronal NFTs have a pattern of deposition through the brain (Braak & Braak 1991) which is used as a hallmark of AD progression at post-mortem examination. Six stages of NFT projection through brain regions were identified and published by Braak and Braak (1991). Stages I and II are transentorhinal stages, as NFTs can only be found in the transentorhinal areas of the temporal lobe by the hippocampus. At this early stage, it is expected that there would not be clear clinical presentation of AD symptoms. Stages III and IV show proliferation of NFT structures in the entorhinal cortex and hippocampus, and are referred to as the limbic stages as the pathology has now spread to parts of the limbic system. Such a spread of NFTs is thought to associate with cognitive impairments in MCI or mild AD. Stages V and VI are the cortical stages as the pathology has now spread to cortical cortex areas, and is associated with significant AD impairments. These stages are used to confirm clinical AD diagnosis at post-mortem, and have been correlated with grey matter atrophy (Whitwell et al. 2008), neuronal numbers, and MMSE scores (Giannakopoulos et al. 2003).

NFTs are formed from aggregated straight filaments, or paired helical filaments, of hyperphosphorylated tau. Tau is a microtubule-associated protein whose function in healthy neurons is to aid fast axonal transport (FAT). FAT is the anterograde (away from the cell body) and retrograde (toward the cell body) movement of membrane-bound organelles such as mitochondria through the neuron, essential for neuronal functioning (Morfini et al. 2002). Tau stabilizes microtubules to provide a track for motor proteins dynein and kinesin to transport organelles along the axon (Lee & Leugers 2012).

Cells which contain NFTs tend to have deteriorated dendrites and a displaced nucleus, showing that NFTs have negative effect upon cell structure and function. Neurons can also be ‘pre-tangle’, containing phospho-tau positive filaments, but having apparently normal cell structure (Augustinack et al. 2002). Tau is hyperphosphorylated in conditions such as AD and other tauopathies, with higher levels of phosphorylation on multiple sites of the tau protein compared to tau in healthy older adults. This decreases affinity for microtubule binding, which decreases microtubule stability (Lu & Wood 1995), and leaves tau fragments free to mis-fold and aggregate (Liu et al 2008). Kinase protein glycogen synthase kinase-3 (GSK-3) regulates anterograde transport as
it phosphorylates kinesin light chains causing them to let go of their cargo. GSK-3 is also able to phosphorylate tau (Sperber et al. 1995), and has been found to be up-regulated in AD patients (Hooper et al. 2008). In vitro experimentation has shown GSK-3 is able to be activated by Aβ, suggesting a potential link between Aβ activity and hyperphosphorylation of tau via GSK-3 (Takashima et al. 1998). Treatment of Aβ in rat hippocampal neurons has also been observed to increase GSK-3 activity and tau phosphorylation (Takashima et al. 1996; Takashima et al. 1998).

Hyperphosphorylated tau may have both a toxic loss of function effect in microtubule regulation, as well as a toxic gain of function from aggregation of tau filaments. Post-mortem tissue stained with phosphorylation-dependent antibodies, showed pre-NFTs phosphorylated at sites which regulate microtubule binding, suggesting that loss of tau function may be the initial damaging process before fibrillation occurs. For instance, pre-tangle cells were stained for anti-body pS262 which is phosphorylated by microtubule affinity regulating kinase (MARK) which is able to detach tau from microtubules (Augustinack et al. 2002). Other studies have also suggested that hyperphosphorylation of tau in the microtubule-binding region occurs early in the development of NFTs (Bramblett et al. 1993; Morishima-Kawashima et al. 1995). Hyperphosphorylation of tau has also been observed to interfere with FAT via a toxic gain of function; addition of tau filaments in vitro inhibited anterograde, kinesin-dependent FAT via activation of GSK-3 (LaPointe et al. 2009; Kanaan et al. 2011).

1.4.5 Cognition and Tau

Post-mortem study has that shown that progressive deposition of NFTs from the limbic to the cortical regions correlates with dementia severity, but this association does not account for much of the clinical variability (Gold et al. 2001). NFT count, but not amyloid deposition, in the CA1 area of the hippocampus was found to correlate with MMSE score in post-mortem examination of AD patients (Giannakopoulos et al. 2003). Levels of NFT in the CA1 also correlated with cognitive decline as determined by the Clinical Dementia Rating score, but a significant variability (approximately 50%) was not explained by this pathology (von Gunten et al. 2006; Giannakopoulos et al. 2007). Recent work in animal models of tau aggregation has been able to attenuate pathology-associated cognitive impairment with pre-treatment with methylene blue (MB).
(Hochgräfe et al. 2015). MB is a dye which is also an antiseptic used to treat urinary tract infections. Application has been found to inhibit tau aggregation \textit{in vivo} (Hosokawa et al. 2012), though the mechanisms behind this are not fully clear. MB has been shown to oxidise cysteine residues of tau, preventing its mis-folding and aggregation (Akoury et al. 2013). Also, MB has been observed to upregulate genes associated with anti-oxidant and anti-inflammatory activity, which may lead to reduced tau aggregation (Stack et al. 2014). However it has also been argued that MB is effective by facilitating the degradation of already aggregated tau (Wischik et al. 1996).

A form of optimised methylene blue is now in Phase three clinical trial, with results expected to be announced 2016 (TauRx 2014). A Phase two trial by TauRx showed promising results in mild-moderate AD patients with treatment of optimised methylene blue. The results of this study indicated a significant effect of treatment in global cognitive score in moderate AD patients, and improvements in CBF compared to placebo in both mild and moderate patients after six months (Wischik et al. 2015).

It can be seen from the work discussed above that the pathologies associated with AD do not fully explain the cognitive symptoms of the disease. Therefore other factors which influence the presentation of AD must be explored.

1.5 Ageing

The main risk factor for AD is age. Age-related vascular diseases such as arteriosclerosis are very common in AD patients (Kalaria 2003) and their potential role in the development of AD and other dementias is unclear. Even in ageing that is cognitively typical, age-related processes often lead to cognitive decline and a reduction in brain function. Healthy elderly participants tend to have slower reaction times and a reduced performance during cognitively effortful tasks than healthy younger participants (Deary et al. 2009). Some neurological changes associated with AD can also occur in healthy ageing. For instance, in the Medical Research Council Cognitive Function and Aging Study (MRC CFAS), post-mortem examination showed approximately a third of cognitively typical individuals had pathologically significant levels of Aβ plaques (Wharton et al. 2011).

It has been argued that age-related deterioration of myelin may be an important contributing factor to AD (Bartzokis 2004). Myelin is reduced in healthy ageing, and
reduced by a greater extent in MCI and AD (Bartzokis 2011). Late-myelinating brain regions, such as the neocortex, are the first areas to show AD pathology (Bartzokis 2004; Braak & Braak 1995), and are structurally more vulnerable than the early myelinating regions, the motor and sensory systems (Bartzokis et al. 2007). Bartzokis et al., (2007) hypothesised that iron released by age-related breakdown of myelin may trigger an increase in Aβ formation. Increased iron levels in the brain have been associated with AD (Zeineh et al. 2015; Lovell et al. 1998) and Aβ formation (Becerril-Ortega et al. 2014). In both healthy functioning and disease states, myelin repair is dependent on the cleavage of neuregulin by BACE1 (a β-secretase), and an age-related increase in BACE1 is thought to correlate with the need for myelin repair in later life (Bartzokis et al. 2007). This increase in BACE1 may therefore be another mechanism which would increase the Aβ formation.

Oligodendrocytes themselves have high energy demands and so high potential for metabolic and oxidative stress. Oxidative stress within the body increases with age. Cellular metabolism creates ROS as a by-product of respiration. These ROS have ‘spare’ ions which cause them to be highly reactive with neighbouring cellular molecules, interfering with their function. Healthy individuals have natural anti-oxidant defence mechanisms to reduce levels of ROS, however these can become overwhelmed, particularly as levels of ROS build-up with age. Markers of oxidative stress are increased in the neurons and mitochondria of AD patients compared to age-matched controls (Castellani et al. 2001; Sultana et al. 2013), and oxidative stress can be triggered by Aβ (Boyd-Kimball et al 2005). Increased oxidative stress also triggers inflammatory processes, leading to increased levels of inflammatory molecules.

The mechanisms by which ageing of the vascular system may influence AD development will be discussed in Section 1.7 Vascular regulation and blood pressure.

1.6 APOE

Presence of the APOE ε4 allele is the biggest risk factor for sporadic AD after age (Tanzi 2012). Around 64% of AD patients have at least one ε4 allele, which is only present in around 30% of non-AD individuals (Corder et al. 1993). The risk of developing AD increases by having one copy of the ε4 allele by 4-fold, and by nearly 14-fold by having two copies (Farrer et al. 1997). The three heritable isoforms of APOE (ε2, ε3 and ε4) differ by single amino acid changes, which have important implications
for structure, and therefore function, of the protein apolipoprotein E (apoE), a cholesterol transporter. The risk of AD associated with APOE genotype is ε4>ε3>ε2. Neuronal apoE is synthesised by astrocytes, and transports cholesterol to neurons to be used as an important component of myelin (Hauser et al 2011). Mice carrying the ε4 allele have been shown to have less dendritic arborisation and reduced excitatory transmission than ε3 carriers (Wang et al 2005). This neuronal dystrophy may contribute to synaptic degradation and impairment of LTP (Koudinov & Koudinova 2001). Carriers of ε4 showed worse outcomes six months after head injury than non-ε4 carriers (Teasdale et al. 1997) suggesting impaired ability for neuronal repair. The genetic risk for AD of the apoE isoforms is inversely correlated with their cholesterol transport efficiency which is: ε2>ε3>ε4 (Michikawa et al. 2000).

Individuals who carry the ε4 allele have reduced apoE in plasma, and a reduction in apoE in the CSF has been measured in AD patients who are also ε4 carriers (Cruchaga et al. 2012). This may imply that, as well as having a less efficient form of apoE, ε4 carriers do not have sufficient levels of apoE available to cope with general white matter repair and upkeep in old age (Poirier 2008). As well as playing a part in the pathogenesis of AD, apoE has been implicated in increased development of CVD and cardiovascular disease (Lopez et al. 2014). In a meta-analysis of 42 studies using MRI scan data by Schilling et al. (2013), apoE ε4 genotype was associated with increased WMH and cerebral microbleeds in the general population, which may contribute to cognitive decline. Increased levels of cholesterol found in the blood of ε4 carriers is likely to be due to differences in apoE structure, and may contribute to CVD (Davignon et al. 1988; Hatters et al. 2006). Presence of one APOE ε4 allele increased the risk of moderate or severe CAA by nearly 3-fold, and by 13-fold in those homozygous for ε4, and was also linked to CAA-associated haemorrhage in a post-mortem study by Greenberg et al., (1995). This relationship was still present after controlling for amyloid plaque burden. Therefore there is an increased risk of haemorrhage and cerebrovascular dysfunction in ε4 carriers which may contribute to its association with AD.

In contrast to APOE’s association with neurodegeneration and cognitive decline, studies have shown that healthy young individuals with APOE ε4 status perform better on cognitive tests than non-ε4 carriers. This has been shown in tests of processing speed (Marchant et al. 2010), attention (Rusted et al. 2013) and executive functioning tasks (Alexander et al. 2007; Marchant et al. 2010). Evidence from Dowell et al., (2012)
suggests that young ε4 carriers have increased white matter, indicating there may be problems with white matter homeostasis in ε4 carriers throughout the lifespan. This may appear advantageous in early life as greater white matter connectivity aids certain cognitive functions, however, as we age, this advantage is overshadowed by the effects of age-related white matter deterioration, and less efficient cholesterol transport.

ApoE has also been associated with AD pathology. In a mouse model, apoE ε4 fragments interacted with tau protein and stimulated its hyperphosphorylation within neurons (Harris et al 2010). Even in those without AD, ε4 is associated with greater Aβ depositions in the brain (Kok et al. 2009). CSF apoE levels correlate negatively with Aβ levels in the brain as measured by PIB in PET scanning (Fagan et al, 2006), and levels of apoE in both plasma and CSF are lower in ε4 carriers (Cruchaga et al. 2012). Therefore, there may be disrupted Aβ regulation in ε4 carriers due to lower levels of apoE (Shinohara et al. 2013). Within the brain, Aβ can bind with apoE protein. This binding site may be competitive for the lipid-binding site of apoE, impairing its cholesterol transport function (Tamamizu-Kato et al. 2008). Presence of apoE may even be necessary for Aβ deposition and aggregation into plaques. Mouse models created from crossing amyloid transgenic mice with an APOE knockout strain, had significantly reduced Aβ deposition, compared to amyloid strains with APOE (Irizarry et al 2000).

Work by Hashimoto et al., (2012) showed that apoE increased Aβ deposition in an isoform dependent manner: ε2<ε3<ε4. ApoE’s anti-inflammatory activity, by converting macrophages to their anti-inflammatory phenotype, also seems to follow the same isoform dependency (Zhu et al. 2012). Therefore Aβ clearance by macrophages may be less efficient in ε4 carriers. In middle-aged offspring of AD patients, significantly higher levels of inflammatory molecules in the blood stream, and increased incidence of hypertension were found when compared to individuals without a genetic link to AD (van Exel et al. 2009). This may suggest that a genetic pre-disposition for AD starts early in life by influencing both vascular and inflammatory systems, even in sporadic AD.

1.7 Vascular regulation and blood pressure

The flow of blood through the blood vessels causes the endothelial cells of the inner vessel walls to be subject to fluid mechanics. The physical effect of blood flowing at
different rates and pressures leads to chemical, physical and epigenetic changes within these cells.

The consistent movement of blood parallel to the endothelial cells of the vessel walls causes shear stress which has regulatory effects. Laminar shear is the normal forward flow of blood which becomes disturbed or oscillatory at bends in the vessel, or at times of poorly controlled blood pressure (Chen et al 2013). Laminar shear stress activates tyrosine kinase vascular endothelial growth factor receptor 2 (VEGFr2) (Jin et al, 2003), which mediates activation of nitric oxide synthesis (eNOS) in the endothelial cells to produce nitric oxide (NO). NO is an important vasodilator and can also inhibit the formation of atherosclerotic plaques by reducing the aggregation of platelets and the formation of adhesion molecules (Iadecola & Davisson 2008). Asymmetric dimethylarginine (ADMA) is an NO inhibitor which has been associated with hypertension (Millatt et al 2003; Kielstein et al 2004). Elevated ADMA levels are also associated with stroke (Yoo et al 2001) and arterial disease (Boger et al 1997), suggesting it may be associated with endothelial dysfunction. Increased ADMA has been observed in plasma of AD patients compared to age-matched controls (Arlt et al 2008), however this has not been found in all studies (Mulder et al 2002; Richardson et al 2014). Fetuin-A (Fet-A) on the other hand may be beneficial for vascular health as it inhibits calcification of vessels (Reynolds et al. 2005). It has also been observed to attenuate the inflammatory response by reducing production of inflammatory molecules and macrophage activation (Wang & Sama 2012). Fet-A has been found to positively correlate with MMSE score in AD patients, suggesting it may have protective properties in AD (Smith et al. 2011).

Transcription factor Kruppel-like factor-2 (KLF-2) is also stimulated by laminar shear stress, leading to anti-inflammatory, anti-coagulant and atheroprotective mechanisms (Boon et al. 2010). For instance, transcription of eNOS gene is upregulated by KLF-2, whereas inflammatory cytokine transcription is downregulated (Parmar et al. 2006). Pro-inflammatory cytokine tumour necrosis factor (TNF)-α is able to limit the expression of KLF-2 mRNA in endothelial cells (Kumar et al 2005), indicating that inflammatory factors may mediate vascular health, as well as being themselves moderated by shear stress. In areas where there is disturbed shear stress, such as branching and turning points in the trajectory of the vessel, there is increased susceptibility to atherosclerotic lesions (Jin et al, 2003). Disturbance of shear stress
activates atherogenic genes in endothelial cells, and induces expression of adhesion molecules and pro-inflammatory chemokines in the vessel wall (Chen et al, 2013). KLF-2 knockout mice were shown to have enhanced diet-induced atherosclerotic plaques, which concentrated at bends in the vessels (Atkins et al 2008).

Laminar shear stress is maintained at a healthy blood pressure. Healthy adults are targeted to have a systolic blood pressure (SBP) of 120mmHg, and a diastolic blood pressure (DBP) of 80mmHg. SBP of 120-130mmHg is classed as healthy, 130-140mmHg is pre-hypertensive, and SBP>140mmHg is hypertensive. For DBP, a measure of over 90mmHg is classed as hypertensive. SBP is the force on the blood vessels during the heart contractions, and DBP is the pressure exerted on the vessels between the cardiac contractions. Blood pressure in healthy adults can often be controlled through life-style changes and there is a range of antihypertensive medications available (NHS 2014). Medications which manipulate blood pressure via the renin-angiotensin system (RAS), angiotensin converting enzyme inhibitors (ACEI) and angiotensin receptor1 blockers (AT1RB) have been found to be protective against the development of cognitive decline. A protective effect on global cognitive test score in AD patients was found in those using a brain-penetrating ACEI for blood pressure control (Ohru et al. 2004). Those taking a non brain-penetrating ACEI did not show this cognitive benefit, suggesting these medications may be neuroprotective independent of their vasoactive effect. ACEIs and AT1RBs increase levels of angiotensin-IV availability in the brain (Fournier et al. 2009; Gard & Rusted 2004), which has been found to improve memory and learning in animal models (Paris et al. 2013).

