UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE

Clinical and Experimental Sciences

STRESS AND ITS IMPACT ON COGNITION IN MILD COGNITIVE IMPAIRMENT

By Rebecca Sussams

Thesis for the degree of Doctor of Philosophy, April 2017

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF MEDICINE
Clinical Experimental Sciences
Doctor of Philosophy

STRESS AND ITS IMPACT ON COGNITION IN MILD COGNITIVE IMPAIRMENT

By Rebecca Sussams

Participants with amnestic Mild Cognitive Impairment (aMCI) do not inevitably show cognitive decline or convert to Alzheimer's disease (AD) supporting the hypothesis that secondary events are crucial in the conversion process. Research suggests that psychological stress is a risk factor for AD. Therefore, we proposed psychological stress will be associated with worsened cognitive decline, a clinical marker of advancing neurodegeneration.

This was a longitudinal observational study assessing the association between the degree of psychological stress and cognitive decline in 134 aMCI participants and 69 control participants. We hypothesised that stress, as measured by the Recent Life Change Questionnaire (RLCQ), would be associated with worsened cognitive decline, as measured by the Free and Cued Selective Reminding Test with Immediate Recall (FCSRT-IR), over an 18 month follow-up period. Other secondary cognitive outcomes included the difference in change of the Montreal Cognitive Assessment score and the Trail Making Test Part B. Exploratory measures of stress included the Perceived Stress Scale and the presence of physical stressors. Hypothesised modulators of the stress response were assessed including mood, neuroticism, social support, and favoured coping style. Biological outcomes included changes in blood levels of inflammatory markers and salivary cortisol.

Objective stressful life events occurring during the course of the study were associated with increased rates of cognitive decline across a range of measures in the aMCI group. Whereas, as predicted, psychological stress was not associated with cognitive decline in the control group. Presence of the ApoE ε4 allele was associated with an increased rate of cognitive decline and increased serum levels of the anti-inflammatory cytokine TGFβ was associated with a slower rate of cognitive decline in the aMCI group. We found that neither measures of mood nor potential modulators of stress exerted a consistent significant influence over rates of cognitive decline in the aMCI group.

List of Contents

List of Tables

List of Figures

UNIVERSITY OF Southampton

Academic Thesis: Declaration of Authorship

I, Rebecca Sussams declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

Stress and its impact on cognition in Mild Cognitive Impairment

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. Either none of this work has been published before submission, or parts of this work have been published as: [please list references below]:

Sussams, R., Schlotz, W., Perry, H., Hopkins, V., Davies, L., Rayner, C., Lewzey, I., Christodoulou, A., MacFarlene, B., Sharples, R., Holmes. 'Systemic Inflammatory Responses to Stress and Its Impact on Cognition in People With Mild Cognitive Impairment' in Alzheimer's Association International Conference. 2013 July 17; Boston, United States. p28.

Sussams, R., Schlotz, W., Perry, H., Holmes. 'Systemic Inflammatory Responses to Stress and Its Impact on Cognition in Subjects With Mild Cognitive Impairment' in PNIRS 20th annual scientific meeting 2013 June 5; Stockholm, Sweden. Abstract 1132.

Signed: .....

Date: .....

Acknowledgements

I would like to express my sincere gratitude to my supervisor, Professor Clive Holmes whose expertise, ongoing support and mentoring has made this PhD achievable. Thank you for keeping me going.

Besides my supervisor, many thanks to Dr Wolff Schlotz for his contributions including guidance with the cortisol sampling procedures and analysis. A further thanks to David Culliford for his guidance with the statistical analysis and to Dr Laurie Lau for his contributions to the cytokine analysis.

I gratefully acknowledge the Alzheimer's Society for funding the study and for allowing me the honour to work with them in this important area of research.

I would further like to express my appreciation to Dr Richard Sharples for his time in clinic and ongoing support. Likewise, I would like to thank Dr Zoe Clough, Sharon Simpson, Janet Craven, Rebecca Weekes, Amelia Abbott and the large group of appreciated colleagues at the Memory Assessment and Research Centre for their valuable contributions.

I am indebted to all the research participants who generously committed their time and effort to participate in this research and made coordinating the study a pleasure.

A very special thank you goes to my family including my brother Brendon as well as Alex, Mibette, Katie, Martine, Avril, and Michael for their never ending encouragement. I am especially grateful to my parents for their time, ongoing support and commitment in helping me through school and teaching me the value of education along the way. Sincere thanks also goes to my friends including Naomi, Kelly H, Hannah, Katy and Debs for always being there. Last but not least, I would like to say a heartfelt thank you to my beautiful daughter, Florence, and to my kind husband, Adam Croucher. Without their precious and continuous support this would not have been possible.

Contribution

I enjoyed assisting Professor Clive Holmes in forming the successful funding bid of £310,000 from the Alzheimer's Society. I subsequently developed the study protocol, Patient Information Sheets and consent forms, study standard operational procedures, reporting forms, and the site files. I further processed and received central ethical approval, liaised with R&D teams at a local level, managed contracting, and led on other study related start-up procedures.

In order to deliver the study on time and to target I identified, set up, trained, and monitored three NHS sites based across Wessex whilst ensuring protocol adherence. At site 1, I performed a full range of study related activities including recruitment, liaising with involved professionals, taking informed consent, administering rater scales, collecting and processing biological samples, and coordinating clinic. I was also the first point of contact for patients and study research personnel.

Upon completing the study, I performed study close down visits at each site and archived study files and related materials. I ensured all aMCI participants, and involved professionals including the General Practitioner, received a detailed report regarding the participant's cognitive performance during the study's follow-up period. Finally, I oversaw data inputting and performed statistical analyses of the data alongside Professor Clive Holmes.

Definitions and Abbreviations

Abbreviation	Definition
ACTH	Adrenocorticotropic hormone
aMCI	amnestic Mild Cognitive Impairment
AD	Alzheimer's disease
APC	Antigen Presenting Cell
ApoE	Apolipoprotein ε
ASIE	Acute Systemic Inflammatory Event
CISS	Coping Inventory of Stressful Situations
CNS	Central Nervous System
CSSS	Chronic Stress Screening Scale
CRH	Corticotropin-releasing hormone
CRP	C-Reactive Protein
DNA	Deoxyribonucleic Acid
EQ-5D	Euro-QoL-5 Domain
FCSRT-IR	Free and Cued Selective Reminding Test with Immediate Recall
GDS	Geriatric Depression Scale
HPA axis	Hypothalamic-Pituitary-Adrenal axis
L	Litre
IFNγ	Interferon gamma
IL1	Interleukin 1
IL4	Interleukin 4
IL6	Interleukin 6
IL8	Interleukin 8

IL10	Interleukin 10
IL13	Interleukin 13
MCI	Mild Cognitive Impairment
MMRM	Mixed Modelling Repeated Measures
MOS-SSS	The Medical Outcomes Study Social Support Survey
MOCA	Montreal Cognitive Assessment
NEO-FFI	NEO-Five Factor Inventory
pg	Picogram
PSS	Perceived Stress Scale
PTSD	Post Traumatic Stress Disorder
PVN	Paraventricular nucleus
RLCQ	Recent Life Change Questionnaire
TH1	T Helper 1
TH2	T Helper 2
TGFβ	Transforming Growth Factor beta
TMT	Trail Making Test
TNF α	Tumour Necrosis Factor alpha
VP	Vasopressin
µl	Microlitre

Chapter 1: Introduction

1.1 Definition of MCI

It is widely recognised that a clinical phase prior to dementia exists where individuals experience gradual cognitive decline. This acknowledgement has led to the concept of Mild Cognitive Impairment (MCI) [1, 2]. MCI has been defined as a transitional period between normal aging and dementia, in which a person demonstrates cognitive decline that is not typical for age [2-4]. The National Institute on Aging and the Alzheimer's Association outlines the core criteria for MCI, which requires concern over a change in cognition, impairment in one or more cognitive domains, preservation of independence in functional abilities, and that the individual is not demented [1].