There are also epigenetic processes regulating vascular health within vascular cells. Genes which promote VEGFr2 and eNOS contain CpG islands, which causes them to be vulnerable to methylation, a process which suppresses gene expression (Chen et al 2013). Global hypermethylation increases with age, and has been associated with cardiovascular disease and increased inflammation (Stenvinkel et al. 2007).

Presence of long-term hypertension has been shown to have a negative impact upon the health of the cerebrovasculature. High blood pressure is associated with cerebral atherosclerosis which can lead to lunar infarcts through occlusion of the small cerebral arteries (Lammie 2002). Cerebral atherosclerosis correlated with systemic blood pressure in post-mortem examination of AD patients, who were found to have greater
arteriosclerosis than age-matched controls in a study by Furuta et al., (1991). Hypertension is also a risk factor for primary intracerebral haemorrhage (PICH) which often co-localize with lacunar infarcts, and stroke (Lammie 2002). Fibrinoid necrosis is a small vessel lesion in the walls of brain arterioles consisting of plasma proteins, fibrin and fibrinogen. They are often co-localized and associated with PICH, and have been observed to be increased in hypertensive patients compared to normotensives (Rosenblum 2008). Atherosclerosis and reduced vessel integrity has been observed in AD patients, which may be caused by or exacerbated by hypertension.

Hypertension has been associated with a reduction in CBF in cross-sectional studies (Jennings et al. 2005), and a decrease over time in CBF has been observed in longitudinal studies of hypertensive patients (Beason-Held et al. 2007; Muller et al. 2012). Animal models of hypertension show vessel injury similar to small vessel disease in humans (Johansson 1999), and have shown resultant cognitive impairment (Hainsworth & Markus 2008). In rhesus monkeys, hypertension was shown to lead to microinfarcts and significant decline in short-term memory performance after one year of hypertension onset (Kemper et al. 2001). This study also found a negative correlation between blood pressure measures and memory scores. Animal studies provide important evidence for the role of hypertension in cognitive impairment and cerebrovascular damage, as the laboratory environment allows control of other vascular and environmental risk factors.

1.7.1 Cerebrovasculature and pathology in AD

The pattern of vascular supply to the brain is unusual as it contains independent capillary beds which are fed from a single arteriole. This causes the deep white matter to be particularly vulnerable to hypoperfusion. The arterioles which supply the deep white matter are long, and can become tortuous with age; tortuosity has been observed to begin as the vessel enters the white matter (Brown & Thore 2011) and is increased in individuals over the age of 50 (Thore et al. 2007). Tortuous vessels have become coiled and bent, increasing their length and increasing the resistance of blood flow. This causes an increase of required pressure to maintain blood flow, and if this blood flow is not maintained, the neurons supplied by this flow are at risk of hypoxia. Therefore age-related changes in blood vessel structure may be lead to an increased vulnerability to low blood pressures. Non-functioning string vessels which have lost their endothelial
cells and cannot transport blood flow are increased in the brain with age (Brown & Thore 2011).

There is evidence of disrupted cerebrovasculature in AD. String vessels for instance are more abundant in AD patients compared to age-matched controls (Challa et al. 2004). Also, reduced blood vessel diameters have been measured in the entorhinal cortex of AD patients (Burke et al. 2014). White matter lesions, CAA, haemorrhage and microvascular degeneration are signs of poor cerebrovascular health and are also common in AD brains (Kalaria et al. 2012). Cerebral atherosclerosis has been shown to be a common comorbidity in AD. A study by Yarchoan et al., (2012) found that 77% AD patients had atherogenic lesions in the Circle of Willis, compared with 47% of controls and 67% with other neurodegenerative diseases. This supports earlier findings by Beach et al., (2007) and Roher et al., (2003) who also found greater severity of atherosclerosis in the Circle of Willis in AD patients, which was associated with greater plaque and NFT deposition. A cross sectional study by Stefanova et al., (2012) found AD patients had a significantly increased prevalence of severe carotid artery stenosis (CAS) when compared to patients with VaD. A longitudinal study found that AD patients with greater baseline CAS had a lower MMSE score after twelve months (Silvestrini et al. 2011). CAS was also found to increase the risk of developing AD in the Baltimore Longitudinal Study of Aging after 14 years of follow-up (Wendell et al. 2012). In this study arterial stiffness correlated with degree of cognitive impairment on tests of memory and learning (Waldstein et al 2008), perhaps suggesting a particular vulnerability for certain cognitive domains related to AD.

A susceptibility to vascular-associated damage in the hippocampus has been suggested from work in animal models (Csiszar et al. 2013; Daniela Carnevale et al. 2012) however translation to humans is less clear. Ageing in humans has been associated with greater blood brain barrier (BBB) permeability in the hippocampus than cortical regions which may contribute to hippocampal dysfunction and atrophy in AD (Montagne et al. 2015). Increased hippocampal perivascular space is also associated with both increased age and hypertension, but this was not associated with cognitive performance in a longitudinal study of elderly community dwellers by Yao et al., (2014). Vascular risk factors and hippocampal shrinkage are also associated with other risk factors for AD such as inflammation (Raz et al. 2014) and depression (Sawyer et al.
CVD was associated with both Aβ plaques and NFTs in AD patients and controls in post-mortem examination (Yarchan et al. 2012). Although the brains of AD patients often have both AD and CVD pathology, it is unclear if the contribution of CVD and AD pathology to cognitive impairment and deterioration is synergistic or additive; does CVD increase Aβ, NFT and other pathologies related to AD, or do CVD and AD pathology independently contribute to clinical symptoms of cognitive impairment? Lo and Jagust (2012) longitudinally studied groups of non-demented, MCI and AD individuals, assessing AD pathology, vascular risk and burden, and cognitive decline. Their findings indicated that cerebrovascular burden and AD pathology are independent in their contribution to cognitive decline and dementia incidence, and that vascular risk does not lead to Aβ distribution or hippocampal atrophy. A significant association was found here between higher WMH and lower glucose uptake in AD patients, suggesting that neuronal vascular burden may lead to reduced synaptic activity. The results from this study suggest that CVD may contribute to dementia via impairing CBF and not directly lead to further AD pathology; CBF has been measured to be 20% lower in AD patients compared to control individuals (Roher et al. 2012).

In contrast, Pluta et al., (2013) argue that brain ischemia resultant from CVD leads to an increase of the cleavage of APP via β- and γ- secretases. Experimental brain injury in animal studies has provided ischemic conditions in the brain, which have been observed to increase expression and activity of β-secretase BACE1 (Chen et al. 2004; Blasko et al. 2004) and γ-secretases (Polavarapu et al. 2008) therefore potentially increasing Aβ peptide formation. Hypoxia-inducible factor-1 increases under acute hypoxia conditions, and is able to react with β- and γ- secretase increasing their expression in cell culture studies (Sun et al. 2006; Zhang et al. 2007). Sun et al., (2006) found that hypoxia in AD transgenic mice potentiated memory impairments compared to normoxic transgenic mice. A decrease in α-secretase activity, a non-amyloidogenic cleaver of APP has also been found after ischemic injury (Pluta et al. 2013; Nalivaevaa et al. 2004). These findings provide a mechanism by which AD pathologies could develop due to hypoperfusion, however it is yet unclear if this occurs in human brains.

Heart and cardiovascular diseases increase the risk of developing AD in later life (Rusanen et al 2014). A longitudinal cohort study by Newman et al., (2005) found that
those with cardiovascular disease, and particularly peripheral artery disease, had higher AD incidence. Accordingly, lifestyle factors which promote vascular health, and those which are detrimental to it, are associated with reduced (Barnard et al 2014) and increased (Nguyen et al 2014) risk of AD respectively.

1.7.2 Blood pressure and AD risk

Hypertension is a major risk factor for stroke, cognitive decline and dementia (Dahlöf 2007). Epidemiological studies have shown hypertension in mid-life to be a risk factor for the development of AD (Stewart et al. 2009; Kivipelto et al. 2001). Qiu et al., (2005) carried out an extensive review of the literature and found that although there was some heterogeneity in the results, there was a consensus suggesting that hypertension in mid-life led to a significant increase in the individuals’ risk for the development of AD later in life. The role of blood pressure measurement in old age was also discussed in this review, and it was found that low blood pressure may be a risk factor for cognitive decline or dementia after 65 years of age. Age-related deterioration of vessels resulting in loss of elasticity causes them to be less able to adapt to fluctuations in blood pressure. This will lead to a reduction in the blood flow to the brain, potentially leading to hypoxia and infarctions.

Further studies have also suggested an association between hypotension and increased risk of cognitive impairment or dementia in old age. For instance, in an elderly population (mean age 80) those who had lower blood pressures were more likely to develop AD (Verghese et al. 2003). Another study (Guo et al. 1997) found that those who were slightly hypertensive over the age of 75 had better cognitive performance, which is supported by the findings of Obisesan et al., (2008) who found that cognitive performance in those ≥80 years was best in those with moderate hypertension. These findings suggest that slight hypertension may be the optimal blood pressure in an older population to allow sufficient cerebral perfusion, however, further investigation is needed.

Orthostatic hypotension (OH) is defined as a drop in at least 20mmHg (SBP) or 10mmHg (DBP) upon going from a lying or sitting position to standing (Freeman et al 2011). It is significantly associated with age (Mehrabian et al. 2010), and is a risk factor for non-lacunar stroke (Yatsuya et al. 2011). OH is associated with markers of atherosclerosis, vessel stiffness and reduced baroreceptor sensitivity (Takahashi et al.
which may contribute to reduced CBF. The relationship between OH and cognitive impairment and dementia however is less clear. Although OH is found in more demented patients than healthy elderly (Sambati et al. 2014; Sonnesyn et al. 2009), in healthy elderly, OH is associated with impaired cognitive performance in some (Mehrabian et al. 2010), but not other studies (Yap et al 2008; Schoon et al 2013). Freidenberg et al., (2013) found that OH could be successfully treated in dementia patients, leading to a reduction in falls, fatigue, dizziness and mental fluctuations; symptoms which could contribute to clinical signs of dementia. The relevance of OH to individual cognitive symptoms within an AD population has not yet been determined.

1.8 Inflammation

Inflammation is a normal, advantageous response by the immune system to acute tissue damage or infection. When the inflammatory response is long-term or chronic, however, this damages healthy tissues and disrupts normal cellular functioning.

The immune response is divided into innate and adaptive immunity. Innate immune cells detect and respond both to externally derived infectious pathogens, and endogenous damaged and apoptotic cells. Adaptive immune cells are T- and B-cells which have adapted to detect and attack specific pathogens of disease. Therefore, the first line of defence is the cells of the innate immune system which detect non-self pathogens and chemical signals of self-cellular damage. Pathogen-associated molecular patterns (PAMPs) are used by pattern-recognition receptors (PRRs) such as Toll-like receptor cells to detect pathogens in the body. This begins a cascade of release and activation of pro-inflammatory molecules and cytokines such as tumour necrosis factor-α (TNF-α), nuclear-factor (NF)-kB, and members of the interleukin (IL) family (McCusker & Kelley 2013). Acting as chemical messengers between immune cells such as T-cells and T-helper cells, cytokines act within a complex cascade, and many are pleiotropic. Certain cytokines (e.g IL-1, IL-6) are generally considered as pro-inflammatory as they stimulate further cytokine release and immune cell activation. Others (e.g IL-4 and IL-10) are considered anti-inflammatory as they inhibit further cytokine production. However, many cytokines appear to have differing effects depending upon their immediate environment.

It was initially presumed that the peripheral immune system was separate from the functioning of the central nervous system (CNS), however, now it is appreciated that
Peripheral and neuroimmune activity are closely linked. An increased level of peripheral inflammation has now been associated with cognitive decline (Mooijaart et al. 2011), dementia (Engelhart et al. 2004; Tan et al. 2007), and depression (Raison et al. 2006). Infusion of lipopolysaccharide (LPS), the outer membrane of gram-bacteria, is often used in human and animal studies to investigate the effects of inflammation. LPS is recognised by PRRs and is able to induce a strong acute immune response.

Induced release of inflammatory cytokines using LPS in healthy human participants has been observed to cause transient low mood and cognitive impairment (Harrison et al. 2009; Reichenberg et al. 2001). In animal studies activation of the immune system can also cause symptoms of social withdrawal, loss of interest in the environment or novel stimuli, loss of appetite and libido, along with fever and excess sleepiness (Hart 1988; Dantzer & Kelley, 2007; Biesmans et al, 2013). This cytokine-induced reaction is known as ‘sickness behaviour’ (Maes et al. 2012), an evolutionally adapted change in motivational state designed to conserve energy during times of infection. Sickness behaviours such as reduction in food intake and sleepiness also help potentiate the fever response by conserving body heat. A raised temperature helps limit pathogen growth and has been observed to reduce mortality in animal studies (Hart 1988). Inappropriate functioning of the sickness behaviour response has been suggested as a cause for malaise depression (Charlton 2000) as an overlap in depressive symptoms and cytokine increase has been observed in humans. It is therefore clear that neuronal processes, and higher functions such as cognition, can be influenced by activation of the immune system, and are likely to involve immune cells and receptors within the brain.

1.8.1 Inflammation in the brain

For such behavioural responses as highlighted above to occur, there must be communication within the brain. Many inflammatory cytokines and their receptors have been measured in the CNS, and have been observed to increase after peripheral injection of pathogens (Szelényi 2001). Microglial cells are classed as the immune cells of the brain, as they are activated by immune proteins and localize with areas of tissue damage and stress. Microglial cells have been observed to survey their local environment in their ‘resting’ state, becoming ‘activated’ during stress or injury, and have been observed to move towards site of injury with the brain (Hanisch & Kettenmann 2007). Nimmerjahn et al., (2005) used a thinned-skull preparation in mice
which expressed enhanced green fluorescent protein in their microglia. Time-lapse recordings made by the authors show microglia migrating towards a lesion made in the cerebrovasculature. In this study, microglia which were at ‘rest,’ were actually shown to be in motion, inserting and withdrawing their arborisations. This mobility allows microglia to monitor the state of the environment of the brain, in order to respond to cell damage and injury. It has been argued more recently that microglia are never at ‘rest’ but shift to different activity states in response to receptor stimulation (Hanisch & Kettenmann 2007). Pro-inflammatory molecules can both activate (Butovsky et al 2006) and be released by microglia (Shieh et al 2014; Kaur et al 2014).

Peripheral induction of LPS is able to activate microglia and impair hippocampal neurogenesis in rats by supressing the survival of new neurons (Ekdahl et al. 2003). Neurotoxic cytokine release from activated microglia was thought to be the cause of impaired neurogenesis and indeed, when microglial activation was supressed, LPS-induced suppression of neurogenesis was attenuated. Wu et al., (2007) were able to attenuate the effect of LPS on neurogenesis in mice by a chronic running exercise condition. Although the exercise did not influence levels of inflammatory factors in the hippocampus, it rescued levels of brain derived neutrophic factor (BDNF) and its receptor, TrkB, which were decreased after LPS-treatment (Wu et al 2007), and are considered important for neuronal survival (Sairanen et al. 2005). This shows the interplay between environmental factors such as exercise, and mechanisms of neuronal health mediated by inflammation.

Experiments have shown that cytokines can also enter the brain by crossing ‘leaky’ areas of the BBB at the circumventricular organs. Cytokines are then able to attach to cytokine receptors on astrocytes (Watkins et al. 1995). This activity may be increased in older adults due to age-related degradation of the BBB, and in hypertensive patients.

1.8.2 Inflammation and cognition

Animal studies have shown that IL-1β is able to influence hippocampal-dependent memory. High density of IL-1 receptors has been detected in the hippocampus (Takao et al. 1990; Cunningham & De Souza 1993), and induction of IL-1 has been observed to inhibit fear conditioning in rats (Rachal Pugh et al. 2001). Similar findings have been observed when IL-1 levels in the hippocampus are raised by peripheral injection of LPS (Nguyen et al. 1998). As in these experiments, IL-1 was raised after the learning
experience, IL-1 is thought to interfere with memory consolidation (Rachal Pugh et al. 2001). The mechanism behind this may be IL-1’s role in LTP. Ross et al., (2003) have shown using hippocampal slice experiments, that under normal conditions, IL-1 is required for LTP, but at high pathological levels of IL-1, LTP is inhibited.