1.2 The relationship between MCI and AD

Research shows those diagnosed with MCI are at a greater risk of converting to dementia [5], with Alzheimer's disease (AD) being the most common cause for converting from MCI to dementia [6]. In general, those diagnosed with MCI develop dementia at a rate of 10-15% per year, whereas the conversion rate for cognitively intact individuals of the same age is only 1-2% per year [4]. However, the MCI population is a heterogeneous group that can differ in both clinical presentation and disease pathology. Subtyping of MCI has helped to reduce this heterogeneity although notably, these subtypes are still debated. Subtyping by aetiology has been widely considered including the criterion for amnestic Mild Cognitive Impairment (aMCI), which was developed by Petersen *et al* in an effort to encapsulate the symptomatic preclinical phase of AD [2, 4]. The authors proposed that those individuals with aMCI primarily show an impairment in episodic memory without the additional

clinical features of AD [2]. Indeed, higher conversion rates from MCI to AD are observed in those characterised as aMCI [7]. The National Institute on Aging and the Alzheimer's Association refer to this state as "MCI due to AD" identifying those who are symptomatic but not demented as a result of accumulating AD neuropathology [1]. Memory impairment is observed in both aMCI and AD however, in AD there is typically a greater number of cognitive domains that are impaired and the activities of daily living and functional abilities are also affected.

Little is known about the direct and indirect costs associated with MCI and the financial burden to society. One study in the USA revealed the average annual medical cost per person was estimated to be substantially higher for those diagnosed with MCI ($6,499) than compared to cognitively intact individuals ($2,969). Increased costs for doctor visits and hospitalisation, and a greater number of prescriptions, were some of the reasons documented for this observation in MCI persons. Overall, the study revealed that medical costs were 44% higher for those diagnosed with MCI [8]. However, the prevalence and economic burden of dementia is far better understood. It has been estimated that 36 million people have dementia worldwide and that this number will double by 2030 due to the phenomenon of an aging population [9]. In the UK, it is predicted that by 2025 there will be over 1 million people living with dementia. Currently, the cost to the UK economy is around £26 billion a year working out at £32,250 per person diagnosed with dementia [10]. Understandably, dementia is a major challenge to health care systems worldwide.

Similar to financial burden, caregiver burden in dementia is well documented however, less is known about the effects of caring for a person with MCI. A recent study found caregiver burden correlated with the different stages of AD, showing burden in mild AD was reported as

more severe than compared to caring for persons with aMCI [11]. Another study found family caregivers spent on average 9 hours a day caring for a loved one with dementia compared to 4 hours caring for those with MCI. Moreover, 44% of dementia caregivers in this study presented with depressive symptoms compared to 27% of those providing care to a person with cognitive impairment [12].

1.3 Prevalence of MCI

Several epidemiological studies have tracked large population cohorts to better identify prevalence of MCI. The Sydney Memory and Ageing Study found in 1037 non-demented community-dwelling participants, aged between 70 to 90 years of age, the overall prevalence of MCI at baseline was 36.7% [13]. However, an examination of the literature suggests that prevalence rates of MCI vary greatly. For instance, a nation-wide survey in South Korea, of those aged 65 or older, revealed a 24.1% rate with aMCI being the most common subtype (20.1%) [14]. In an Italian cohort study of 2,337 people aged over 65, a 21.6% MCI prevalence rate was identified with 63.2% diagnosed with aMCI [15]. Whereas, in the USA the Mayo Clinic Study of Aging found among 1,969 participants, aged 70 to 89 years, a 16% prevalence rate with only 11.1% fitting the Petersen criteria for aMCI [16]. In Germany, Busse and colleagues found in a longitudinal cohort of 1045 participants, aged 75 and over, a prevalence rate ranging between 3 to 20% depending on the MCI criterion used [17]. Thus there is suggestion that prevalence variance partly depends on the operational criteria used.

1.3.1 Conversion rates in different populations

Large scale epidemiological studies further show there is a higher conversion rate from MCI to AD when research is based in a memory clinic

setting than compared to studies based in the community. For instance, the annual conversion rate from MCI to dementia has been estimated at 4.2% for community based research [18] whereas in a clinical setting the conversion rate typically lies between 10-15% [19]. Clinic based research may benefit from greater access to medical records including neurological examinations, imaging results, and psychiatric history. Therefore, the validity of a MCI diagnosis made in a clinical research setting may be more accurate due to the diagnosis characteristically being made by a trained clinician drawing on several lines of evidence. Furthermore, those who seek memory services may demonstrate a more persistent or prominent memory complaint than compared to those seeking no support in the community.

1.3.2 Demographic risk factors

Longitudinal cohort studies are helpful in identifying risk factors that influence conversion rates from MCI to AD and illuminating protective mechanisms that may delay conversion. Aging is a well-established aetiological factor for the onset of AD [14, 16, 17], with prevalence rates doubling every 5 years after the age of 60 [20, 21]. Another well documented risk factor for both MCI and AD is a history of depression, as evidenced by two recent meta-analysis studies [22, 23]. In particular, research suggests a history of depression in men increases the risk of AD [24, 25]. Furthermore, a history of depression has also been shown to increase the likelihood of conversion from MCI to dementia [26].

A broad range of other factors increase the risk of cognitive decline, MCI and AD in older age including hippocampal atrophy [27], and key vascular risk factors such as smoking [14, 28, 29], diabetes mellitus [28, 30, 31], high blood pressure [31-34], atherosclerosis [35], high cholesterol [31],

stroke [32, 36, 37] and heart disease [38, 39]. Higher conversion rates from MCI to AD have further been observed in those showing evidence of in vivo brain amyloid load on imaging, a well-known hallmark of AD [40] as well as in those carrying the ApoE ε4 allele [5, 13, 41, 42], an allele found in approximately 16 % of the general population [43]. However, it is important to highlight that those who are homozygous for ε4 do not always convert to AD, with approximately 50% still dementia free by the age of 90 [41].

Interestingly, both meta-analysis and large-scale studies identify gender as a risk factor, with an increased likelihood of developing MCI if you are male whereas being female increases the risk of AD [13, 16, 21, 26, 44]. For instance, among a cohort of 1,969 Olmsted County residents, aged 70 to 89 years, older men were at greater risk of MCI. These findings remained unchanged after controlling for a range of demographic and clinical variables [16]. However, caution should be applied when interpreting findings due to other studies showing being female increases the risk of MCI [45, 46]. The reason for these conflicting findings remain unclear, highlighting the need for further research to better understand such variability. However, differing diagnostic criteria, neuropsychiatric assessments, and sampling strategies may partially account for the variance seen.

It has long been documented that women are at a significantly increased risk of AD than men [21]. The gender difference may in part be attributed to women living longer than men, and more likely to report diabetes, a known dementia risk factor [47]. However, recent findings also reveal women are two times more likely to develop AD when carrying the ApoE ε4 gene variant than compared to male carriers, who only showed a marginal increased risk of AD [48]. Similarly, mid-life hypertension in women, but

not in men, is associated with an increased risk of dementia [49]. Of interest, the Women's Health Initiative Memory study found in women aged 65 years and over, those receiving HRT experienced worse cognition and an increased risk of AD in the future [50]. Overall, findings suggest there are gender specific risk factors for MCI and dementia.

Whilst understanding risk factors is important, identifying possible protective factors could potentially prolong healthy living free of cognitive decline and dementia. Alcohol has been shown to possibly exert a protective influence [51], alongside a more physically active lifestyle [52, 53] being married [54], and having a Mediterranean type diet [55, 56]. Notably, longitudinal cohort studies show those diagnosed with aMCI do not inevitably demonstrate cognitive decline or convert to AD [5, 40]. Findings such as these support the hypothesis that secondary events are crucial in the conversion process.

Psychosocial risk factors are emerging from the literature as significant predictors of cognitive decline including a low level of education [14, 44, 57], social isolation [16, 58], and a personality prone to experience distress [59]. Psychosocial risk factors could be considered attractive as they can potentially be modified without the need for costly pharmaceutical intervention. Recent research from the Alzheimer's Research UK, featuring analyses from the Office of Health Economics, reveal that delaying the onset of dementia by five years would reduce dementia cases by a third and save the economy £21 billion by 2050 [60]. By identifying and managing modifiable risk factors it may be possible to promote neuro-protection and consequently postpone dementia onset.

Psychological stress is starting to emerge as a potential risk factor for cognitive decline in aMCI patients, onset of AD, and overall AD progression [61-63]. It is known that the experience of psychological stress initiates a complex multi-system physiological response, principally involving the Hypothalamic-Pituitary-Adrenal axis (HPA axis) and the immune system. These systems are implicated in AD pathogenesis and will be discussed in more detail later on.

1.4 Underlying biology of MCI and AD

1.4.1 Cortical pathology in MCI and AD

There are well-known pathological changes associated with the development of AD including the presence of extracellular deposits of cortical amyloid plaques and intracellular neurofibrillary tangles (NFT) [64-66], which are associated with one another. In concert with these key pathological changes is atrophy of certain brain areas most prominently observed in the hippocampus [67], a decrease in synaptic plasticity [64, 68, 69], a reduction in acetylcholine levels of the cholinergic system [70], and widespread neuro-inflammation [64]. Accumulating evidence suggests systemic and central inflammation plays a significant role in the development and progression of AD. Neuro-inflammatory pathology found includes activated microglia, the presence of complement proteins, up-regulated inflammatory signalling, and increased pro-inflammatory cytokine expression associated with Aβ plaques and NFT [66, 68, 71-77].

The cortical pathology of MCI has been less well defined but imaging and post-mortem studies suggest MCI to be an intermediate stage between normal aging and AD. For instance, amyloid burden has been quantified as 84% in AD patients, 45% in MCI patients, and 23% in cognitively intact controls [78]. Research further shows a stepwise reduction in hippocampal volume from healthy aging, MCI to AD [79] and that hippocampal atrophy steadily increases during the conversion process from MCI to AD [80-83]. Moreover, patients with MCI show significantly increased tau protein in plasma levels compared to that of controls [84] but still demonstrate significantly lower CSF p-tau levels compared to patients with AD [85]. Furthermore, neurofibrillary pathology found in the entorhinal cortex, hippocampus, and amygdala of MCI patients is shown to increase during the conversion process from MCI to AD [86]. As in AD, markers of

increased inflammation have also been found in MCI but not in cognitively intact control participants [87-92].

1.4.2 Endocrine dysregulation in MCI and AD

Numerous studies have documented an association between HPA axis dysregulation and cognitive impairment. For instance, elevated cortisol levels have been found in the presence of long-term cognitive impairment in older participants [93-98]. Furthermore, a study involving 1140 community dwelling adults found those exhibiting elevated cortisol levels performed worse across a range of cognitive domains. This association remained significant after the authors controlled for depression, age, educational status, and medical conditions such as stroke history, diabetes, and hypertension [28, 97]. The same authors further found a gene-cortisol interaction with the presence of one or more ApoE ε4 alleles leading to a stronger association between cortisol and worse cognitive performance [97]. However, the question of whether a dysregulation of the HPA axis is causal or a consequence of neurodegenerative processes can clearly not be clarified by studying the general population alone.

In a recent study, increased cortisol levels were associated with MCI [99] and worse cognitive performance in those diagnosed with MCI [100]. In AD, a number of clinical studies suggest a hyper-secretion of cortisol [101-103] that seems exacerbated by the presence of ApoE ε4 [104-106] and is accompanied by accelerated cognitive decline [107, 108]. A longitudinal study following 51 healthy participants for 5-6 years, annually measuring cortisol levels and performing MRI scans, found those who experienced increased cortisol exposure were at increased risk of reduced hippocampal volume over time [95]. A recent study demonstrated those MCI participants (of AD type) who presented with higher CSF cortisol levels at

baseline were more likely to cognitively and clinically decline [109]. Thus, it seems plausible that elevated cortisol exposure, due to HPA dysregulation, may potentiate MCI and AD progression providing partial support for the glucocorticoid cascade theory [110].

The glucocorticoid cascade theory postulates hippocampal atrophy, initiated by the pathological processes associated with AD, dysregulates negative feedback control of the HPA axis. This leads to hypercortisolemia which becomes neurotoxic and further contributes to neurodegeneration that subsequently accentuates HPA axis dysregulation. Indeed, HPA axis dysregulation has been observed in a small but interesting study using dexamethasone (DEX) administration in MCI participants. The administration of DEX should lead to the suppression of cortisol production however, those with MCI demonstrated significantly increased cortisol levels after 0.5mg DEX administration in comparison to the control group [111]. The authors concluded that MCI participants demonstrated normal basal cortisol levels but a dysregulated HPA axis feedback. However, findings from other investigations into the relationship between cortisol and cognition have largely been inconsistent [61] dampening support for the glucocorticoid cascade theory. For instance, several studies assessing cortisol levels in control, MCI and AD populations have found elevated levels present only in AD participants [105, 112]. Although the cause underlying this observed difference in cortisol exposure is unknown, results such as these have led some to suggest that HPA axis dysregulation is an end result of AD pathological processes, not casual in the conversion of MCI to AD.