TNF-α may also play a role in modulating LTP, as pathological levels have been observed to inhibit early-LTP in hippocampal slices (Butler et al. 2004). Rats treated with 3,6’-dithiothalidomiade, an inhibitor of TNF-α synthesis, showed some rescue of LPS-induced memory impairment (Belarbi et al. 2012). In this study, 3,6’-dithiothalidomiade was observed to normalize levels of TNF-α and its receptors to non-inflammatory levels.

The potential role of IL-2 in cognition requires further investigation (McAfoose & Baune 2009). Deficits in working memory have been observed in patients receiving IL-2 treatment (Capuron et al 2001), and peripheral levels of IL-2 are associated with cognitive impairments in schizophrenia (Asevedo et al 2014). Mouse models are currently being used to attempt to elucidate the mechanisms behind IL-2 and cognitive performance (Petitto et al 2012).

The work summarized above suggests that high levels of pro-inflammatory cytokines in the hippocampus may lead to memory deficits, at least in animal models. It is known that hippocampal-dependent memory is particularly problematic for AD patients, showing signs of deficits in pre-clinical and early stages of the disease. Pro-inflammatory genotypes have been associated with smaller hippocampal volumes (Raz et al 2014), and animal models have suggested an association between increased neuronal inflammation, cognitive performance and age (Bardou et al. 2013; Chen et al. 2008). The role of inflammation in AD specifically is discussed below.

1.8.3 Inflammation in AD

*In vitro* work has shown microglia can be activated by Aβ to release pro-inflammatory cytokines such as TNF-α and IL-6, as well as pro-inflammatory complement proteins. Within an AD mouse model, Aβ load was reduced by induction of vasoactive intestinal peptide (VIP) which is known to have anti-inflammatory effects (Song et al 2012). VIP reduces the release of TNF-α, IL-1β and IL-6 from microglia by activating VIP receptors (Delgado et al 2002). Interestingly, VIP receptor activity is reduced in the cortex (Joo et al 2005), and VIP mRNA has been found to be reduced in the
hippocampus, of aged animals (Vela et al 2005) perhaps contributing to a pro-
inflammatory state in an aged brain. Mouse models of familial AD have also shown that
microglia physiology changes with age, becoming less efficient at clearing amyloid
deposits, but remaining able to produce pro-inflammatory molecules (Hickman et al.
2008). Dystrophic microglia cells have been observed in aged brains to co-localize with,
and precede, deposits of NFTs throughout the cortex. This may signify dystrophic
microglia as a causal factor of AD pathology, as microglia degeneration precedes the
onset of neurofibrillary pathology (Serrano-Pozo et al. 2012; Streit et al. 2009). Levels
of activated microglia have been observed to be increased in AD patients compared to
age-matched controls (Cagnin et al. 2001), suggesting an increase in neural immune
activity. Further, recent advances in imaging techniques have allowed the
characterization of microglial activation in vivo in AD patients (Cagnin et al. 2001;
Schuitemaker et al. 2013; Edison et al. 2008). This undoubtedly has provided further
evidence to the involvement of inflammatory mechanisms in the aetiology of AD.

Activation of the complement cascade is an important part of the immune response,
and stimulates recruitment of pro-inflammatory cytokines. Aβ has been observed to
bind to C1, the first protein complex of the complement cascade made up of enzymes
C1r, C1s and C1q (Webster et al. 1997). This triggers the complement pathway which
ends in cell lysis via the membrane attack complex (MAC). If the complement cascade
is unregulated, this will lead to over expression of pro-inflammatory cytokines and
inappropriate activation of MAC (Bonifati & Kishore 2006). The formation of MAC on
the membrane of a neuron causes an influx of Ca^{2+}, Na^+ and water into the cell, leading
to cell dysfunction and death. It is thought that immune activation early in amyloid
deposition may assist in the removal of inappropriate peptides and protein aggregates,
and therefore be beneficial, whereas chronic neuroinflammation may be toxic by
continuing the complement pathway (Prokop et al. 2013). Proteins along the stages of
the complement cascade (C1-C9) have also been observed in AD brains localized to Aβ
plaques and NFTs (Shen & Meri 2003). This may show the immune system reacting to
remove aggregate protein deposits in AD. Microglia activity is also regulated by
complement proteins (Bonifati et al, 2007). It has been suggested that aberrant
activation of microglia by an over-active complement system, could also lead to
synaptic destruction. Developmental synaptic pruning is performed by phagocytosis by
the microglia, and this has been found to be mediated by complement protein
complexes C1-C3 (Stevens et al 2007). Activation of these complexes by Aβ may cause them to signal synaptic elimination which would contribute to the synaptic loss seen in AD patients (Stephan et al 2012).

Levels of peripheral and central immune activity have been shown to be increased in AD patients. For instance, Maes et al. (1999) observed that serum levels of IL-6 were higher in AD patients than age-matched controls, and that TNF-α production in stimulated immune cells from these patients was also increased. Also, higher levels of TNF-α, and lower levels of complement regulatory protein and BDNF have been measured in AD patients compared to age-matched controls (Gezen-Ak et al 2013). Patients with acute events of systemic inflammation, associated with increased TNF-α, showed greater ADAS-COG decline after six months (Holmes et al. 2009), suggesting increased peripheral immune activity can have a negative effect upon disease progression. CRP is elevated in response to IL-6, and is associated with increased risk of coronary heart disease (Ridker 2003), diabetes (Pradhan 2001) and atherosclerosis (Bian et al. 2014), all conditions associated with AD. CRP has previously been measured to be lower in AD patients than in healthy controls (Yarchoan et al. 2013), but within an AD population, those with the highest CRP levels have poorer dementia rating scores (O’Bryant et al. 2010) and MMSE score (Nilsson et al. 2011). It will be interesting to see if CRP correlates with scores in other cognitive domains. Increased levels of IL-2 are also associated with greater cognitive decline in AD patients (Leung et al. 2013). Increased levels of IL-1β have been measured in AD populations, but this has not been found in all studies (Swardfager et al. 2010). Previous work has suggested that levels of IL-1β, IL-6 and TNF-α do not differ between AD patients taking ACEIs, and those who are drug-naïve (Richardson et al. 2013).

A meta-analysis concluded that there is no difference in IL-10 levels between AD and controls (Swardfager et al. 2010). However, within an AD population, higher IL-10 levels have been associated with greater decline on MMSE (Leung et al. 2013). IL-10 overexpression has been recently been associated with increased amyloid pathology and cognitive symptoms in a mouse model of AD (Chakrabarty et al. 2015).

Long term use of non-steroidal anti-inflammatory drugs (NSAIDs) due to autoimmune conditions such as arthritis results in a decrease in the risk of developing AD compared to the normal population (McGeer & McGeer 2013). However unfortunately, a Cochrane review concluded that use of NSAID treatment does not improve symptoms
of AD once it occurs (Jaturapatporn et al. 2012). A randomized control trial in MCI patients investigated the usefulness of anti-inflammatory COX-2 inhibitor rofecoxib for preventing dementia incidence after four years. However, in this study rates of AD diagnosis were greater in the treatment trial than in the placebo (Thal et al 2004). More recently, COX-2 inhibitors have been associated with cardiovascular problems due to their hypertensive effect (Grosser et al 2006) however this is controversial (Katz 2013). Also, studies are now suggesting a neuroprotective role for COX-2 expressed in vascular cells at the site of neuronal injury (An et al 2013). The complicated influence of COX-2 inhibitors and other anti-inflammatories upon the cerebrovascular system is far from understood.

1.9 Blood pressure and inflammation

Evidence suggests a close relationship between vascular and inflammatory systems. Increased levels of pro-inflammatory cytokines, such as IL-6, have been measured in humans with hypertension (Chae et al 2001; Stumpf et al. 2005). IL-6 stimulates release of intercellular adhesion molecule-1, which is known to be involved in the development of atherosclerosis and myocardial infarction (Ridker 2003). An atherosclerosis-associated inflammatory response involves increased permeability of the endothelial cell layer and accumulation of T-cells and macrophages. These cells in turn release pro-inflammatory molecules, sustaining the inflammatory response (Wierda et al. 2010).

Increases in inflammatory molecules due to hypertension have been observed in the cerebrovasculature in animal studies. The paraventricular nucleus (PVN) of the hypothalamus contains angiotensin type 1 (AT₁) receptors which are stimulated by angiotensin II (Ang-II), a potent vasoconstrictor. The PVN has an excitatory effect upon the renal sympathetic nervous system which induces renin release (Ferguson et al. 2008). Renin release is part of the renin-angiotensin system (RAS) pathway, which leads to a production of Ang-II, an increase in blood volume and blood pressure. Increases in blood volume and pressure stimulate vessel stretch receptors, which increases gene activation within the PVN, suggesting a positive feedback cycle connecting neural control of blood pressure and the RAS. Clinically, Ang-II is associated with essential hypertension as it is produced by the RAS as a vasoconstrictor to increase blood pressure when a drop in perfusion is detected by the kidneys. AT₁
receptor blockers are used to dampen this effect of Ang-II on the vasculature and the resultant increase in blood pressure.

Ang-II input to the PVN also triggers activation of microglia and pro-inflammatory cytokines. TNF-α and IL-6 mRNA levels in the PVN were increased in Ang-II treated mice (Ganta et al. 2005). Mice which were immune deficient due to T-cell deficiency, showed resistance to Ang-II induced hypertension (Guzik et al. 2007; De Ciuceis et al. 2005). In a study by Sriramula et al., (2008) Ang-II administration increased blood pressure and salt intake in wild-type mice, but had no effect in TNF-α⁻/⁻ mice. These effects were induced in the knock-out mice after treatment with recombinant TNF-α, suggesting that pro-inflammatory TNF-α is required for the hypertensive effects of Ang-II. These animal findings show that the inflammatory and vascular systems are strongly linked, and that manipulation of one will cause effects upon the other.

ROS activate microglia, which induces production of inflammatory cytokines (Choi et al. 2012). Therefore processes increasing ROS and oxidative stress can be thought of as potentially pro-inflammatory. Ang-II increases mRNA of NADPH oxidase (Gao et al. 2005) leading to increased ROS, and the increased blood pressure from Ang-II administration was found to be ameliorated by superoxide dimutase (SOD), a ROS scavenger (Zimmerman et al. 2002). Increased levels of ROS superoxide anion (O₂⁻) are present in hypertensive rats (Kishi et al. 2004) which were found to impair NO-induced vasorelaxation; this effect can be interrupted by an O₂⁻ scavenger (Xu et al. 2002). Pro-inflammatory TNF-α downregulates the synthesis of NO (Kim et al. 2001) and increases oxidative stress (Kuwano et al. 2008). Age-related pro-inflammatory increases in ROS can damage cerebral blood vessels, BBB and glial function, making the brain more vulnerable to AD-like phenotypes such as protein mis-folding and synaptic degradation.

1.9.1 Blood pressure and inflammation in ageing and AD

There is now increasing recognition of the vascular influences on AD development and progression. Epidemiological studies show that vascular risk factors such as diabetes, smoking and hypertension are also risk factors for AD. AD patients tend to have higher levels of arteriosclerosis and CAA than age-matched controls. There are also high levels of cardiovascular disease in AD populations, and worse cardiovascular disease has been associated with increased AD severity. For instance, in the Nun study, it was found that
in those with AD pathology, a greater amount of vascular lesions in the brain increased the likelihood of cognitive impairments (Snowdon et al. 1997).

Animal models have shown both an inflammatory and neurodegenerative response to models of hypertension. In a study by Carnevale et al., (2012a) hypertension in mice caused a phenotype typical of AD in the brain; cerebral hypoperfusion which was followed by an increase in activated microglia, which was then followed by an increase in Aβ. Further study by this group showed an increase in oxidative stress in the cerebrovasculature in a mouse model of hypertension, which led to an up regulation of receptor for advanced glycation end products (RAGE) mRNA and its ligands. RAGE controls the transport of Aβ across the BBB (Deane et al. 2003) and its up-regulation caused an influx of Aβ from the blood stream and into the parenchyma, where it could disrupt neuronal function. The hypertensive mice in this study were found to be impaired in object recognition and spatial memory when compared to sham-treated mice, which are cognitive domains affected in early stages of AD (Carnevale et al. 2012b). Also using a hypertensive mouse model, Gentile et al., (2009) found that hypertension caused BBB deterioration in the cortex and hippocampus, which was associated with increases in Aβ deposition. The cortex and hippocampus are particularly affected in AD both with pathology and associated cognitive impairments. Krstic et al., (2012) has shown immune challenge in AD transgenic mice was able to trigger and propagate the deposition of both Aβ and tau AD pathology.

HT: hypertension; CBF: cerebral blood flow; Aβ: amyloid-beta
1.10 Study Rationale

Previous studies have suggested that long-term hypertension may have a detrimental effect upon cognition in old age (Yaffe et al. 2014). It has also been established that mid-life hypertension is a risk factor for the development of AD (Qiu et al. 2005). Current hypertension in older adults however, may have beneficial effects upon performance on cognitive tasks (Liu et al. 2013), particularly those in the attentional domain (Gifford et al. 2013). The impact current blood pressure may have on cognition in AD patients however is not fully understood. Blood pressure variability has been associated with declines in global cognition (Lattanzi et al. 2014), but effects upon different cognitive domains are only beginning to be assessed. A detrimental effect of increased DBP has been found on tests of executive function in AD patients (Goldstein et al. 2005; Lamar et al. 2010), but other cognitive domains require further investigation.

Increased levels of inflammatory markers have been associated with poorer global cognitive performance in AD patients (Holmes et al. 2009). However, it is unknown whether inflammatory markers associate with non-global tests of cognition. A negative effect of inflammation upon mood has been established in healthy volunteers (Harrison et al. 2009) but its role in emotional appraisal has not been assessed. Essential hypertension commonly occurs in patients with alexithymia (Jula et al. 1999), and a reduction in emotional appraisal has been observed with carotid stimulation (Makovac et al. 2015). Therefore a synergistic effect of high blood pressure and high inflammatory status may lead to a reduction in emotional appraisal in AD patients. A deficit in emotional recognition has been observed in AD patients in some (Spoletini et al. 2008; Weiss et al. 2008) but not all studies (Burnham & Hogervorst 2004). Inflammatory status and blood pressure may be confounding variables which could explain discrepancies within the literature.

As well as emotional appraisal, inflammation and blood pressure may have interactive effects upon other cognitive domains within an AD population. Animal studies have suggested mechanisms by which hypertension and increased inflammation can lead to increased depositions of AD pathology and cognitive impairment (Carnevale et al. 2012; Krstic et al. 2012; Krstic & Knuesel 2013).
Current treatments for AD are at best symptomatic and help some of the patients for a limited period of time as medications often lose their effects once the disease has progressed beyond the moderate stages (Gillette-Guyonnet et al. 2011). Therefore further understanding of other factors which influence cognition in AD may lead to further treatment or preventative options. Hypertension and inflammation appear to be important risk factors and modulators of AD neuropathology. Hence this study will attempt to add to existing knowledge by studying hypertension and inflammation’s effect on cognition in AD separately and synergistically. The hypothesis section is presented below followed study methodology. The results and discussion of blood pressure on cognitive presentation is presented in section four, the role of inflammation is presented in section five, and the combined role of both is discussed in section six.
2. Hypothesis

Evidence increasingly shows a link between mid-life high blood pressure and AD. A link has also been established between inflammation, cognitive impairment and AD. Findings from animal studies suggest that a reciprocal relationship between inflammation and hypertension may be a causal factor in the development of the cognitive impairments observed in AD patients. Evidence from non-AD human studies however, suggests that in older people blood pressure positively associates with cognitive performance. Based on findings in the literature so far, it was hypothesised for this study that a history of hypertension would be associated with a poorer cognitive performance in AD patients, whereas paradoxically, current blood pressure as quantified by MAP would positively associate with cognitive performance. It was also hypothesised that there would be a negative association between inflammatory markers, ADMA and cognition, but a positive association between Fet-A and cognitive performance. It was further hypothesised that there would be a synergistic relationship between inflammatory and vascular disease markers and cognitive score in mild AD patients.
3. Methods

3.1 Patient sample and recruitment

All participants had been diagnosed with mild/mild-moderate AD according to NINCDS-ADRDA criteria at time of testing. Participants were recruited from Sussex Partnership NHS Foundation Trust Memory clinics. To ensure patient protection and confidentiality, patients were first asked if they would be happy to speak to researcher (LN) about a research project into blood pressure and AD by their clinician. If they agreed, they were briefed about the study by the researcher and given an Information Sheet (see Appendix 1). The patient and caregiver were then given at least 48 hours to read the Information Sheet, before the caregiver was contacted by the researcher by telephone. This telephone conversation gave caregivers an opportunity to ask questions and then the decision was made whether or not to participate in the study. If patient and caregiver agreed to take part, a date was then set for the researcher to visit for data collection.

3.1.2 Ethics

Governance for this study was approved by Sussex Partnership NHS Foundation Trust (Ref: 5025-2013). Ethical approval was obtained from National Research Ethics Service (NRES) Fulham, London (Ref: 13/LO/0478).

3.1.3 Data Collection Procedure

Participants could choose to be seen for data collection either at the memory clinic or at their home. Data was collected by the researcher LN for each participant at one time point. All data collection sessions lasted 60-90 minutes and followed the same procedure.

Participant and caregiver were briefed at the start of the session to remind them of the purpose and procedure of the study. Then the Consent Form (see Appendix 2) was filled out and signed by all parties.
3.2 Blood pressure measurements

After the consent process, blood pressure was taken sitting after resting for at least ten minutes, and then taken once after standing up for one minute. An automatic sphygmomanometer was used. Two further blood pressure measurements were taken following the same procedure, one during and one after the cognitive testing session.

3.3 Cognitive testing

Prior to design of the study, the researcher (LN) liaised with a patient and public involvement (PPI) group at Sussex Partnership NHS trust. The Lived Experience Advisory Forum (LEAF) is a group of service users, patients and family members who work with clinicians and academic partners to develop patient involvement in research. Patients with a diagnosis of AD and their caregivers advised that a range of cognitive domains not usually studied in AD patients may be an important part of this research study. Deficits in emotional control and communication, prospective memory and concentration were reported to have a particular impact upon patients and caregivers. This meeting had an influence on the choice of cognitive tests used in this study. LEAF were also consulted throughout this study on aspects of recruitment and dissemination.

3.3.1 National Adult Reading Test (NART)

The NART (Nelson, 1982) requires the participant to read aloud a set of 45 irregular words, which are scored as correct or incorrect. It is used as a measure of pre-morbid intelligence (Bright et al. 2002) as reading of irregular words is thought to be preserved in early dementia. McGurn et al., (2004) found significant correlations between NART score at age 80 and IQ score from age 11 in both demented and non-demented individuals, confirming the NARTs applicability in dementia populations. The NART is used in this study to exclude participants which show atypical cognition prior to their diagnosis. The NART word list is in Appendix 3.
3.3.2 Prospective memory task

To assess PM a computerised card-sort task was used (Rusted et al. 2009). This task assesses event-based non-focal prospective memory, giving a measure of both reaction time and accuracy. This task is divided into a baseline condition and then a prospective memory (P) condition which has an additional PM demand. For the baseline condition, the participant is presented with a standard deck of 52 playing cards one at a time on the computer screen. The participant is required to sort the cards into their suits by pressing the corresponding key on the keyboard. Only Hearts and Spades are represented on the keyboard and participants were asked to ignore the Clubs and Diamonds. For each trial, the reverse of the playing card is seen first for 1000ms and then the face of the card is presented for 750ms. Once the full deck has been shown, this is the end of the baseline condition. For the P condition, two 52 decks of cards are shown one at a time as described above. As well as sorting by suit, participants are now required to perform an additional action; for every number ‘7’ card they see they must press the space bar.

Before beginning the baseline condition participants completed a short practice trial which was not analysed to ensure there were no problems with visual acuity or task comprehension. Once the baseline condition was completed, participants were given instructions for the P condition. They repeated the instructions back to the researcher before commencing. Accuracy and reaction time of responses were recorded by Eprime1 software.

This test of PM has previously been used in both healthy elderly and AD populations (Farina et al. 2013). It is therefore suitable to assess differences within an AD population, which may be attributable to variations in blood pressure and inflammation.

3.3.3 Emotional Recognition

A computerised task was used to assess accuracy and reaction time when identifying facial emotions. Patients were first presented with a face with a neutral expression which then animated to form an emotion. Participants were required to identify which emotion out of Ekman’s six universal emotions: Happy, Sad, Angry, Surprise, Disgust, or Fear was being represented. The list of emotions was present on the screen throughout the experiment. To ensure no confliction from memory or attention
impairment, participants were asked by the researcher ‘Which emotion is this?/Which emotion do you think this is?’ after every expression presentation. The participant was able to replay the animation if they wished. Once the participant had responded by clicking on the emotion label with the mouse, the task moved on to the next face.

Emotions used were the six universal emotions identified by Ekman et al., (1969). These emotions and their corresponding facial expressions are thought to be cross-culturally expressed and identifiable.

Visual emotional appraisal requires further investigation in AD patients as there are inconsistencies within the literature (Spoletini et al. 2008; Weiss et al. 2008; Burnham & Hogervorst 2004). This study will investigate if blood pressure and inflammatory variations may explain these inconsistencies. This test is suitable for an AD population as it is not dependent upon attention or memory ability.

3.3.4 Selective attention

The Map Search task from Tests of Everyday Attention (TEA) (Robertson et al. 1996) was used here. The participant was required to search for symbols (petrol stations) on a printed coloured map. The maximum score was 80 and they had two minutes to find as many as they could amongst other visual distractors. Visual ability to see the symbols was checked before beginning the task, and participants had access to a magnifying glass. The number of symbols found in minute one and minute two were recorded by the researcher. This test has been validated for use in AD populations (Robertson et al. 1996).

3.3.5 Subjective Prospective and Retrospective Memory Questionnaire (SPRMQ)

This is a set of sixteen questions on the participants’ own retrospective and prospective memory ability (Smith et al. 2000). For example, participants were asked, ‘Do you repeat the same story to the same person on different occasions?’ Participants were asked to answer questions on a Likert-Scale from ‘1’ (never) to ‘5’ (all the time). The original study by Smith et al (2000) reported an increased number of memory failures in a group of patients suffering from AD compared to young and elderly participants without a dementia diagnosis. This questionnaire has been recently used in other AD
populations (Farina et al. 2013). Total response on prospective memory questions was correlated with performance on PM task. SPRMQ is listed in Appendix 4.

3.4 Blood Sample Collection

5mls of blood was collected from each participant at the end of the cognitive testing session. Venepuncture performed by researcher (LN) from the medial cubital vein. The tourniquet round the upper arm was removed once the needle was inserted to ensure minimal stasis. All samples were transported to the laboratory at room temperature in HTA approved packaging.

Blood samples were collected in vacutainers and allowed to clot for three hours. The sample was then centrifuged at 2000g for 10mins. Then the serum was extracted and the spin was repeated. Serum was then extracted and stored in cryovials, labelled, and stored at -80°C until analysis.

The remaining pellet was destroyed in chlorine solution and disposed of according to HTA guidelines.

3.5 Clinical data

Once written consent was obtained from participant, clinical notes from memory assessment services were used to gather MMSE scores (Appendix 5) and a list of current medications. Whether or not the participant had a history of hypertension was also determined here.

3.6 Blood sample analysis

Pro-inflammatory cytokines (CRP, IL-1β, IL-2, IL-6, and TNF-α), anti-inflammatory cytokines (IL-10), and markers of vascular damage (Fetuin-A and ADMA) were measured in serum samples.

Concentrations of the target proteins and dimethylarginine were quantified by specific enzyme-linked immunosorbent assay (ELISA) kits carried out according to the manufacturer’s instructions. Standards and samples were run in duplicate, and an
average optical density was calculated for each sample. Concentration of the target proteins could then be calculated from the standard curve created from the kit standards. Standard curves are shown in Figure 1, Figure 2, Figure 3 and Figure 4. ELISA plates with standards and samples were read at an absorbance of 450nm.
Figure 1. Standard curves for pro-inflammatory markers IL1β, IL-2 and IL-6 (pg/ml). $R^2$ for relationship between concentration and absorbance is shown.
Figure 2. Standard curves for pro-inflammatory markers CRP and TNF-α (pg/ml). $R^2$ for relationship between concentration and absorbance is shown.
Figure 3. Standard curve of anti-inflammatory marker IL-10 (pg/ml). $R^2$ for relationship between concentration and absorbance is shown.
Figure 4. Standard curve of ADMA and Fet-A concentration (ng/ml). $R^2$ for relationship between concentration and absorbance is shown.
3.7 Statistical analysis

The Shapiro-Wilks test of normality was carried out on all data produced in this study (see sections 4-6). Subsequent statistical analysis tests performed were determined by results of this test. Pearson’s R was used to determine strength of association between current blood pressure and cognitive test performance. Spearman’s Rho was used on data that failed Shapiro-Wilks test of normality (see sections 4-6). 95% confidence intervals were calculated using Fisher’s z transformation for correlation coefficients to provide an estimate of the range of certainty for correlation coefficients. ANOVAs were used to compare means, and multivariate analysis was used to investigate possible interaction effects of predictor variables. Outliers and non-responders on cognitive tests and ELISA measurements were removed from analysis. All statistical analysis performed in IBM SPSS Statistics Version 22.

3.7.1 Sample size calculation

Sample size calculations were performed to ensure clinically significant differences in cognitive performance and blood pressure could be detected (Rosner 2010). Means (Mu1 and Mu2) and standard deviations (S.D) available from previous literature were used to calculate the necessary N for a power of 0.8. These are shown in Table 1. Statistical calculations were approved by BSMS statistician Dr S. Bremner.
<table>
<thead>
<tr>
<th>Study</th>
<th>Measurement</th>
<th>Mu1</th>
<th>Mu2</th>
<th>S.D</th>
<th>Power</th>
<th>Required N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glodzik et al (2014)</td>
<td>MAP</td>
<td>97.0</td>
<td>87.3</td>
<td>9.8</td>
<td>0.8</td>
<td>32</td>
</tr>
<tr>
<td>Lattanzi et al</td>
<td>SBP variability</td>
<td>5.8</td>
<td>13.9</td>
<td>5.9</td>
<td>0.8</td>
<td>20</td>
</tr>
<tr>
<td>Kuo et al (2004)</td>
<td>SBP</td>
<td>123</td>
<td>146</td>
<td>16.3</td>
<td>0.8</td>
<td>24</td>
</tr>
<tr>
<td>Farina et al (2013)</td>
<td>Reaction time for PM task</td>
<td>666</td>
<td>834</td>
<td>220</td>
<td>0.8</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Accuracy for PM task</td>
<td>0.97</td>
<td>0.62</td>
<td>0.33</td>
<td>0.8</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>SPRMQ</td>
<td>37.32</td>
<td>59.16</td>
<td>13.66</td>
<td>0.8</td>
<td>14</td>
</tr>
</tbody>
</table>

Table 1. Sample size calculations for blood pressure and cognition using data from previous literature in AD patients.

As can be seen in Table 1, an N of 54 is required to detect significant differences in cognitive tasks used in this study. To allow for potential drop-out of participants, an additional fifteen percent were recruited. There no withdrawals from the study following consent, and a final sample size of 61 was achieved which satisfied statistical requirement.

### 3.8 Demographic data

<table>
<thead>
<tr>
<th>N</th>
<th>Average age at testing</th>
<th>Gender ratio (M:F)</th>
<th>Average years of education</th>
</tr>
</thead>
<tbody>
<tr>
<td>61</td>
<td>80.1 (6.7)</td>
<td>31:30</td>
<td>10.4 (1.5)</td>
</tr>
</tbody>
</table>

Table 2. Demographic data of participants. Standard deviations are given in parentheses.
4. Blood pressure and cognitive performance

4.1 Foreword

A history of hypertension may contribute to differences seen in cognitive performance in the elderly. Hypertension in mid-life has been found to be a risk factor for later-life cognitive decline and AD (Qiu et al. 2005). Mechanisms by which a long-term hypertensive state can causally lead to an increased risk for AD have been uncovered by animal studies. CVD, as measured by WMH in brain imaging, has been associated with hypertension (Gąsecki et al. 2013), as has reduced CBF (Jennings et al. 2005) and greater BBB permeability (Vasilevko et al. 2010). Animal models have suggested hypertension, whether induced mechanically by aortic constriction, (Gentile et al. 2009), via infusion of angiotensin-II (Csiszar et al. 2013), or by high salt intake (Díaz-Ruiz et al. 2009) increases deposition of AD pathology, Aβ plaques and NFTs. Further work has shown hypertension is able to lead to both protein deposition and cognitive impairment in mice (Carnevale et al. 2012b).

The evidence linking hypertension in older adults and cognition however, is mixed. In older age groups, a lower blood pressure may be worse for cognitive performance (Pandav et al. 2003; Kahonen-Vare et al. 2013). Obsisesan et al., (2008) found an inverse correlation between cognition and blood pressure in participants 60-80 years old, however, this relationship was reversed in participants over 80. It may be that a history of hypertension is detrimental for cognition, but slight hypertension in old age may be necessary for sufficient cerebral perfusion, and therefore show a benefit (de la Torre 2002).

A recent meta-analysis by Gifford et al., (2013) suggested that certain types of cognition may be differentially affected by blood pressure. Here, blood pressure inversely correlated with performance on tests of memory and language, but a positive correlation was found for attention tasks. The participants in these studies were middle-aged to young-old (50-70 years of age), and without a dementia diagnosis. Attentional problems within the visual domain are particularly associated with AD and may contribute to deficits in encoding memory as discussed in Section 1.3.1 Memory and attention, and this is likely to be related to the cholinergic system (Lawrence & Sahakian 1995). Any further effect of blood pressure within an AD population requires
investigation. Work is only beginning to investigate the role of high or low blood pressure in those who already have a diagnosis of AD, and researchers have identified the need for further research in AD patients (Yuan et al. 2015). One study so far in Poland showed AD patients had lower blood pressure than the general population, and that blood pressure did not associate with changes in global cognitive performance after four year follow-up. This contradicts an earlier study however, which found high SBP predicted greater decline in global cognition in AD patients (Mielke et al. 2007), and a study in African-American AD patients which associated hypertension with worse executive function score (Goldstein et al. 2005). The role blood pressure may play in emotional appraisal has not yet been investigated in AD patients. Alexithymia, a neurological condition characterised with emotional dampening is associated with essential hypertension (Jula et al. 1999). Therefore, hypertension in AD patients may be associated with improved performance on attention tasks, but poorer emotional appraisal compared to those without hypertension.

4.2.1 Methods

Blood pressure measurements and cognitive tests were performed as detailed in Methods Section 3. Methods To summarise, blood pressure was taken sitting and standing using an automatic sphygmomanometer around the upper arm. This measurement was repeated three times throughout the testing session so that an average could be calculated. Cognitive tests were performed as detailed in 3.3 Cognitive testing.
4.4 Results

4.4.1 Current blood pressure

61 participants had their blood pressure and cognitive performance measured. The average age was 80.1, with a range of 65 to 96 years. Average blood pressure readings from participants are shown in Table 3.

<table>
<thead>
<tr>
<th>SBP sitting (1&lt;sup&gt;st&lt;/sup&gt;)</th>
<th>SBP sitting (2&lt;sup&gt;nd&lt;/sup&gt;)</th>
<th>SBP sitting (3&lt;sup&gt;rd&lt;/sup&gt;)</th>
<th>SBP sitting mean</th>
<th>SBP standing (1&lt;sup&gt;st&lt;/sup&gt;)</th>
<th>SBP standing (2&lt;sup&gt;nd&lt;/sup&gt;)</th>
<th>SBP standing (3&lt;sup&gt;rd&lt;/sup&gt;)</th>
<th>SBP standing mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>144</td>
<td>144</td>
<td>144</td>
<td>144</td>
<td>140</td>
<td>138</td>
<td>138</td>
<td>139</td>
</tr>
<tr>
<td>DBP sitting (1&lt;sup&gt;st&lt;/sup&gt;)</td>
<td>DBP sitting (2&lt;sup&gt;nd&lt;/sup&gt;)</td>
<td>DBP sitting (3&lt;sup&gt;rd&lt;/sup&gt;)</td>
<td>DBP sitting mean</td>
<td>DBP standing (1&lt;sup&gt;st&lt;/sup&gt;)</td>
<td>DBP standing (2&lt;sup&gt;nd&lt;/sup&gt;)</td>
<td>DBP standing (3&lt;sup&gt;rd&lt;/sup&gt;)</td>
<td>DBP standing mean</td>
</tr>
<tr>
<td>84</td>
<td>83</td>
<td>83</td>
<td>83</td>
<td>84</td>
<td>84</td>
<td>84</td>
<td>84</td>
</tr>
</tbody>
</table>

Table 3. Blood pressure readings standing and sitting/mmHg in mild AD patients.

It can be seen in Table 3 that blood pressure was consistent across readings, leading to an average blood pressure of 144/83mmHg when sitting, and 139/84mmHg when standing. Mean arterial pressure (MAP) can be calculated from the SBP and DBP using the formulae:

\[
\text{MAP} = (\text{SBP-DBP}) \times \frac{1}{3} \text{DBP}
\]

A paired t-test showed that there was a significant difference between SBP when sitting and SBP standing \(t(60) = -2.510, \ p = 0.015\). There was no significant difference between DBP sitting and standing, or MAP when sitting and standing \((p > 0.05)\).
Therefore the effect upon cognition of MAP sitting, and SBP standing decline was assessed.