However, results from animal models suggest that hypersecretion of glucocorticoids do play a role in the development or maintenance of AD. Rodent experiments show the equivalent administration of stress-levels of

glucocorticoids result in subsequent increased Aβ formation and an augmentation of Tau accumulation. The authors concluded elevated glucocorticoid exposure accelerated neurofibrillary tangles (Green, Billings et al. 2006). Data from other studies generate similar findings, showing stressful conditions and glucocorticoid exposure, both independently and in combination with one another, induce hyperphosphorylation of Tau in the hippocampus and prefrontal cortex [113, 114]. The administration of glucocorticoids in combination with a stress condition has also been shown to drive Aβ production [115]. Therefore, drawing on clinical and animal model data, the question of whether HPA axis dysregulation is causal, a failing protective mechanism against increasing pathological neuroinflammation, a neurotoxic contributor, or simply a result of neurodegenerative processes remains inconclusive [105]. Overall, it is plausible to suggest that a dysregulated neuroendocrine system paired with mounting AD pathology may result in a neurotoxic pro-inflammatory environment.

1.4.3 Systemic inflammation in MCI and AD

Under normal conditions, acute inflammation is a protective response that facilitates healing and recovery. However, accumulating evidence links systemic inflammation, and inflammation in general, over time with increased risk of cognitive decline and AD [116]. A well supported hypothesis postulates that neurodegeneration is caused by or exacerbated by the over-activation of the Central Nervous System (CNS) resident macrophages, called microglial cells, which form the brain's innate immune response. Under normal conditions, these cells constantly scavenge for plaques and damaged neurons quickly responding to any homeostatic challenges [117, 118]. Following an immune challenge, microglial cells transform from a rested state into a morphologically activated form leading to the increased synthesis of potentially neurotoxic

molecules including pro-inflammatory cytokines [118, 119]. This up-regulated expression of pro-inflammatory cytokines leads to increased reactive oxygen species levels and subsequent neuronal cell death [116, 117]. Importantly, this inflammatory response is short lived and controlled by a range of regulatory mechanisms to prevent unnecessary neuronal damage [120]. However, from early on in AD, microglia cells are proposed to exist in a persistently primed state where they are partially activated. A breadth of research demonstrates increased markers of microglial activation in MCI and AD participants [66, 121-125].

However, CNS innate immunity should not be considered in isolation with a number of plausible pathways proposed that would potentially allow a systemic inflammatory event to stimulate a CNS inflammatory response [126]. Thus, new systemic inflammatory insults could act as a secondary trigger that rapidly activate primed microglia in the CNS [116, 117, 126]. This in turn leads to the exaggerated release of pro-inflammatory cytokines. Elevated levels of pro-inflammatory cytokines would consequently exacerbate the existing neurotoxic environment in the AD brain leading to further oxidative neuronal cell damage [117]. It is suggested that potentially low levels of inflammation may be sufficient to trigger primed microglia in this way [127].

1.4.3.1 Clinical studies

In an effort to examine the link between cognition and inflammation extensive research has studied how inflammatory markers relate to MCI and AD. A number of studies have shown that elevated levels of pro-inflammatory cytokines in the blood and CSF, including IL1β, IL6, IL8, CRP, TNFα, and IFNγ, are associated with worse cognitive functioning in older people [128-133], alongside an increased risk of MCI [13, 89-92] and AD [39, 90, 92, 134-143]. However, some studies, in contrast, have found no

or mixed differences between AD participants and control groups [144, 145]. A recent meta-analysis has concluded that AD is accompanied by increased periphery concentrations of inflammatory markers including IL6, TNFα, and IL1β [138] although it should be noted, that cross-sectional data is limited and cannot confirm whether this is cause or effect. More persuasive support is drawn from longitudinal studies (discussed later) which suggest a pro-inflammatory phenotype and activated signalling system is associated with increased risk of developing both MCI and dementia later in life [71, 146-151] and can predict conversion from MCI to AD several years before [89, 152, 153].

1.4.3.2 Autopsy studies

Further support indicating an association between inflammation and AD pathology comes from autopsy research, which shows a direct link between AD pathological hallmarks and neuroinflammation. This includes activated microglia in reaction to Aβ deposits [72, 123], activated microglial in neuritic plaques [121], membrane attack complex (C5b-9) immunoreactivity to plaques and NFTs [64], IL18 RNA expression in association with plaques [154] and increased IL1β production in key brain regions including the hippocampus [122]. Increased MHC II molecule expression has also been associated with early AD that inversely correlated with cognitive testing [73].

1.4.3.3 Imaging studies

Evidence drawn from imaging research further suggests an association between increased inflammation and neurodegeneration. PET imaging studies have shown microglial activation in MCI participants [40] and in those with AD [155]. In an AD group of participants, a 20-35% increase in microglial activation was inversely correlated with MMSE scores and

associated with a two-fold increase in amyloid load [66]. A much larger MRI study of 350 participants found IL6 and TNFα to inversely correlate with whole brain volume as well as the entorhinal cortex and ventricular volume [125]. Findings such as these are echoed in similar research showing a marked association between activated microglia and AD pathology [124].

1.4.3.4 Genetic studies

Genetic studies link an established risk gene for MCI and AD, ApoE ε4 [13, 156, 157], to the spontaneous and induced pro-inflammatory cytokine IL1β production [158]. In addition, the presence of other common genetic variations, such as IL1A allele and polymorphisms for pro-inflammatory cytokines IL1, TNFα, and IL6, have been associated with a dose-dependent risk of both early and late onset AD [159-161]. Finally, convincing data has now come from large genome wide association studies which have identified rare (with large effect) and common (with small effect) gene variants in a range of immune genes including TREM2, CR1 and clusterin as risk factors for AD [162-164].

Summary

In conclusion, there are multiple avenues of research that strongly suggest the important role of systemic inflammation in the development of MCI and AD, and that systemic and CNS inflammation should be considered together. However, the specific role that inflammation plays seems complex and not fully understood. For instance, recent studies implicate varying inflammatory biomarkers in the different types and stages of dementia [165, 166] and pro-inflammatory cytokines associated with the development of AD have at times been discordant [129, 130, 152, 166-170]. Although well-known methodological errors may partially account for

the considerable variance observed, further research is required to clarify whether the inflammation is cause or effect.