Tests of normality were performed on MAP sitting, SBP decline and age. SBP decline and age were found to be normally distributed by Shapiro-Wilk test ($p = 0.426$ and $p = 0.881$, respectively) with no outliers. One outlier was removed from MAP giving an N of 60, to achieve normality on Shapiro-Wilk test ($p = 0.470$).

In this population there was no significant correlation as determined by Pearson’s R test, between SBP decline and MAP with age shown in Figure 5, $p > 0.05$.

Figure 5 scatterplot showing relationship between age and blood pressure variables SBP decline and MAP.
4.4.2 Blood pressure history

Clinical notes were reviewed to assess if participants had a history of hypertension, defined here as a diagnosis of hypertension recorded in patients’ GP notes. Long-term prescriptions of anti-hypertensive medication for blood pressure control were also recorded. Forty-four percent of participants had a history of hypertension. There was no significant difference in current blood pressure between those with and without a history of hypertension as determined by one-way ANOVAs (Table 4) suggesting blood pressure was successfully controlled in patients with a history of hypertension.

<table>
<thead>
<tr>
<th>Current pressure</th>
<th>Average (S.D)</th>
<th>F</th>
<th>Sig. (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP decline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of hypertension</td>
<td>7.62 (17.70)</td>
<td>1.209</td>
<td>0.279</td>
</tr>
<tr>
<td>No history</td>
<td>1.95 (13.28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of hypertension</td>
<td>103.53</td>
<td>0.926</td>
<td>0.343</td>
</tr>
<tr>
<td>No history</td>
<td>99.95 (9.84)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. No significant differences in current blood pressure between those with and without a history of hypertension.

4.4.3 Cognitive score data

The average responses for the cognitive tests are shown below, and their association with age and gender explored.

4.4.3.1 NART
Average NART score was 38.90, out of a maximum of 45, with a standard deviation (S.D) of 7.33. NART score did not correlate with age \((r = 0.27, \text{N} = 61, p = 0.836)\), and there was no significant effect of gender as determined by ANOVA: for males \((M = 38.58, \text{S.D} 7.81)\), and females \((M = 39.25, \text{S.D} = 6.89); F(1,57) = 0.121, p = 0.730\).

### 4.4.3.2 Prospective memory

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Prospective Memory</th>
<th>Baseline</th>
<th>Prospective Memory</th>
<th>Cost of PM in Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy/Proportion Correct</td>
<td>Accuracy/Proportion Correct</td>
<td>Reaction Time/ ms</td>
<td>Reaction Time/ ms</td>
<td>Reaction Time/ ms</td>
</tr>
<tr>
<td>0.65 (0.23)</td>
<td>0.52 (0.27)</td>
<td>832.78</td>
<td>884.07</td>
<td>147.22</td>
</tr>
<tr>
<td>(200.22)</td>
<td>(205.97)</td>
<td>(130.28)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Average response by AD patients on prospective memory task; reaction time in milliseconds and accuracy. S.D in parentheses.

The average responses for the PM task are shown in Table 5. The proportion of correct responses on this task showed no significant relationship with age in the baseline \((r = -0.182, \text{N} = 46, p = 0.226)\) or P condition \((r = 0.068, \text{N} = 46, p = 0.724)\). At baseline, reaction time did not correlate with age \((r = 0.220, \text{N} = 46, p = 0.142)\), nor did it during the P trials \((r = 0.136, \text{N} = 46, p = 0.366)\). As cost of PM trial was not normally distributed, Spearman’s Rho was used to determine that there was no significant correlation with age \((\text{Rho} = 0.317, \text{N} = 28, p = 0.101)\).

A significant effect of gender was found on this task. Males were faster than females in the P trials. For the P condition males had a mean reaction time of 820.09 seconds \((\text{S.D} = 193.61)\), and females had a mean reaction time of 960.23 seconds \((\text{S.D} = 198.15); F(1,44) = 5.853, p = 0.020\). The male participants were more accurate in the baseline condition. The mean score for males was 0.72 \((\text{S.D} = 0.22)\), and females had a mean score of 0.58 \((\text{S.D} = 0.23); F(1,44) = 4.448, p = 0.041\). A non-parametric test was carried out to check if there was a significant difference in cost to reaction time between males and females. A Mann-Whitney U test showed males had significantly less cost
than females ($U = 49.00$, $p = 0.025$). Therefore males performed better on this task of PM.

### 4.4.3.3 SPRMQ

<table>
<thead>
<tr>
<th>Response Total</th>
<th>Response Prospective</th>
<th>Response Retrospective</th>
</tr>
</thead>
<tbody>
<tr>
<td>35.6 (9.9)</td>
<td>16.9 (5.4)</td>
<td>18.7 (5.9)</td>
</tr>
</tbody>
</table>

Table 6 showing total average responses on subjective memory questionnaire Likert-Scale.

The average response for the subjective memory questionnaire is shown in Table 6. Age significantly associated with total response score ($r = -0.440$, $N = 55$, $p = 0.001$) (Figure 6), prospective response ($r = -0.400$, $N = 55$, $p = 0.002$) (Figure 7), and retrospective response ($r = -0.381$, $N = 55$, $p = 0.004$) (Figure 8), suggesting less report of memory failure with increasing age.

![Figure 6. Scatterplot showing relationship between age and subjective memory failure rating in AD patients.](image-url)
Figure 7. Scatterplot showing relationship between age and prospective memory rating in AD patients.

Figure 8. Scatterplot showing relationship between age and retrospective memory rating in AD patients.
There was no effect of gender on this task. ANOVA showed no difference between males (M = 34.8, S.D = 9.5), and females (M = 36.4, S.D = 10.6), $F(1,53) = 0.343, p = 0.560$ in total response score.

The possibility of a correlation between scores on the P trials of the PM task and prospective memory failures questionnaire was assessed, but not found ($r = -0.065$, $N = 55$, $p = 0.757$) as can be seen in Figure 9.

Figure 9. Scatterplot showing no relationship between prospective memory score and subjective prospective memory rating in AD patients.
4.4.3.4 Emotional recognition

<table>
<thead>
<tr>
<th>Emotional domain</th>
<th>Reaction time/ s</th>
<th>Accuracy / proportion correct</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fear</td>
<td>26.97 (20.08)</td>
<td>0.40</td>
</tr>
<tr>
<td>Surprise</td>
<td>19.01 (6.51)</td>
<td>0.78</td>
</tr>
<tr>
<td>Happy</td>
<td>17.50 (5.67)</td>
<td>0.78</td>
</tr>
<tr>
<td>Sad</td>
<td>28.10 (19.71)</td>
<td>0.37</td>
</tr>
<tr>
<td>Anger</td>
<td>22.41 (11.11)</td>
<td>0.68</td>
</tr>
<tr>
<td>Disgust</td>
<td>21.84 (8.42)</td>
<td>0.68</td>
</tr>
<tr>
<td>Total</td>
<td>21.66 (6.52)</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Table 7. Average response times and accuracy of AD patients for the different Ekman emotions in an emotional recognition task.

The average responses for the emotional recognition task are shown in Table 7. There was no gender difference on reaction time for this task (all \( p > 0.05 \)). However in terms of proportion correct, in the happy trials males were more accurate (M = 0.87, S.D = 0.31) than females (M = 0.67, S.D = 0.41), as determined by ANOVA \( F(1,59) = 4.399, p = 0.040 \). This was also true for the surprise domain where males (M = 0.91, S.D = 0.49), and females (M = 0.65, S.D = 0.45), \( F(1,59) = 4.923, p = 0.030 \). Age did not correlate with response on this task (\( p > 0.05 \)).

4.4.3.5 Selective attention

The total possible score on this test is 80. The average score achieved by the participants was 42.98, with a S.D of 21.5. There was no significant effect of gender as determined by ANOVA. Males scored M = 43.6, S.D = 20.3, and females scored M = 42.1, S.D = 23.4, \( F(1,53) = 0.066, p = 0.798 \). There was also no association with age (\( r = -0.156, N = 55, p = 0.256 \)).
4.4.4 Cognitive score and blood pressure history

ANOVA's were performed to assess whether there were significant differences on cognitive test results between those with and without a history of hypertension (Table 8).

<table>
<thead>
<tr>
<th>Task</th>
<th>Average (S.D)</th>
<th>F</th>
<th>Sig. (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>History of hypertension/ No history</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NART</td>
<td>36.53 (7.56)</td>
<td>0.448</td>
<td>0.509</td>
</tr>
<tr>
<td>PM Baseline accuracy</td>
<td>0.68 (0.26)</td>
<td>0.808</td>
<td>0.377</td>
</tr>
<tr>
<td>Baseline accuracy</td>
<td>873.15 (200.44)</td>
<td>0.378</td>
<td>0.544</td>
</tr>
<tr>
<td>RT</td>
<td>820.41 (225.83)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P Baseline accuracy</td>
<td>0.32 (0.13)</td>
<td>0.400</td>
<td>0.533</td>
</tr>
<tr>
<td>P RT</td>
<td>803.38 (159.62)</td>
<td>0.834</td>
<td>0.370</td>
</tr>
<tr>
<td>SPRMQ</td>
<td>37.87 (8.58)</td>
<td>0.015</td>
<td>0.905</td>
</tr>
<tr>
<td>Emotion recognition task/ accuracy</td>
<td>0.72 (0.16)</td>
<td>0.172</td>
<td>0.681</td>
</tr>
<tr>
<td>Emotion recognition task/ RT</td>
<td>22.02 (7.31)</td>
<td>0.033</td>
<td>0.857</td>
</tr>
<tr>
<td>Attention task</td>
<td>35.57 (19.27)</td>
<td>2.238</td>
<td>0.147</td>
</tr>
</tbody>
</table>

Table 8. No significant differences on cognitive test performance of AD patients with and without a history of hypertension.
4.4.5 Cognitive score and current blood pressure

4.4.5.1 NART

There was no correlation between the NART score and blood pressure measures at time of testing. For SBP decline, $r = 0.068$, $N = 61$, $p = 0.607$ and for MAP, $r = -0.157$, $N = 61$, $p = 0.240$.

4.4.5.2 Prospective memory

A significant correlation was found between reaction time at baseline and SBP decline as shown in Figure 10 ($r = 0.357$, $N = 46$, $p = 0.015$; 95% CI 0.11 to 0.56) suggesting a slower reaction time with greater decline in SBP when standing. For MAP there was no relationship ($r = 0.216$, $N = 46$, $p = 0.150$).

Figure 10. Scatterplot showing relationship between SBP standing decline and reaction time in AD patients.
In the PM trials, reaction time on correct responses did not correlate with SBP decline ($r = 0.012, N = 42, p = 0.938$) or MAP ($r = -0.156, N = 42, p = 0.324$).

A cost of PM intention can be calculated by subtracting the average reaction time during baseline from the average reaction time during the PM trial. 42 participants had data available for this analysis. Cost of reaction time was not correlated with SBP decline ($r = 0.098, N = 42, p = 0.539$) or MAP ($r = 0.178, N = 42, p = 0.261$).

This task also gives a measure of accuracy for baseline sort card trials and PM trials. In the baseline, accuracy was not correlated with SBP decline ($r = 0.109, N = 42, p = 0.491$) or MAP ($r = 0.087, N = 42, p = 0.584$). For the PM trials, there was no correlation between accuracy and SBP decline ($r = -0.183, N = 24, p = 0.391$) or MAP ($r = 0.047, N = 24, p = 0.828$).

4.4.5.3 SPRMQ

For this task, a total response score of memory failures was checked for an association with blood pressure. This was also done separately for the retrospective and prospective memory questions. For the total questionnaire Pearson’s R found no significant correlation between memory score and SBP decline ($r = 0.160, N = 61, p = 0.242$) and MAP ($r = -0.138, N = 61, p = 0.315$).

The retrospective memory score showed no correlation with SBP decline ($r = 0.123, N = 61, p = 0.371$) and MAP ($r = -0.138, N = 61, p = 0.316$).

Looking at just the prospective memory questions, SBP decline did not correlate with memory score ($r = 0.163, N = 61, p = 0.235$). There was also no correlation with MAP ($r = -0.105, N = 61, p = 0.445$).

As there is a significant association of age on this task, a hierarchical multiple regression was used to determine if there is a relationship with blood pressure when age is also entered into the model. Model 1 looked at the effect of age on total questionnaire score and found that age explained 44% of the variance, which was significant at the 0.05 level ($p = 0.001$). The addition of the blood pressure variables to Model 2 added only 5.5% of variance to the questionnaire total score, which was not significant ($p = 0.163$). The variance for Model 2 was 49.9%, $p = 0.002$. The standardised β values for the hierarchical regression are shown in Table 9.
<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Standard error B</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>87.827</td>
<td>14.684</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.652</td>
<td>0.183</td>
<td>-0.440</td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>101.077</td>
<td>17.620</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.628</td>
<td>0.181</td>
<td>-0.424</td>
</tr>
<tr>
<td>SBP decline</td>
<td>0.151</td>
<td>0.083</td>
<td>0.240</td>
</tr>
<tr>
<td>MAP</td>
<td>-0.156</td>
<td>0.119</td>
<td>-0.174</td>
</tr>
</tbody>
</table>

Table 9. Multiple hierarchical regression analysis of variance on the effect of blood pressure on subject memory rating when age is controlled for.

### 4.4.5.4 Emotional recognition

This task gives a measure of accuracy and reaction time for correct emotional recognition. SBP decline did not correlate with accuracy on any of the trials. Relationship with total accuracy is shown in Figure 11.

![Figure 11](image.png)

Figure 11. Scatterplot showing no relationship between accuracy on emotional recognition task and SBP standing decline in AD patients.
There was also no association found between SBP decline and reaction time as seen in Figure 12.

![Figure 12. Scatterplot showing lack of relationship between SBP standing decline and reaction time on emotional recognition task in AD patients.](image)

No relationship was found between MAP and accuracy or reaction time as shown in Figure 13 and Figure 14.

![Figure 13. Scatterplot showing lack of relationship between MAP and accuracy on emotional recognition task in AD patients.](image)
There was an effect of gender on the results of two trials in this task; accuracy of surprise and happy faces. Therefore a hierarchical regression model was created to see if there was a significant interaction of blood pressure and gender on these results.

For the happy trial, only 9.7% of the variance in accuracy was given by a model of blood pressure variable when gender was controlled for and this was not significant ($p = 0.543$). Standardised $\beta$ values for the hierarchical regression are shown in Table 10. For the surprise trial, a model of blood pressure when controlling for gender explained 14.3% of the variance in accuracy which was not significant ($p = 0.152$). Standardised $\beta$ values for the hierarchical regression are shown in Table 11.

Figure 14. Scatterplot showing lack of relationship between MAP and reaction time on emotional recognition task in AD patients.
<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Standard error B</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>1.075</td>
<td>0.145</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>-0.204</td>
<td>0.093</td>
<td>-0.277</td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>0.892</td>
<td>0.474</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>-0.237</td>
<td>0.098</td>
<td>-0.322</td>
</tr>
<tr>
<td>SBP decline</td>
<td>0.003</td>
<td>0.003</td>
<td>0.111</td>
</tr>
<tr>
<td>MAP</td>
<td>0.002</td>
<td>0.005</td>
<td>0.064</td>
</tr>
</tbody>
</table>

Table 10. Multiple hierarchical regression analysis of variance in accuracy on happy trials of emotional recognition task when controlling for gender.

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Standard error B</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>1.184</td>
<td>0.191</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>-0.270</td>
<td>0.122</td>
<td>-0.278</td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>-0.112</td>
<td>0.560</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>-0.332</td>
<td>0.120</td>
<td>-0.342</td>
</tr>
<tr>
<td>SBP decline</td>
<td>0.010</td>
<td>0.004</td>
<td>0.402</td>
</tr>
<tr>
<td>MAP</td>
<td>-0.003</td>
<td>0.005</td>
<td>-0.099</td>
</tr>
</tbody>
</table>

Table 11. Multiple hierarchical regression analysis of variance on accuracy of surprise trials when gender is controlled for.
4.4.5.5 Selective attention

For a measure of selective attention the Map search task from the Tests of Everyday Attention was used. There was no correlation between SBP decline as seen in Figure 15 ($r = -0.058$, $N = 61$, $p = 0.677$) and attention score. There was however, a significant correlation between attention score and MAP ($r = 0.270$, $N = 60$, $p = 0.046$; 95% CI 0.02 to 0.49) as seen in Figure 16.

Figure 15. Scatterplot showing lack of relationship between SBP decline when standing and attention score in AD patients.

Figure 16. Scatterplot showing relationship between MAP and attention score in AD patients.
4.5 Discussion

This study assessed the relationship between blood pressure and performance on different cognitive domains within an AD population. The results of this study suggest a positive association between current MAP and attention, whereas a greater standing SBP decline was associated with a slower reaction time. Gender may influence the ability for emotional appraisal, and age may have a negative association with memory awareness.

The hypothesis that current blood pressure would positively associate with cognitive performance in mild AD patients was partly supported in this study. It was found here that MAP significantly associated with score on a visual attention task, but not scores on other cognitive tasks. The hypothesis that a history of hypertension would be associated with poorer cognitive performance was not supported in this study.

For a computerised prospective memory task and an emotional recognition task, males were quicker and more accurate than females. Males generally outperform females on visual tasks (Li & Singh 2014) and so a high amount of visual tasks may have led to an unintended bias in this study. Although changes in oestrogen levels in older females are thought to lead to an increased risk for the development of AD (Li et al. 2014), the role gender plays in cognitive performance within an AD population is less studied.

Due to the negative effect of long-term hypertension on the brain, it was expected that there would be significantly worse cognitive performance in those with a history of hypertension compared to those without a history of hypertension. Using medical history in AD patients could be useful in predicting clinical course, and may account for individual differences in cognitive performance seen in AD populations. However, as can be seen in Table 8, there were no significant differences found. This may be due to the fact that their hypertension was successfully controlled as shown in Table 4.

It may be that the prospective memory task used here was particularly difficult for this population, as some participants were excluded from analysis due to lack of correct responses, leaving an N of 46. This may have limited the ability to detect an association with blood pressure, which was not found to be significant in this study. Indeed this gives a statistical power of only 0.4 to detect significance. This task has been previously used in an AD population by Farina et al., (2013) where the authors also had to remove
participants from analysis due to outliers. The high exclusion rate may have limited the ability to compare this prospective memory score to the subjective prospective memory awareness rating, as no significant correlation was found. Impairments in memory awareness in AD patients has been noted when their subjective memory ratings are compared to those given by their caregiver (Ott et al 1996), therefore, validation of this computerised prospective memory test in AD by comparing caregiver rating and actual memory performance may be useful in future. AD patients have previously been found to be impaired on memory awareness when their rating is compared to actual memory performance (Oyebode et al. 2007), but there is much variation between patients (Feher et al. 1994). For the subjective questionnaire there was less report of memory failure with increasing age, which may imply that there is less awareness of memory failures with age. A decrease in cognitive awareness with increasing age has been previously reported in healthy adults (Palmer et al. 2014) and in AD patients (Derouesné et al. 1999).

This task also gave a measure of reaction time in the baseline condition. This is reaction to a visual cue, where the participant had to respond by pressing the correct button on the keyboard. It was found that an increase in SBP decline associated with a longer reaction time. Frewen et al., (2014) found significantly longer reaction times in those with orthostatic hypotension (OH) than those without in a non-demented elderly sample. OH has been associated with cognitive impairment and dementia, including AD (Mehrabian et al. 2010). This study suggests that a SBP decline may be associated with cognitive impairments even if a sufficient decline is not reached for a diagnosis of OH.

In the emotional recognition task, there was a higher accuracy for happy and surprised faces. These trials also had the smallest reaction time to correct response. This is to be expected as a cognitive bias for positive stimuli has previously been documented in elderly participants (Johnson & Whiting 2013). A negative association between fearful faces and blood pressure was expected from previous work in young healthy volunteers (Makovac et al. 2015), although this is the first study to assess this in an elderly AD population. As the effect found in Makovac et al., (2015) was dependent upon an increase in parasympathetic activity, this effect may be lost in elderly populations. An impairment of the baroreceptor response has been observed in elderly participants, specifically, a decreased parasympathetic and increased sympathetic vascular response (Aharon-Peretz et al. 1992; La Rovere & Pinna 2014).
In opposition to historical hypertension, having a current low blood pressure may be detrimental to cognition in elderly patients. A positive association was found here between MAP and selective attention. This supports the findings of Gifford et al., (2013) who found a small positive correlation between attention and blood pressure in healthy older adults. There has been less investigation into the effect blood pressure may have on attention performance in AD patients. Work from the Levey group of Emory University has found a detrimental effect of increased DBP on tests of executive function in AD patients (Goldstein et al 2005; Goldstein et al 2008; Lamar et al 2010), but attention was not found to be significantly effected in these studies. Methodological differences may explain discrepancies between this study and the previous findings. For instance Goldstein et al., (2008) used hypertension as categorical data (Yes/No) which may have masked the effect of actual blood pressure readings. Goldstein et al., (2008) however, did include a calculation of Pearson’s R with SBP and DBP as continuous variables on their population of 74 AD patients. No significant association was found here between blood pressure and attention score, but a different type of attention was assessed. In the current study, the task focused on visual selective attention, however, Goldstein et al., (2008) used an auditory task. Further work detailing the effects of blood pressure on various types of attention, would be useful to see if there is truly an impact of blood pressure on visual attention within an AD population.
5. Inflammation and cognitive performance

5.1 Foreword

Increased neuroinflammation has been associated with the aetiology of AD from both animal studies (Krsticevic & Knuesel 2013), and population studies in humans (Kok et al. 2011). These findings are supported by earlier work in human post-mortem studies, suggesting a causal link between AD and neuroinflammation. Yasojima et al., (1999) for instance, found significantly greater levels of complement system proteins, including those at the end of the signalling pathway, in brain tissue from AD patients compared to controls. The complement mRNA was co-localised with Aβ and NFT pathology, suggesting that the pathology may be able to initiate an inflammatory response. PET scanning which can be used to measure levels of microglial activation in AD patients has shown an increased inflammatory response in comparison to those without dementia (Schuitemaker et al. 2013). Edison et al., (2008) found a negative correlation between MMSE and microglial activation, but not amyloid load, in AD patients, showing a possible impact of inflammation on cognitive symptoms independent from amyloid burden. A peripheral blood biomarker for inflammatory status in AD patients is now sought after.

Increased levels of peripheral inflammatory markers have been observed in AD patients (Koyama et al. 2013; Swardfager et al. 2010; Remarque et al. 2001) suggesting a pro-inflammatory state, however there are inconsistencies in the literature. For instance, Leung et al., (2013) found increased levels of IL-6 and IL-10 to be associated with greater cognitive decline, although IL-6 is considered pro-inflammatory and IL-10 is an anti-inflammatory cytokine. Julian et al., (2015) however, did not find any relationship between inflammatory markers IL-1β, IL-6 or TNF-α and cognitive status in AD patients. Meta-analyses by Swardfager et al., (2010) and Koyama et al., (2013) concluded that peripheral inflammatory markers are increased in AD. However, when assessing the studies included in analysis, it is clear that there are wide variations in the cytokines associated with AD, and the concentrations observed.

Increased levels of inflammatory markers have been negatively associated with cognitive performance separately from AD. For instance, Wright et al., (2006) found increased levels of IL-6 were negatively associated with MMSE score in older adults
without dementia. Also, increased CRP has been associated with poorer retrospective memory and attention performance in healthy older adults (Teunissen et al. 2003). Contrastingly, high levels of CRP were associated with poorer performance on attention but not retrospective memory tasks in the PROSPER study (Mooijaart et al. 2011). An investigation by Dik et al., (2005) however, found no association between CRP, IL-6 and tests of global cognition, retrospective memory and processing speed. Therefore the effect of inflammatory markers on different types of cognition requires further investigation. The results of Edison et al., (2008) suggest that inflammation may provide an additional cognitive burden within AD patients. This is supported by the findings of Holmes et al., (2009) which found increased cognitive decline in AD patients who suffered a systemic inflammatory event. This shows that peripheral immune activation is able to affect cognition within AD.

5.2 Methods

A blood sample was taken from the participants as detailed in Methods Section 3.4 Blood Sample Collection. Concentrations of pro-inflammatory markers (CRP, IL-1β, IL-2, IL-6, and TNF-α), an anti-inflammatory cytokine (IL-10), Fetuin-A, and a marker of vascular health (ADMA) were analysed as detailed in Section 3.6 Blood sample analysis.
5.3 Results

5.3.1 Pro-inflammatory cytokines

Two outliers were identified in IL-6 by being greater than two standard deviations from the mean. These were removed from further analysis.

Average concentrations of pro-inflammatory markers in pg/ml with outliers removed are shown in Table 12.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>N</th>
<th>Range</th>
<th>Mean (S.D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>42</td>
<td>2.44-25.64</td>
<td>13.34 (6.39)</td>
</tr>
<tr>
<td>IL-2</td>
<td>42</td>
<td>10.50-56.67</td>
<td>29.76</td>
</tr>
<tr>
<td>IL-6</td>
<td>40</td>
<td>5.14-13.38</td>
<td>9.08 (1.969)</td>
</tr>
<tr>
<td>CRP</td>
<td>42</td>
<td>132.57-906.62</td>
<td>744.75</td>
</tr>
<tr>
<td>TNF-α</td>
<td>42</td>
<td>212.5-758.33</td>
<td>467.52 (162.05)</td>
</tr>
</tbody>
</table>

Table 12 Concentrations of pro-inflammatory markers (pg/ml) in serum samples taken from mild AD patients

Shapiro-Wilks Tests of normality were performed on the concentrations of pro-inflammatory markers. IL-2 and CRP were found to be non-normally distributed ($p < 0.000$), and so non-parametric tests were used on these data sets. No relationship was found with age and any of the markers except TNF-α; Pearson’s R = 0.374, N = 34, $p = 0.029$. Scatterplots with Pearson’s R and Rho values, and corresponding $p$ values, are shown in Figure 18.
Independent sample T-test and Mann-Whitney U test showed there were no significant differences for any of the measures.

Figure 18. Scatterplots showing relationship between age and pro-inflammatory markers from serum samples from mild AD patients. Pearson’s R, Spearman’s Rho and p values are displayed.

The average concentrations for males and females are shown in Table 13 and Table 14. Independent sample T-test and Mann-Whitney U test showed there were no significant differences for any of the measures.
Table 13. T-tests showing no differences between average concentrations of pro-inflammatory markers between male and female mild AD patients.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Gender</th>
<th>Mean</th>
<th>F</th>
<th>t</th>
<th>Sig. (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>M</td>
<td>14.58</td>
<td>2.631</td>
<td>1.288</td>
<td>0.115</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>11.77</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>M</td>
<td>9.65</td>
<td>0.670</td>
<td>1.029</td>
<td>0.420</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>8.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>M</td>
<td>467.43</td>
<td>1.64</td>
<td>-0.004</td>
<td>0.205</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>467.64</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 14. Mann-Whitney U test showing no differences in pro-inflammatory markers between male and female AD patients.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Gender</th>
<th>Mean</th>
<th>U</th>
<th>Sig (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>M</td>
<td>27.22</td>
<td>124.50</td>
<td>0.537</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>32.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>M</td>
<td>764.83</td>
<td>72.00</td>
<td>0.610</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>717.38</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.3.1.1 Relationship between pro-inflammatory cytokines and cognitive performance

Pearson’s R and Spearman’s Rho were performed to assess whether there was a relationship between inflammatory status and cognitive test performance.

Prospective memory

This test gives a measure of reaction time for both the baseline and P condition. No relationship was found between pro-inflammatory markers and baseline reaction time, or reaction time during P trials, as shown in Figure 19 and Figure 20.

Figure 19. Scatterplots showing lack of relationship between pro-inflammatory markers and reaction time in AD patients. Pearson’s R, Spearman’s Rho and p values are displayed.
No relationship was found here between pro-inflammatory markers and accuracy on PM trials of the prospective memory task as shown in Figure 21.
The subjective retrospective and prospective memory questionnaire asked the participant to rate their own everyday memory performance. This gives a measure of total memory rating, retrospective memory rating and prospective memory rating.

**Figure 21.** Scatterplots showing lack of relationship between prospective memory performance and pro-inflammatory markers in AD patients. Pearson’s R, Spearman’s Rho and $p$ values are displayed.

**SRPMQ**

The subjective retrospective and prospective memory questionnaire asked the participant to rate their own everyday memory performance. This gives a measure of total memory rating, retrospective memory rating and prospective memory rating.
A significant negative relationship was found between total memory rating and IL-1β \( (r = -0.381, N = 34, p = 0.034) \), and TNF-α \( (r = -0.495, N = 34, p = 0.005) \). No relationship was found between the other markers of inflammation and memory rating.

For prospective memory rating, a significant negative relationship was found with IL-1β \( (r = -0.413, N = 34, p = 0.021; 95\% CI 0.18 \text{ to } 0.6) \) and TNF-α \( (r = -0.466, N = 34, p = 0.008; 95\% CI 0.24 \text{ to } 0.64) \). No relationship was found between the other markers of inflammation and prospective memory rating. Relationship between IL-1β and memory ratings is shown in Figure 22.

For retrospective memory rating, a significant negative relationship was found with TNF-α \( (r = -0.416, N = 34, p = 0.020; 95\% CI 0.18 \text{ to } 0.61) \). No relationship was found between the other markers of inflammation and retrospective memory rating. Scatter plot showing the relationship between memory ratings and TNF-α is shown in Figure 23.

Figure 22. Scatterplot showing relationship between IL-1β serum concentrations and subjective memory ratings in mild AD patients.
Due to the positive relationship between age and TNF-α, and the relationship between memory rating and age reported in Section 3.4.3.3, hierarchical multiple regressions were performed to determine if there was a relation with memory rating when age is controlled for. For total memory rating, Model 1 assessed the effect of age on questionnaire score and found that age explained 19.5% of the variance, which was significant at the 0.05 level ($p = 0.013$). The addition of the TNF-α to Model 2 added 12.7% of variance to the questionnaire total rating score, which was significant ($p = 0.030$). The total variance for Model 2 was 32.1%, $p = 0.004$. The standardised $\beta$ values for the hierarchical regression are shown in Table 15.

Figure 23. Scatterplot showing relationship between TNF-α serum concentration and subjective memory ratings in mild AD patients.
Table 15. Multiple hierarchical regression on the effect of TNF-α on total memory rating when controlling for age in mild AD patients.

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Standard error B</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>87.827</td>
<td>19.850</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.652</td>
<td>0.247</td>
<td>-0.440</td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>81.842</td>
<td>18.726</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.439</td>
<td>0.249</td>
<td>-0.297</td>
</tr>
<tr>
<td>TNF-α</td>
<td>-0.024</td>
<td>0.010</td>
<td>-0.384</td>
</tr>
</tbody>
</table>

Table 16. Multiple hierarchical regression on the effect of TNF-α on prospective memory rating when controlling for age in mild AD patients.

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Standard error B</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>42.473</td>
<td>10.910</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.319</td>
<td>0.136</td>
<td>-0.400</td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>39.383</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.209</td>
<td>0.138</td>
<td>-0.262</td>
</tr>
<tr>
<td>TNF-α</td>
<td>-0.012</td>
<td>0.006</td>
<td>-0.368</td>
</tr>
</tbody>
</table>

For prospective memory rating, Model 1 assessed the effect of age on questionnaire score and found that it explained 16% of the variance which was significant; \( p = 0.026 \). Adding TNF-α to Model 2 explained an additional 11.7% of the variance which was significant \( p = 0.042 \). The total variance for Model 2 was 27.7% which was significant \( p = 0.011 \). The standardised β values for the hierarchical regression are shown in Table 16.
For retrospective memory rating, Model 1 assessed the effect of age on questionnaire score and found it explained 11.6% of the variance which was significant ($p = 0.035$). The addition of TNF-α in Model 2 explained an additional 8.7% of the variance which was not significant ($p = 0.085$). Total variance explained by Model 2 was 17.7% which was significant; $p = 0.025$. The standardised β values for the hierarchical regression are shown in Table 17.

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Standard error B</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>45.354</td>
<td>12.072</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.333</td>
<td>0.150</td>
<td>-0.381</td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>42.425</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.229</td>
<td>0.156</td>
<td>-0.262</td>
</tr>
<tr>
<td>TNF-α</td>
<td>-0.012</td>
<td>0.006</td>
<td>-0.318</td>
</tr>
</tbody>
</table>

Table 17. Multiple hierarchical regression on the effect of TNF-α on retrospective memory rating when controlling for age in mild AD patients.

**Emotional recognition**

No relationship was found here between pro-inflammatory markers and reaction time or accuracy on an emotional recognition task as shown in Figure 24 and Figure 25.
Figure 24. Scatterplots showing lack of relationship between reaction time on emotional recognition task and pro-inflammatory markers. Pearson’s R, Spearman’s Rho and p values are displayed.
No relationship was found here between selective attention score and pro-inflammatory markers as shown in Figure 26.
Figure 26. Scatterplots showing lack of relationship between pro-inflammatory markers from serum and selective attention score in mild AD patients. Pearson’s R, Spearman’s Rho and p values are displayed.
5.3.2 Anti-inflammatory cytokine

Concentrations of IL-10 are shown in Table 18. One outlier was removed from IL-10 analysis as it was greater than two standard deviations from the mean. Once this outlier was removed, Shapiro-Wilks test showed IL-10 dataset was not normally distributed $p = 0.013$.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>N</th>
<th>Range</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10</td>
<td>38</td>
<td>6.94-366.67</td>
<td>25.76</td>
</tr>
</tbody>
</table>

Table 18 showing average values of IL-10 (pg/ml).