1.5 The human stress response

There is a longstanding debate surrounding the definition of stress. Definitions that incorporate both physical and psychological stress are arguably the most helpful. Physical stress has been defined as a physiological challenge to homeostasis whereas, psychological stress has been defined as the perception of a challenge to homeostasis [171]. The stressor is defined as the stressful event itself, such as being a victim of crime, whereas the term 'stress' is the body's physiological response to the stressor [172]. The effect of stress on health outcomes has attracted considerable attention. Overall, the stress response is recognised as a life promoting mechanism whilst simultaneously possessing the potential to exert adverse effects on health. A distinction between acute and chronic stress may help determine when the effects of stress cease being beneficial and instead becomes harmful to health. Acute stress is proposed to continue for a matter of minutes or hours whereas chronic stress is suggested to remain over weeks, months, or potentially years [173].

1.5.1 Acute stress

Walter Cannon was one of the earliest researchers attempting to define stress and introduced the concept of homeostasis. Cannon proposed that acute stress is a necessary state enabling the body to successfully adapt to a changing environment that could potentially threaten homeostasis [174]. As part of this process, the brain appraises each potential threat and subsequently engages relevant neural networks and physiological systems. Physiological adaptation such as this enables an optimal physiological

state for situations that require a 'fight or flight' reaction [174]. Shortly after, Hans Selye [172] proposed the General Adaption Syndrome (GAS). GAS emphasised stressors to provoke a life-promoting non-specific physiological response. Therefore, all stressors initially activate the immune and endocrine system in the same way. Selye was one of the first to suggest that exposure to chronic stress caused long-term adaptive changes resulting in exhaustion and disease. Dhabhar and McEwen's more recent model also views acute stress to be beneficial, ultimately promoting survival, whereas exposure to chronic stress is proposed to be potentially harmful. However, Dhabhar and McEwen challenged Seyle's theory suggesting stressors do not provoke a non-specific physiological response but rather, initiates bidirectional effects on immunity including simultaneous immunosuppression and inflammation depending on the type of stressor [175, 176]. For instance, the acute stress response thought to be beneficial is also proposed to potentially exacerbate existing autoimmune and inflammatory disorders [177].

Dhabhar and McEwen propose that during acute stress the body prepares the cardiovascular, musculoskeletal, neuroendocrine, and immune system for either a 'fight or flight' response [176, 178]. This response includes the production of pro-inflammatory cytokines [176, 179], an immunoenhancing response thought to prepare the body to challenges (wounding or infection) that are likely to occur from a stressful encounter [176]. During acute stress this pro-inflammatory response can be further enhanced by the presence of cortisol [175, 178, 180, 181]. However, if stress is prolonged and becomes chronic then, as in diseases such as rheumatoid arthritis, a continuation of the pro-inflammatory response is likely to be harmful [182]. A meta-analysis spanning across 30 years supports the proposed association between the experience of a stressor and a pro-inflammatory response [179]. Additionally, the

immunosuppressive effects of stress have also been well documented [183, 184].

The goal to restore homeostasis following acute stress [174] generated the complimentary concept termed allostasis. Allostasis is a multi-pathway process first introduced by Sterling and Eyer [185] that allows the body to quickly mount a response to physiological, environmental or psychological challenges. During allostasis, physiological concentrations of mediators including glucocorticoids and cytokines change in order to allow the body to successfully adapt to stress [186, 187]. Overall, the goal of allostasis is to maintain constancy by temporarily changing the body's set physiological parameters [185, 188]. Once the threat has passed this response is immediately terminated returning the body to a pre-determined homeostatic equilibrium. In essence, allostasis enables the body to quickly and successfully respond to adverse events for a temporary period of time.

1.5.2 Chronic stress

A chronic stressor can take many forms including bereavement, caregiving for a loved one, childhood abuse, and ongoing job strain. Theory and research suggest that when the allostatic response is forced to persist, for example in response to chronic stress, the body is subjected to prolonged chemical imbalances and elevated physiological states e.g. chronically increased blood pressure [182, 188-190]. Consequently, the adaptive benefit of these initial life promoting changes become outweighed by the cumulative costs of prolonged exposure to stress mediators. Over time, this state is suggested to become harmful to health and is termed 'allostatic load' by McEwen and colleagues (Fig. 1) [182, 186, 189, 190].

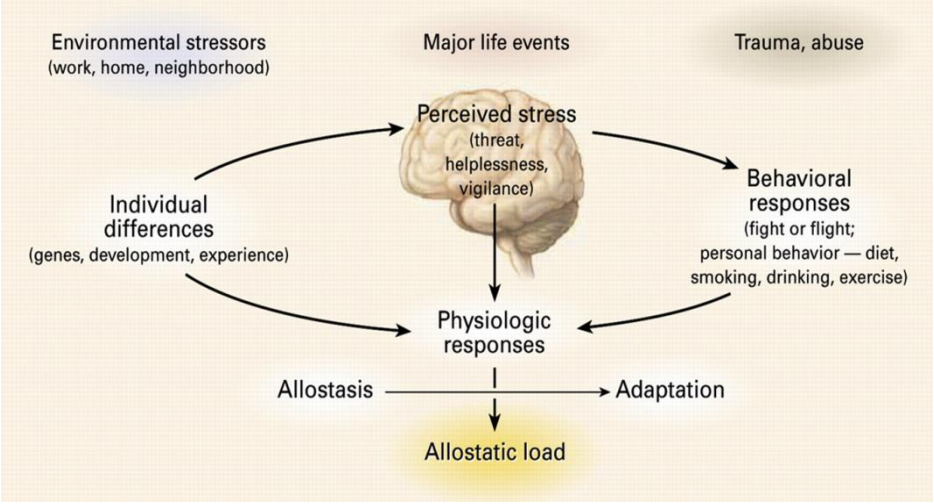


Figure 1. Human physiological stress response

Derived from McEwen 2007 [191], the above diagram demonstrates the dynamic physiological stress response in humans. Central to allostasis is the consideration of individual differences and behaviour modulating adaptation and the potential allostatic load state.

Research findings tentatively reveal an association between chronic stress and negative health outcomes [175, 192]. For instance, chronic stress is an identified risk factor for coronary heart disease [193], cardiovascular disease [194-197], diabetes [194], atherosclerosis [198, 199], and reactivation of herpes virus [200].

1.6 Modulators of the stress response

Individual characteristics and health related behaviours are suggested to influence allostatic load and should be considered [188]. A range of vulnerability factors have been studied to identify which may significantly influence interpretation of stressful events and subsequently increase the risk of adverse health outcomes [190]. Overall, findings suggest factors including personality, coping style and social support are important mediators in determining the duration and magnitude of the physiological stress response [175, 191].

1.6.1 Personality

Extensive research has identified a relationship between personality and our experience of stress at several levels. For instance, personality may alter the type of coping strategies chosen and influence the physiological processes occurring between appraisal of events and subsequent response [201-205]. In particular, the personality trait 'neuroticism' has been linked with individual proneness to experience distress [205]. Those who are more neurotic react to stress with more anger and depression [203], display stronger reactions to recurrent problems [201], and report experiencing stressful life events more frequently [205] than compared to those scoring low in the trait. Neuroticism has been associated with negative health outcomes including increased risk of death from cardiovascular disease [206]. Therefore, it is feasible to assume that those with a neurotic personality style may be subjected to prolonged levels of stress hormones. Indeed, neuroticism has been associated with higher concentrations of cortisol [207-209] and increased IL6 levels [210]. Whereas a more positive affect personality style is associated with lower levels of cortisol and inflammatory markers [211]. However, findings are inconsistent with a recent meta-analyses showing neuroticism was not related to inflammatory markers [212].

Furthermore, distress prone personalities have been linked with a greater risk of memory impairment. For example, cohort studies show neuroticism to be associated with cognitive impairment [213], MCI [214], increased rate of cognitive decline [215], and AD [216]. Several recent meta-analysis provide additional support for a relationship to exist between higher levels of neuroticism and greater risk of cognitive decline and dementia [217-219]. However, it is not yet understood whether distress proneness is an early neuropsychiatric symptom of mounting AD pathology or alternatively, an independent risk factor [216]. Although the association between distress proneness and increased risk of dementia has been

shown to be independent of AD neuropathology [59]. In addition, a cohort study showed the link between neuroticism and cognitive impairment was evident 25 years prior to cognitive impairment [213]. It is unlikely that AD neuropathology would have influenced personality this early on. Altogether, findings tentatively suggest that proneness to life time distress may increase risk of dementia. However, current research provides little insight into the underlying mechanisms responsible for this association.

1.6.2 Social Support

Another factor thought to influence our perception of stress is social support, which refers to the degree in which people have access to resources including relationships and emotional support [220]. A lack of social support is emerging as an important psychosocial modulator of health. For instance, research findings link social isolation with raised inflammatory markers including elevated CRP [221] and greater IL6 levels [222, 223]. Furthermore, loneliness has been associated with other stress mediators including a higher cortisol awakening response [224]. In contrast, increased social support has been linked to lower levels of IL6 and Natural Killer cells [225]. These results provide cautious support that social support influences physiological systems.

The notion that social support may buffer the effects of stress on health outcomes has attracted considerable interest. Surprisingly, few studies have investigated the interaction between social support and risk of dementia. However, early findings tentatively hint that reduced social support may be predictive of cognitive decline and onset of dementia [58, 149]. Dickinson and colleagues further found that a combination of decreased social support and exposure to stress was associated with cognitive decline in older adults over a 1 year follow-up period [226]. In

addition, loneliness has been independently associated with progression from MCI to dementia in 93 MCI patients [227]. More recently, loneliness was identified as a significant risk factor for dementia amongst a cohort of 7867 people in China, 393 of which converted to dementia during a 3 year follow-up period [228]. Such findings suggest further investigation into the impact of social support on AD pathogenesis is warranted.

1.6.3 Coping styles

The concept of coping refers to the cognitive and behavioural efforts used to manage, reduce, or control stress [229]. Three broad dimensions of coping have been proposed that include problem-orientated coping, emotion-orientated coping, and avoidance [230]. Individuals choosing a problem-orientated coping style try to change the situation, often by deciding upon and following a plan of action. Whereas those choosing an emotion-orientated coping style try to adjust their thoughts and feelings in response to the problem. The third coping style is avoidant, which involves the individual trying to evade the problem typically by using distraction techniques. However, there are a number of copying styles proposed and inconsistent findings fuel debates regarding which types of coping style effectively reduce stress.

Nevertheless, there is some agreement that employing emotion-focused coping strategies are more likely to be associated with poorer mental and physical health outcomes, including greater perceived stress and depression [202, 231-235]. Moreover, research looking at the impact of coping in response to stressors on physiological parameters yield significant associations between positive strategies and reduced pro-inflammatory markers [236] and cortisol exposure [237]. However, other studies have shown individual differences should be considered [238] and

in some cases, no interactions have been observed [239]. Therefore, the impact of coping style on health parameters, including those that may act as potential risk factors for dementia, remains inconclusive.

1.7 Biological underpinning of stress response

1.7.1 The HPA axis

The endocrine system plays an important role in maintaining homeostasis. The network between the hypothalamus, the pituitary, and the adrenal gland constitutes the HPA axis, which is a crucial pathway for the stress response [240] (Figure 2). The HPA axis triggers and regulates circulating basal levels of glucocorticoids, primarily cortisol, over a 24 hour period. This circadian rhythm varies, rising at differing time points including just before waking and then subsequently falling during the course of the day [241]. Glucocorticoids perform many actions including suppressing the immune system, altering memory formation and inhibition of bone and muscle growth [242]. Consequently, the HPA self-regulates the secretion of glucocorticoids within narrow limits during this sleep-wake cycle to protect the body from the potentially harmful effects of prolonged chemical imbalances [243, 244].

There are two general operational states of the HPA axis. The first operates under unstressed conditions where basal levels of cortisol are partially permissive in action, preparing the body's homeostatic defence mechanisms for anticipated action such as a stressful event [244]. The second state occurs in reaction to real or perceived stress. The HPA axis activates a cascade of hormones to form the stress response, resulting in elevated glucocorticoid levels [245]. Initially, the hypothalamus receives inputs from the limbic structures including the amygdala and pre-frontal

cortex, which appear to activate the HPA axis in response to psychological and physical stressors [243, 246]. The subsequent HPA response will vary in magnitude and duration depending on the nature and intensity of the stressor [110, 187, 243, 247-250]. Glucocorticoids negatively feedback to terminate this response once the stressor has passed [175].

1.7.1.1 Acute stress and the HPA axis

Acute stress activates the HPA axis via stimulation of neurons in the paraventricular nucleus (PVN) located in the hypothalamus [244] followed by transcription of corticotropin-releasing hormones (CRH) and vasopressin (VP) in the PVN. CRH and VP are subsequently released into the pituitary portal circulation via the median eminence. From here, these peptides travel to corticotrope cells situated in the anterior pituitary gland. CRH then binds with corticotrope cells triggering release of adrenocorticotropic hormones (ACTH) into peripheral circulation. VP potentiates the effects of CRH on ACTH synthesis. Once in circulation, ACTH's main target is the adrenal cortex which is responsible for the subsequent release of glucocorticoids [244, 245].