IL-10 had no relationship with age, $\text{Rho} = -0.059$, $N = 38$, $p = 0.723$. There was also no significant difference between males and females for IL-10 ($\text{Mann-Whitney } U = 142$, $p = 0.326$).
5.3.2.1 Relationship between anti-inflammatory cytokine and cognitive performance

Prospective Memory

No relationship was found between IL-10 and reaction time or accuracy on prospective memory task as shown in Figure 27.

Figure 27. Scatterplots showing lack of relationship between IL-10 and prospective memory performance in mild AD patients. Pearson’s R, Spearman’s Rho and p values are displayed.
SRPMQ

There was no relationship between IL-10 and the total memory rating (Rho = -0.043, N = 34, p = 0.810). There was also no relationship between IL-10 and prospective (Rho = -0.065, N = 3, p = 0.714) and retrospective (Rho = -0.027, N = 34, p = 0.879) memory rating.

Emotional recognition

The relationship between IL-10 and emotional recognition was assessed. Spearman’s Rho showed there was no relationship between IL-10 and accuracy (Rho = 0.284, N = 34, p = 0.104) or reaction time (Rho = 0.182, N = 33, p = 0.311).

Selective attention

No relationship was found between selective attention and IL-10. The relationship is shown in Figure 28.

![Figure 28](image.png)

Figure 28. Scatterplots showing lack of relationship between IL-10 and selective attention task performance in mild AD patients. Pearson’s R, Spearman’s Rho and p values are displayed.
5.3.3 Fetu-in-A and asymmetric dimethylarginine

Asymmetric dimethylarginine (ADMA) and Fet-A concentrations were analysed in serum samples. ADMA is an NO inhibitor which has previously been associated with hypertension (Millatt et al 2003; Kielstein et al 2004). Increased ADMA has been observed in plasma of AD patients compared to age-matched controls (Arlt et al 2008), however this has not been found in all studies (Mulder et al 2002; Richardson et al 2014). Its potential relationship with cognitive symptoms is yet to be assessed.

Fet-A has previously been found to positively correlate with MMSE score in AD patients (Smith et al. 2011). Fet-A may be beneficial for vascular health as it inhibits calcification of vessels (Reynolds et al. 2005). It has also been observed to attenuate the inflammatory response by reducing production of inflammatory molecules and macrophage activation (Wang & Sama 2012).

The average concentrations of ADMA and Fet-A in serum samples are shown in Table 19. Shapiro-Wilks test showed that Fet-A and ADMA were not normally distributed ($p < 0.000$).

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Mean</th>
<th>Range</th>
<th>Mean M/F</th>
<th>U</th>
<th>Sig ($p$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADMA</td>
<td>2.78</td>
<td>2.09-3.65</td>
<td>2.89/2.63</td>
<td>124.50</td>
<td>0.077</td>
</tr>
<tr>
<td>Fet-A</td>
<td>0.38</td>
<td>0.01-1.02</td>
<td>0.386/0.387</td>
<td>128</td>
<td>0.357</td>
</tr>
</tbody>
</table>

Table 19. Average values of ADMA (μmol/l) and Fet-A (g/l) concentrations in total sample of mild AD patients, and in males and females. Mann-Whitney U test showed no significant difference between males and females.

Spearman’s Rho showed no correlation between ADMA and age, $Rho = 0.257$, $N = 39$, $p = 0.115$. Fet-A had no relationship with age $Rho = 0.135$, $N = 36$, $p = 0.432$. 

108
5.3.3.1 Relationship between cognitive performance and ADMA and Fet-A.

Prospective memory

No relationship was found between ADMA concentrations and reaction time on prospective memory test in the baseline or PM condition. There was also no relationship between ADMA and prospective memory accuracy, as shown in Figure 29.

![Figure 29. Scatterplot showing lack of relationship between ADMA serum concentrations and performance on prospective memory task in mild AD patients. Pearson’s R, Spearman’s Rho and p values are displayed.](image)

Figure 30 shows there was no relationship between Fet-A and prospective memory task.
SRPMQ

No relationship was found between ADMA concentrations and response on total and prospective memory ratings questionnaire. A significant negative relationship was found here between ADMA and retrospective memory rating. Relationships are shown in Figure 31.

For Fet-A concentrations, there was no relationship with the total memory rating (Rho = 0.103, N = 32, p = 0.576). There was also no relationship between Fet-A and prospective (Rho = 0.173, N = 32, p = 0.344) and retrospective (Rho = 0.056, N = 32, p = 0.759) memory rating.

Figure 30. Scatterplots showing lack of relationship between Fet-A and prospective memory performance in mild AD patients. Pearson’s R, Spearman’s Rho and p values are displayed.
Figure 31. Scatterplots showing relationship between ADMA serum concentration and subjective memory rating in AD patients. Pearson’s R, Spearman’s Rho and $p$ values are displayed.

Rho = -0.309
$p = 0.071$

Rho = -0.206
$p = 0.235$

Rho = -0.343
$p = 0.044$
CI = 0.1 to 0.55
Emotional recognition

No relationship was found between ADMA and an emotional recognition task (Figure 32.)

Spearman’s Rho was used to find there was no relationship between Fet-A concentration and accuracy (Rho = -0.151, N = 32, p = 0.409) or reaction time (Rho = -0.130, N = 31, p = 0.486).

Figure 32. Scatterplots showing lack of relationship between ADMA serum concentrations and reaction time and accuracy on an emotional recognition task in AD patients. Pearson’s R, Spearman’s Rho and p values are displayed.
Selective attention

There was no relationship between ADMA, or Fet-A and selective attention score, as shown in Figure 33 and Figure 34.

![Scatterplot showing lack of relationship between ADMA concentrations and selective attention score in mild AD patients. Pearson’s R, Spearman’s Rho and p values are displayed.](image1)

**Figure 33.** Scatterplot showing lack of relationship between ADMA concentrations and selective attention score in mild AD patients. Pearson’s R, Spearman’s Rho and p values are displayed.

![Scatterplot showing lack of relationship between Fet-A concentrations and selective attention score in mild AD patients. Pearson’s R, Spearman’s Rho and p values are displayed.](image2)

**Figure 34.** Scatterplot showing lack of relationship between Fet-A concentrations and selective attention score in mild AD patients. Pearson’s R, Spearman’s Rho and p values are displayed.
5.3 Discussion

The relationship between cognitive performance and inflammatory markers in AD patients was investigated. The relationship between cognition and two markers of vascular health was also assessed.

The hypothesis that inflammation would be negatively associated with cognitive performance was partly supported in this study. Pro-inflammatory markers IL-1β and TNF-α were negatively associated with reports of memory failures, but not scores on other cognitive tests. It was hypothesised that there would be a negative association between ADMA and cognition, and a positively association between Fet-A and cognitive performance. This was partly supported by the finding of a relationship between retrospective memory rating and ADMA concentration. Fet-A concentrations were not associated with cognitive performance in this study.

The pro-inflammatory markers assessed here were IL-1β, IL-2, IL-6, TNF-α and CRP. The range found for IL-1β 2.44-25.64 pg/ml, was similar to the range found in AD patients by Richardson et al., (2014). The range for IL-2 10.50-56.67 pg/ml was similar to that of Leung et al., (2013). For IL-6, a range of 5.14-13.38 pg/ml found here is close to the means reported in Licastro et al., (2003) and Bonotis et al., (2008). For CRP and TNF-α the ranges of 132.57-906.62 pg/ml and 212.5-758.33 pg/ml respectively were higher than previously reported in AD patients, however, the range for TNF-α does overlap with the one reported by Leung et al., (2013).

There was no relationship between pro-inflammatory markers and prospective memory performance. However, a negative relationship was found between prospective memory rating and both IL-1β and TNF-α concentrations, suggesting less report of memory failures with increasing levels of inflammation. The same relationship was also found for retrospective memory rating. As relationships were found between age and TNF-α, and age and memory ratings, hierarchical regression analyses were performed to check for interactive effects. It was found that for total memory rating, TNF-α explained 32.1% of the variance when age was controlled for which was significant, \( p = 0.004 \). For prospective memory rating, when controlling for age, TNF-α explained 27.7% of the variance which was significant \( p = 0.011 \). For retrospective memory rating, TNF-α explained 17.7% of the variance when controlling for the effect of age,
which was significant, $p = 0.025$. Therefore a high inflammatory status may be associated with impairments in cognitive awareness within AD.

With regards to emotional recognition or selective attention, no relationship was found here with pro-inflammatory markers.

IL-10 was assessed here as an anti-inflammatory marker. Ranges measured in this study were 6.94-366.67 pg/ml for IL-10. The values for IL-10 overlap with those reported by Leung et al., (2013) and Rota et al., (2006) for AD patients. No relationship was found between cognitive test results and IL-10 in this study.

ADMA and Fet-A were measured here as biomarkers for vascular health. A range was found here of 2.09-3.65 μmol/l for ADMA and 0.01-1.02 g/l for Fet-A. The mean concentration for Fet-A was similar to that reported by Smith et al., (2011) in a sample of AD patients. A relationship was found between retrospective memory rating and ADMA concentration, but no other cognitive measure. Increasing ADMA levels were correlated with less report of memory failure, which may show there is less awareness of memory performance with increasing ADMA level. Fet-A levels were not associated with cognitive performance in this study.

It was found here that increasing concentrations of pro-inflammatory markers were associated with less report of memory failures. This relationship remained significant when the association with age was controlled for. This may suggest that both increasing age, and increasing pro-inflammatory status associated with AD, is independently associated with a decrease in memory performance awareness. This should be investigated further by assessing the relationship between pro-inflammatory markers, especially TNF-α and IL-1β and caregiver rating of memory awareness and patient memory performance. If a relationship between inflammation and memory awareness is confirmed, then pro-inflammatory biomarkers may be useful in identifying which patients could benefit from further memory awareness training, and coping strategies for cognitive failures.

An investigation in elderly participants without dementia by Miralbell et al., (2013), found a negative association between retrospective verbal memory and ADMA concentrations, and, as in the current study, no association with measures of speed or attention. It would be interesting to see if there is a relationship between retrospective memory performance and ADMA within an AD population in a future study. ADMA is used as a marker of vascular health as it is an NO inhibitor (Millatt et al. 2003; Kielstein
et al. 2004) therefore increasing levels may represent poorer vascular health. Indeed, increased ADMA has been associated with increased risk of stroke (Yoo et al 2001) and subarachnoid haemorrhage (Jung et al. 2007), showing it is able to have a negative impact within the cerebrovasculature. Further work is needed to refine its association with AD, and AD cognitive symptoms. It would also be useful to determine in longitudinal studies if high ADMA levels are able to predict faster decline in AD due to its association with cerebrovascular damage.
6. Blood pressure, inflammation and cognitive performance

6.1 Foreword

A close reciprocal relationship between inflammatory processes and the vascular system has been suggested. Mechanisms by which this relationship may influence cognition and AD are now being investigated.

Increased levels of pro-inflammatory molecules have been measured in patients with hypertension (Chae et al. 2001; Stumpf et al. 2005). Hypertension causes physical changes within the walls of blood vessels, which is able to initiate an inflammatory response. An inflammatory state may also increase the risk for hypertension development. Pro-inflammatory molecules were observed in middle-aged patients who later developed hypertension (Sesso et al. 2003; Engstrom et al. 2002).

Animal studies have suggested that a combination of hypertension and increased inflammation increases the risk for AD. For instance, Carnevale et al., (2012b) observed increased neuroinflammation in hypertensive mice which preceded Aβ deposition. These physiological changes were accompanied by memory impairments. In humans, van Exel et al., (2009) observed that the middle-aged offspring of sporadic AD patients had greater blood pressures and pro-inflammatory responsiveness than those without a genetic link to AD. Therefore, there may be a predisposition to AD, even in sporadic patients, via vascular and inflammatory mechanisms.

Although a history of hypertension may lead to an increased risk for AD, lower blood pressures in older age has now been identified as a risk factor (Guo et al. 1999; Qiu et al. 2003; Verghese et al. 2003). Inflammation is thought to be increased in AD, however there is much variability between patients (Swardfager et al. 2010; Koyama et al. 2013). The impact that different concentrations of peripheral inflammatory markers and different blood pressures may have upon the presentation of AD is unknown.
6.2 Methods

Blood pressure was measured in AD patients as described in Section 3.2 Blood pressure measurements. Cognitive tests were performed as detailed in 3.3 Cognitive testing. A blood sample was taken as detailed in Methods section 3.4 Blood Sample Collection. Concentrations of pro-inflammatory cytokines (IL-1β and TNF-α), and a marker of vascular health (ADMA) were analysed as detailed in Section 3.6 Blood sample analysis.
6.3 Results

6.3.1 Interactive effect of inflammation and blood pressure on cognitive performance

SPRMQ

Due to the effects of both pro-inflammatory markers and ADMA on subjective retrospective memory ratings, hierarchical regression analyses were performed. A model of ADMA when controlling for age, significantly predicted the retrospective memory rating, $F(2,32) = 3.445, p = 0.044$, explaining 17.7% of the variance.

A model was created to assess the effects of both ADMA and TNF-α on retrospective memory rating when controlling for age. The model explained 24.4% of the variance in memory rating which was just significant, $p = 0.05$. 
6.4 Discussion

Markers of vascular health may have interactive effects with markers of increased inflammation upon cognitive presentation. ADMA and TNF-α serum concentrations were found to have a cumulative impact on retrospective memory rating. This partly supports the hypothesis that there would be a synergistic relationship between inflammatory and vascular disease markers and cognitive score in mild AD patients.

ADMA may be useful as a biomarker of endothelial dysfunction (Bouras et al. 2013; Perticone et al. 2010) and is associated with atherosclerosis (Xia et al. 2015). Increased ADMA levels have been previously associated with AD (Arlt et al. 2008) and with lower memory performance in elderly participants without dementia (Miralbell et al. 2013). Increased levels of TNF-α have previously been associated with greater global cognitive decline in AD patients (Holmes et al. 2009). This is the first study to assess the relationship between vascular and inflammatory markers and memory awareness. A lack of awareness of memory failures has previously been observed in AD (Mårdh et al. 2013) but there is much variability between patients. This study suggests that inflammatory state and vascular health may be confounding factors for memory awareness. Mechanisms of meta-cognition in healthy elderly and AD patients are being uncovered (Thomas et al. 2013), which may lead to the development of cognitive strategies to help those who lack awareness of memory performance. Further study evaluating the usefulness of cognitive strategies in AD patients with high levels of vascular disease and inflammation would be useful.
### Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Correlation</th>
<th>Relative effect (95% CI)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP and visual attention</td>
<td>Positive</td>
<td>$r = 0.270, p = 0.046$</td>
<td>This result suggests a correlation between increased blood pressure and increased visual attention performance in this patient population</td>
</tr>
<tr>
<td>SBP decline and reaction time</td>
<td>Positive</td>
<td>$r = 0.357, p = 0.015$</td>
<td>As the decline from sitting to standing in SBP increased, so did reaction time</td>
</tr>
<tr>
<td>ADMA and retrospective memory awareness</td>
<td>Negative</td>
<td>Rho = -0.343, $p = 0.044$</td>
<td>Retrospective memory awareness is poorer in those with increased ADMA concentrations</td>
</tr>
<tr>
<td>IL-1β and retrospective memory awareness</td>
<td>Negative</td>
<td>$r = -0.416, p = 0.020$</td>
<td>Retrospective memory awareness is poorer in those with increased IL-β concentrations</td>
</tr>
<tr>
<td>IL-1β and prospective memory awareness</td>
<td>Negative</td>
<td>$r = -0.413, p = 0.021$</td>
<td>Prospective memory awareness is poorer in those with increased IL-β concentrations</td>
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<tr>
<td>TNF-α and prospective memory awareness</td>
<td>Negative</td>
<td>$r = -0.466, p = 0.008$</td>
<td>Prospective memory awareness is poorer in those with increased TNF-α concentrations</td>
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Table 20: Summary of key findings

This study aimed to assess the association between blood pressure, blood biomarkers of vascular health, inflammation, and the cognitive symptoms of AD patients. There were several hypotheses in this study.
The aetiology of AD has recently been associated with changes in blood pressure. Mid-life hypertension has been identified as a risk factor for AD (Shah et al. 2012; Qiu et al. 2005) whereas in later life hypotension has now been identified as a risk factor (Verghese et al. 2003). The role blood pressure plays in the general health of an ageing population is still being elucidated. It appears that the guidelines for young and middle-age adults of a target blood pressure of 120/80mmHg, are not the same as those which must be given to older age groups. It is now considered best practice to not aggressively treat high blood pressure to an SBP of below 140 mmHg in those over the age of 60 (James et al. 2014). Therefore, there appears to be a shift in optimal blood pressure ranges as people enter old age. This is likely due to known age-related changes in vessel stiffness (de la Torre 2012), and a decrease in parasympathetic activity and baroreceptor response (Aharon-Peretz et al. 1992; La Rovere & Pinna 2014). Tortuous and string vessels in the cerebrovasculature appear after middle-age, which have a greater resistance to blood flow and reduced flexibility (Brown & Thore 2011). This may also lead to a hypertensive state being necessary for sufficient cerebral perfusion.