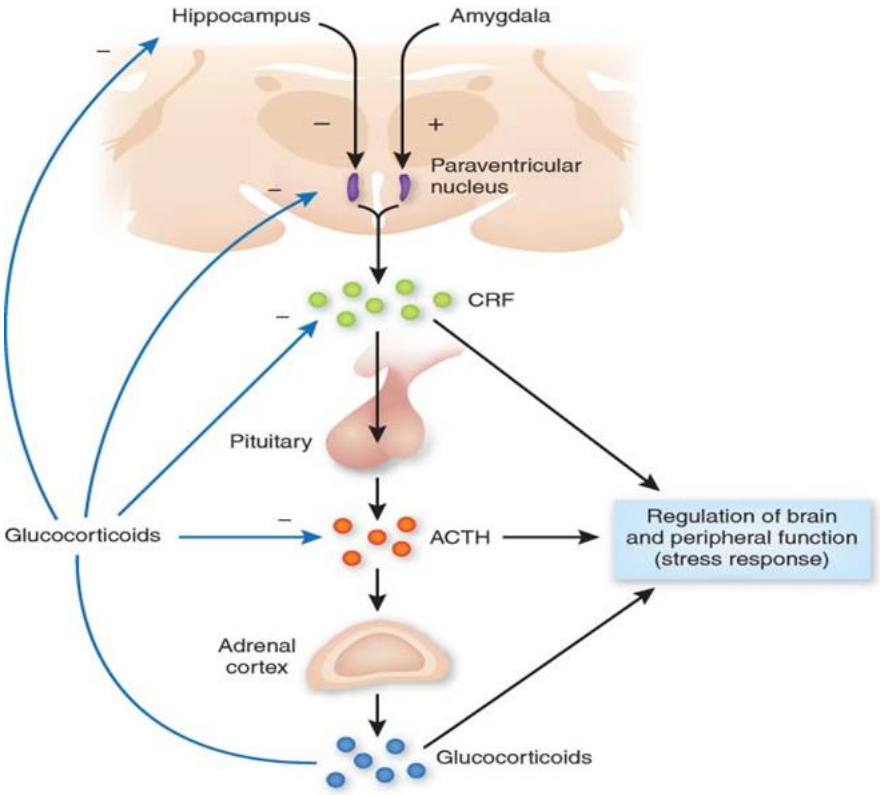


Figure 2. HPA axis in response to stress

The above simplified diagram (derived from Hyman 2009 [248]) demonstrates HPA axis activation during a stress response. Principally, the HPA axis is under the excitatory control of the amygdala and the inhibitory regulation of the hippocampus. Overall, the release of glucocorticoids is coordinated and regulated by the hypothalamus. The hypothalamus coordinates processes falling between the perception of stress and a homeostatic response [176, 182, 190, 251]. Glucocorticoids are proposed to negatively feedback at three different levels of the HPA axis to exert regulatory control.

A large body of research demonstrates that acute psychological stress triggers HPA activation and release of glucocorticoids, principally cortisol [104, 244, 252-258]. Glucocorticoids are proposed to then orchestrate the "fight or flight" response by modulating a range of processes including mobilising energy and raising breathing rate and heart rate. Importantly, glucocorticoids act as a negative feedback loop, via the hippocampus and

other sites, terminating the response once the stressor has passed [243, 247, 248, 251, 257, 259, 260].

1.7.1.2 Chronic stress and the HPA axis

In contrast to acute stress, which is thought to be life promoting, chronic stress is suspected to drive the development of disease [244, 261, 262]. Prolonged and potentially impaired adaptive changes to the endocrine and immune system seem likely to act as the link between stress and disease.

Postnatal and juvenile animal models partially support clinical findings suggesting chronic stress is associated with long-term HPA axis alterations, including a flattened cortisol diurnal rhythm and greater CRH production [263-266]. Moreover, animal models show chronic stress downregulates hippocampal glucocorticoid receptor expression signifying stress modulates structures at a genomic level [263-265, 267-270].

Clinical studies show chronic stress is associated with long-term dysregulation of the HPA axis [271, 272], increased expression of CRH and VP [262, 267], and hypo and hyper-secretion of cortisol [104, 273]. For instance, early life maltreatment has been associated with flattened morning cortisol exposure in mid adult life [274]. Likewise, a large scale longitudinal study, consisting of 1,055 participants aged 63 and above, found late life stress was associated with elevated cortisol secretion in the morning whereas, early life stress was associated with hypo-secretion of morning cortisol [104]. Cohen et al observed lower socioeconomic status, a known chronic stressor, was associated with increased cortisol exposure during the evening [275]. Similarly, adolescents living in deprived conditions exhibit long-term elevated basal glucocorticoid levels in later life [268]. Comparable results were found in an earlier study conducted by

Heim and colleagues in which the experience of childhood maltreatment was associated with a hyperactive HPA axis in response to new stressors [271]. However, a more recent population based longitudinal study based in Switzerland tracking 796 older adults, aged 65 and above, found no association between reported lifetime stress and cortisol measures [98]. Overall, findings are mixed but suggest a differential association may exist between the timing of stressors and long-term HPA axis alterations.

Cortisol and glucocorticoid receptors play an important biological role in regulation the immune system.

1.7.2 The immune system

The immune system orchestrates a response against stress, trauma, debris and pathogens via the combination of an innate and adaptive immune response [276]. The innate immune response includes the migration of phagocytic cells, such as macrophages or microglial cells in the brain, that travel to the site of trauma where they engulf and degrade their target. This generated response is known as inflammatory and also includes the release of messenger molecules released by macrophages and microglia cells called cytokines. The effects of cytokines are broad in nature and can modulate neuronal cell function to either facilitate regeneration or neurodegeneration [277]. Essentially, cytokines are considered as either pro-inflammatory (including IL1, IL6 and TNFα) or as anti-inflammatory (including IL4, IL10, IL13 and TGFβ) [179, 277].

In comparison to the innate immune response, the adaptive immune response takes longer to mount a defence and is characterised by greater specificity. Adaptive immunity requires an antigen to be presented by

antigen presenting cells, such macrophages, before being activated. When activated, there are two divisions of adaptive immunity called humoral and cellular. Humoral (Th2) immunity responds with the secretion of antibodies whilst cellular (Th1) immunity involves the activation of cytotoxic lymphocytes [179]. A range of different cytokines guide and amplify the response with the balance between Th1 and Th2 being tightly regulated in order to maintain homeostasis [175]. When Th1 is the dominant response it suppresses Th2 and vice versa through the release of cytokines and binding of glucocorticoids [259].

1.7.2.1 The immune response to acute stress

The body's inflammatory response to challenge is rapid and initially protective facilitating healing through a range of processes including the mobilisation of immune cells [278]. However, persistent inflammation can become harmful to healthy tissue and is therefore regulated both by the release of anti-inflammatory cytokines and glucocorticoids. Dhabhar and McEwen [176, 178, 190] suggest that secretion of cytokines is fundamental to the initial 'fight or flight' response aiding optimal adaptation to adverse conditions.

According to Dhabhar and McEwen, stress triggers a biphasic response whereby immune suppression can coincide with an inflammatory response depending on a range of determining factors including acute versus chronic stress [118, 179, 279, 280]. In essence, acute stress enhances immunity whereas chronic stress is thought to suppress parts of the immune system. Extensive research documents an upregulation of inflammatory markers including IL6, TNFα, and CRP [179, 281-284], and leukocyte redistribution [178] in response to acute psychological stressors. For instance, elevated IL6 serum levels were observed in a study of 122 healthy young adults who had recently experienced a negative acute social

interaction [285] whilst Dickerson et al found elevated TNFα serum levels in a group of 39 healthy young women who had undergone an acute social stress task [279].