In regards to blood pressure and cognitive performance within an AD population, a standing decline in SBP was found to positively correlate with a measure of reaction time, suggesting slower reaction times with a greater change in SBP when going from sitting to standing. OH is associated with greater vessel stiffness (Takahashi et al. 2015) and may show a failure of the vessels to respond to changes in posture and pressure, which could lead to a reduction in CBF. A decrease in CBF has been associated with poorer cognitive performance in AD patients (Binnewijzend et al. 2013). Freidenberg et al., (2013) successfully treated OH in dementia patients, which lead to a reduction in falls, fatigue, dizziness and mental fluctuations; symptoms which could contribute to the clinical symptoms of AD. The relevance of OH to individual cognitive symptoms within an AD population has not yet been determined, but this current study suggests that SBP decline may have a negative impact upon reaction time even if the decline is not severe enough to reach a diagnosis of OH.

In this study, MAP was found to positively correlate with performance on a visual selective attention task. Previous research in older adults without cognitive impairment found cognitive performance to be impaired by presence of hypertension, but that tasks in the attention domain were spared from this effect (Saxby et al 2003). Also, a meta-analysis by Gifford et al., (2013) found a positive association between blood pressure
and attention in older adults without dementia. Therefore it seems that attentional tasks show a different relationship with blood pressure compared to those of other cognitive domains. The findings of Madden et al., (1998) suggest that the relationship between attention and blood pressure is mediated by age, as the authors found a detrimental effect of hypertension on attention performance in middle-age adults, but not in those over 60 years. In patients with a cognitive impairment disorder such as AD, damage to the cerebrovasculature is a common co-morbidity. In stroke patients, increased blood pressure was used to increase cerebral perfusion, which led to improved cognitive performance (Hillis et al 2003). Similar mechanisms may underly the finding of an advantage of current hypertension for attentional tasks in those with AD. In cross-sectional studies of cognitive performance in older adults such as those included in Gifford et al., (2013) it is not possible to rule out that some of the participants in analysis did not have pre-clinical AD, has it is known to have a slow, hidden onset. Therefore longitudinal studies in middle aged adults would be useful in determining whether this positive relationship between attention scores and blood pressure are present for the general population, as well as those with AD.

Markers for vascular health, ADMA and Fet-A, were measured and correlated with cognitive performance. ADMA is a NO inhibitor and so high levels are thought to associate with impairments in blood vessel function. As NO is an important vasodilator, increased levels of ADMA may impair vessel flexibility and responsiveness to changes in blood pressure. Fet-A is multi-purpose protein which has been found to have vaso-protective effects (Reynolds et al. 2005). It has previously been found to positively associate with MMSE score in AD patients (Smith et al. 2011).

It was found in this study that serum concentrations of ADMA negatively associated with retrospective memory ratings, suggesting that patients with higher levels of ADMA may be less aware of memory failings in the retrospective domain. Previous studies have indicated that ADMA levels in the blood are higher in AD patients than in age-matched controls (Selley 2003; Richardson et al. 2014), but this is one of the first studies to assess the relationship between serum ADMA concentrations and cognitive performance in AD patents. Arlt et al., (2008) did find a relationship between cognitive impairment and ADMA levels in the CSF, but did not find a relationship with plasma ADMA in their study. It may be that a measure of global cognitive performance will not show a relationship with ADMA levels, as there is a selective vulnerability for
retrospective memory and memory awareness. An investigation by Miralbell et al., (2013) found that ADMA concentrations in older adults without dementia negatively correlated with performance on a retrospective memory task, but not performance on tasks of attention, visual spatial skills or processing speed. A further study assessing the association of ADMA and retrospective memory performance in AD patients would be useful to confirm this selective association. No association was found between Fet-A and the cognitive scores in this study. It may be that associations found with global cognition are lost when looking at the individual cognitive domains included in this study.

A pro-inflammatory state is associated with ageing and AD (Swardfager et al. 2010). Markers associated with pro-inflammatory and anti-inflammatory activity were measured in serum samples of AD patients to assess their relationship with cognitive performance. Pro-inflammatory markers IL-1β and TNF-α were found to negatively correlate with a measure of subjective memory rating. This suggests that higher levels of inflammation, or a greater pro-inflammatory state, may be associated with impaired memory awareness. Previous work has found increased levels of pro-inflammatory markers, particularly TNF-α and IL-1β to be associated with poorer cognitive performance in AD patients (Holmes et al. 2009; Leung et al. 2013). This study is one of the first to find a relationship with inflammation and memory awareness. This relationship was also found with ADMA concentrations and so there may be a synergistic effect. Combined analysis of ADMA and TNF-α concentration showed they had a significant effect upon retrospective memory rating when controlling for the effects of age.

As well as the synergistic effects that inflammation and markers of vascular health, and inflammation and blood pressure may have on cognition, this study shows that gender and age also influence cognitive symptoms within an AD population. Males outperformed females on a test of prospective memory, and were more accurate on happy and surprise trials of the emotional recognition task. This may be due to the reported advantages that older males have on tasks involving the visual domain (Li & Singh 2014). Benke et al., (2013) however, also found that male AD patients outperformed females on a memory task. Male and female AD patients may require different treatment strategies such as cognitive stimulation therapies, and so it is important to assess gender differences. A decrease in oestrogen levels in post-
menopausal women has recently been associated with an increased risk of cognitive impairment (Daniel 2013), which may, at least in part, explain the differences in AD presentation.

A decrease in memory failure reporting was found to be associated with increasing age, which may suggest a decrease in memory awareness in older AD patients. It is important to identify which patients may over-estimate their cognitive performance as this may require different coping strategies for themselves and their caregivers. Relating an age-related decline in memory awareness with memory performance and caregiver rating would be an important future study.

Current drug treatments offered to AD patients provide only short-term relief of cognitive symptoms. They are often associated with side effects (Small et al. 2005; Masuda 2004), and in some patients show no cognitive benefit at all (Foster & Plosker 1999; Rogers & Friedhoff 1996). There are other treatment options however, which have been associated with a slowing of the progression of AD, and may be implemented alongside prescribed medication. Lifestyle factors such as adherence to the Mediterranean diet, seem to be able to lower the risk of developing AD, and is associated with reduced mortality in an AD population (Scarmeas et al. 2006). Exercise and physical activity has also been found to slow the functional and cognitive decline of AD patients (Farina et al. 2014). The benefit these interventions have within AD may be due to their positive effects upon both the cardiovascular and inflammatory systems.

7.2 Future Studies

The results of this study merit further investigation into the role of blood pressure and inflammation on the cognitive symptoms of AD. This and other studies (Gifford et al. 2013) have found measures of blood pressure and markers of inflammation to have different relationships with different types of cognition. Increasing appreciation of the varying relationships between cognitive domains and physical variables may aid symptom management for age-related cognitive deficits. Aging is accompanied by many changes to the vascular and inflammatory systems which may be causal contributors to age-related cognitive decline.

Here, a positive correlation was found between MAP and a visual attention task. As the ability to attend successfully to both auditory and visual stimuli plays an
important role in other forms of cognition, exploring the factors influencing this in AD patients could lead to symptom management strategies. Validation of the association between ADMA and retrospective memory awareness in AD populations could also lead to further understanding of physical variables which influence one of the hallmark characteristics of the condition. A novel finding of a synergistic relationship between a marker of pro-inflammatory activity, ADMA and retrospective memory awareness could be explored in more detail in a longitudinal study to investigate the possibility of interventional measures to manage cognitive impairments in AD.

8. Concluding remarks

This study assessed the effect of blood pressure and biomarkers of inflammation and vascular health upon cognition in AD patients. The results produced here add to the body of evidence linking vascular and inflammatory systems to AD and cognitive impairment. It was found here that current blood pressure positively associated with performance on an attentional task, suggesting that the findings by Gifford et al., (2013) in healthy older adults, may also relate to those with AD. Contrary to the hypothesis, a history of hypertension diagnosis was not found to have an effect upon cognition. This gives a positive message of hypertension in middle-age, however, suggesting that it may not have significant consequences upon cognition later in life if successfully controlled. This study found a synergistic negative effect of TNF-α and ADMA upon memory awareness. This novel finding of an association between inflammation, vascular health and cognitive awareness requires further investigation. Identifying which AD patients may lack awareness of their cognitive performance due to increased inflammation and vascular damage, is important for developing coping strategies for themselves and their caregivers.

It is clear that the reciprocal relationship of vascular and inflammatory systems contribute to cognition and AD. Further work into this relationship may provide treatment strategies for AD patients. Longitudinal studies would now be useful to identify patients who may have a faster clinical progression due to vascular and inflammatory factors.
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Appendices

Appendix 1 Information sheet

Sussex Partnership NHS Foundation Trust

Researcher: Lucy Nelson

Cognitive Treatment and Research Unit
Grove House
Southview Road
Crowborough East Sussex
TN6 1HB

01273 877876

Tel: 01892 603107
Fax: 01892 60311

Blood Pressure, Inflammation and Alzheimer's disease

We would like to invite you to take part in our research study. Before you decide, it is important for you to understand why the research is being done and what it will involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish.

Part 1 tells you the purpose of this study and what will happen to you if you take part.

Part 2 gives you more detailed information about the conduct of the study. Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Part 1
What is the purpose of the study?
This study is designed to look at the role of blood pressure and inflammation on the progression of Alzheimer's disease. We hope that information from this study will increase our understanding of Alzheimer's disease, and how it is affected by blood pressure and inflammation. Problems with blood vessels have been linked to the development of Alzheimer's disease and other dementias.

**Why have I been chosen?**

You have been invited to take part because you are aged 60 or over and have a clinical diagnosis of Alzheimer's Disease.

**Do I have to take part?**

No. It is up to you to decide whether or not to take part. If you do, you will need to sign a consent form. If you decide to take part you are still free to withdraw from the study at any time without giving a reason, and without your treatment being affected.

**What will happen to me if I take part?**

If you agree to take part, you will sign a consent form and you will be interviewed with your caregiver for about one hour. During the interview we will record data about your age, gender, years in education and past medical history and current medications. Most of these will be obtainable from your medical notes. We will also measure your blood pressure when you are sitting down and standing up. We will then ask you questions to assess your current memory and attention. This is in the form of questionnaires and tasks; for example, one of the tasks is looking at faces on a computer screen to see if you can tell what emotion they are showing, and another task is looking for symbols on a map. Finally we will ask you to give a blood sample of 15mls. About a year later we will contact you to let you know the findings of the study. It is again up to you to decide whether you wish to be contacted about this.

**What do I have to do?**

If you are happy to take part we will interview you for about an hour with your caregiver. We would also ask your permission for us to contact your GP for past blood pressure measurements and medications. If you have received an MRI scan as part of your diagnostic procedure we would also ask your permission for us to look at that data. You will not undergo any further scanning for the purpose
of this study. Any information received is handled in confidence and kept secure.

**What are the other possible disadvantages and risks of taking part?**

If you do participate in the study, and if we find anything unexpected we will ask your permission to contact your GP for further advice.

There is no risk involved with the study, however there may be minimal discomfort when the blood sample is taken. You may also experience some slight bruising around the area afterwards.

**Will there be any benefits from participating in this study?**

There is no immediate clinical benefit to you from this study and we do not anticipate that the results will benefit you directly. However the information we gather from this study may potentially inform the future understanding of the progression of Alzheimer’s disease, and treatment of people with Alzheimer’s disease.

**What if there is a problem?**

We do not anticipate any problems during this study. If you have any complaints or concerns during this study, please do not hesitate to contact us or the independent Patient Advisory Liaison Services on 01737 231958 or email pals@sash.nhs.uk.

**Will my taking part in the study be kept confidential?**

Yes. All information about you is handled in confidence. The details are included in Part 2.

Contact Details

Lucy Nelson. Email: l.nelson@bsms.ac.uk

Medical Research Building
Brighton and Sussex Medical School
University of Sussex
Brighton
BN1 9PS
Part 2:

What will happen if I don’t want to carry on with the study?

This is absolutely fine. You can withdraw from the one hour interview at any time, and you do not need to give a reason for doing so. Any information collected from you and your samples would be destroyed.

What if there is a problem?

We do not anticipate any problems during this study. Providing a blood sample can cause some discomfort, but every effort will be taken to minimise this.

Complaints

If you have a concern about any aspect of the study, you should ask to speak to Lucy Nelson (information above). If you remain unhappy and wish to complain formally, you can do this by calling the Complaints Office of the Sussex Partnership NHS Trust (please call 01903843026).

Harm

The Sussex Partnership NHS Trust has insurance in place to cover its legal liability should any harm arise from this study.

Will my taking part in this study be kept confidential?

Yes. All information gathered during this study is kept strictly confidential and stored securely at Brighton and Sussex Medical School. All electronic data from the study will be coded and anonymised. This means that there will be no way of relating your personal details with your data held on computer or your blood sample.

What will happen to any samples I give?

We will collect around 5mls of blood. All samples will be handled, stored and disposed of according to national guidelines. The samples will be frozen until analysis at the end of the study. Immediately after analysis in the laboratory any remaining sample will be disposed of. Your name will not appear on the blood tubes and you will not be identified by anyone else other than the research team.

The blood sample will be used for laboratory testing to look for molecules that have been linked to inflammation and general vascular health. These tests cannot be used for any diagnosis or treatment.
Will any genetic tests be done?

Yes. Part of the blood sample will be used to test for a type of the APOE gene. This gene is involved with controlling the movement of cholesterol in the body. Previous research suggests that this gene may be linked to Alzheimer’s disease and other disorders. However it cannot be used to test if someone will get or has Alzheimer’s disease. We will not look at any other gene type.

What will happen to the results of the study?

All results will be anonymised. Once the study is completed, data will be analysed and written up for publication in scientific journals. We will also send a lay summary of the results to you, if you wish. The results will also be sent to the service user group that has advised on this study

Who has organised and funded this study?

The study is organised and funded by Brighton and Sussex Medical School and University of Brighton.

Who has reviewed this study?

This study has been extensively reviewed by Research and Development at Sussex Partnership NHS Foundation Trust, University of Brighton Doctoral College, Brighton and Sussex Medical School and is approved by the NRES Committee London - Fulham.

Thank you for taking the time to read this information sheet

Chair: John Bacon CB
Rodrigues Working in partnership with Brighton & Hove City Council, East Sussex County Council and West Sussex County Council

19/04/2013. Version 2

REC Reference: 13/LO/0478
Appendix 2 Consent Form

Cognitive Treatment and Research Unit
Grove House
Southview Road
Crowborough
East Sussex
TN6 1HB

Tel: 01892 603107
Fax: 01892 603115

CONSENT FORM

Title of Project: Blood Pressure, Inflammation and Alzheimer's disease. Please initial box

I confirm that I have read and understood the Information Sheet and had the chance to ask questions about the study. I am satisfied with the answers I have been given.

I understand that my participation in this study is voluntary and that I am free to withdraw at any time without giving a reason, and without my medical care being affected in any way.

I understand that relevant sections of my clinical records and MRI scan data will be looked at by the research team or external regulatory bodies. I give permission for my medical notes and data to be used for this purpose.

I give permission to the research team to contact my GP/surgery for relevant information.

I understand that a sample of my blood will be taken and kept for analysis at end of study. I understand that my name will not appear on the blood sample.

I understand that my blood sample will be used for genetic testing of the APOE gene.

I agree to take part in the above study.

I agree to be contacted in one year to consider whether I wish to take part in a one-time only follow-up interview.
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CONSENT FORM

VERSION NUMBER 2 DATE 19/04/2013 REC Reference: 13/LO/0478
### Appendix 3 NART

**NART Marking Sheet**

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2 Do you fail to recognize a place you have visited before?
3 Do you fail to do something you were supposed to do a few minutes later even though it is there in front of you, like take a pill or turn off the kettle?
4 Do you forget something you were told a few minutes before?
5 Do you forget appointments if you are not prompted by someone else or by a reminder such as a calendar or diary?
6 Do you fail to recognize a character in a radio or television show from scene to scene?
7 Do you forget to buy something you planned to buy, like a birthday card, even when you see the shop?
8 Do you fail to recall things that have happened to you in the last few days?
9 Do you repeat the same story to the same person on different occasions?
10 Do you intend to take something with you, before leaving a room or going out, but minutes later leave it behind, even though it is there in front of you?
11 Do you mislay something that you have just put down, like a magazine or glasses?
12 Do you fail to mention or give something to a visitor that they were asked to pass on?
13 Do you look at something without realizing you have seen it moments before?
14 If you tried to contact a friend or relative who was out, would you forget to try again later?
15 Do you forget what you watched on television the previous day?
16 Do you forget to tell someone something you meant to mention a few minutes ago?