1.7.2.2 The immune response to chronic stress

In contrast to acute stress, chronic stress is proposed to shift the physiological response towards immunosuppression dampening down the original acute pro-inflammatory response. This is a plausible and defensive measure protecting the body from unnecessary harm. For instance, a significant interaction between chronic stress and poor antibody response to virus vaccinations has been documented [183], in addition to susceptibility to upper respiratory infectious disease [184, 286], and lower natural killer cell activity [287]. However, chronic stress has further been associated with a pro-inflammatory state including elevated levels of pro-inflammatory peripheral cytokines [280, 287-291] and an upregulation of CD8+ T cells accompanied by a reduction in Cluster of Differentiation 4 (CD4+) helper T cells [225]. The chronic stress state of PTSD is characterised by HPA axis dysregulation and an upregulated pro-inflammatory status [273, 292, 293]. Overall, findings support the hypothesis that chronic stress induces multiple effects on the immune system that can result in either an immunosuppressive or pro-inflammatory state (see Figure 3).

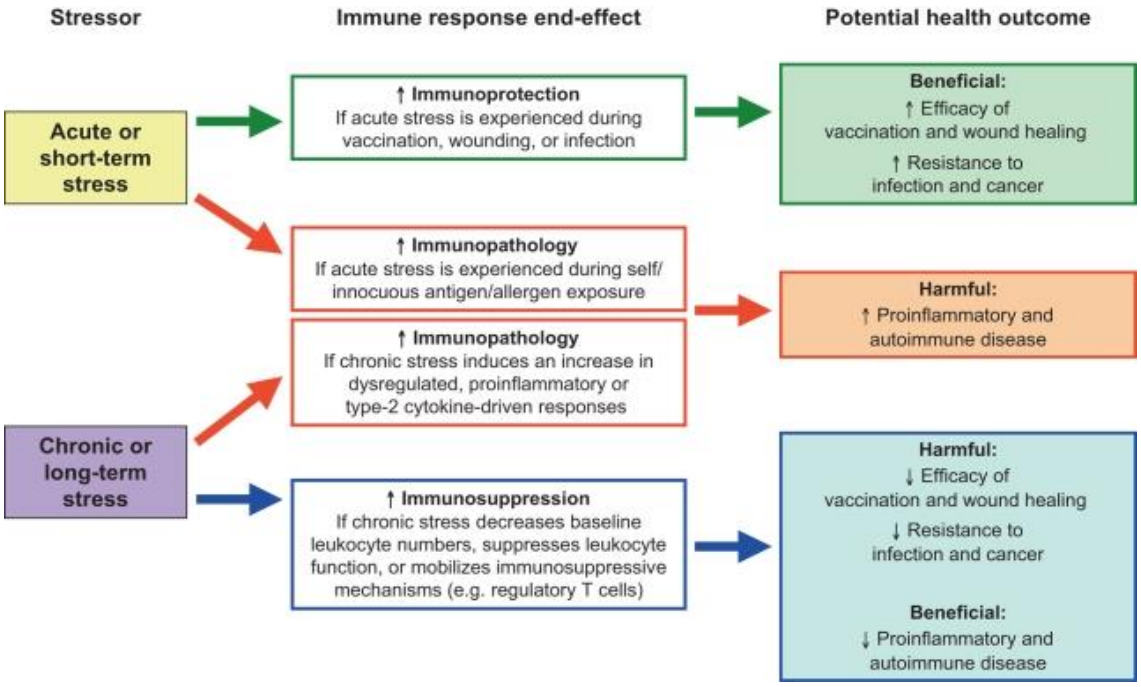


Figure 3. Multi system response to stress

The above diagram (derived from Dhabhar 2009 [175]) depicts the interaction between stress, the immune response and subsequent health outcomes. Dhabhar explains that acute stress enhances immunoprotective responses. However, when acute stress is experienced in combination with existing immune activation, pro-inflammatory or autoimmune disorders may be exacerbated. Thus, chronic stress is proposed to result in an anti-inflammatory or pro-inflammatory response that can exacerbate inflammatory and autoimmune diseases.

Comparable to neuroendocrine research findings, early life adversity is associated with disturbed immune functioning later in life. For instance, self-reports of childhood sexual abuse has been significantly associated with raised plasma levels of CRP and IL6 during adulthood. The association remained significant after adjustment for potential confounders including smoking and BMI [294]. Likewise, a birth cohort study identified those who had experienced maltreatment during childhood were at a significantly increased risk of raised CRP levels 20 years later [289]. This effect was independent of stress during adulthood, health status and health

behaviours. Another large study following 15,357 participants found that those reporting childhood trauma between 1996 and 1997 were at significantly increased risk of an autoimmune disease in 2005. The likelihood of hospitalisation also increased with the accumulative number of childhood traumatic events recorded [177]. Moreover, Gouin and colleagues found IL6 to be amplified in 130 adults who reported a history of child abuse in response to daily stressors [280]. The Emory Twin study consisting of 482 individuals also found an association between early life trauma and raised CRP levels during adulthood. However, this association was largely explained by familial factors suggesting that the family environment may act as a mediator increasing the risk of both trauma and an inflammatory phenotype later in life [295]. However, overall, research suggests that chronic stress can alter the microenvironment towards a pro-inflammatory state [118].

1.7.3 Bidirectional communication between the HPA axis and Immune system

As previously discussed, stressful events stimulate both the immune system and the HPA axis resulting in the release of cytokines and glucocorticoids. Bidirectional communication exists between the systems to ensure each system responds proportionately to challenges [118, 190, 259] (Figure 4). Physiologic concentrations of glucocorticoids are well known to negatively regulate the pro-inflammatory immune response to prevent overreaction [190, 259, 296], potentiate cytokine expression of acute phase proteins [297], upregulate pro-inflammatory cytokine receptor expression [298], promote either a Th1 or Th2 response [245], and influence levels of inflammatory cytokines 10 years later [299]. In the brain, CRH has been shown to bind to microglia and subsequently modulate neuroinflammation [300], increase TNFα expression, and promote proliferation of microglia [301, 302].

On the other hand, the immune system stimulates the HPA axis through secretion of pro-inflammatory cytokines [303] with cytokine receptors identified at all levels of the HPA axis (Silverman, Pearce et al. 2005). More specifically, IL1, IL6, TNFα, IFNγ, IL2 have all demonstrated an ability to stimulate the HPA axis in both animals and humans [259, 304] and cytokine levels are shown to impact upon glucocorticoid receptor expression [298]. Thus, it seems that the HPA axis and immune system form a negative feedback loop whereby the immune system stimulates the HPA axis that in turn releases glucocorticoids that subsequently dampens down the initial immune response [175]. Hence it is likely that dysregulation of either system will result in a chemical imbalance [305]. For instance, a cross-sectional study in a population of coronary heart disease patients found those who were depressed presented with increased CRP levels in the presence of lower cortisol levels [306].

When we examine how the two systems respond to stressors we find mixed and complex results. In a small study of 15 women, those who had been assaulted in the previous 24-72 hours presented with elevated pro-inflammatory cytokine levels (IL6, CRP and IFNγ), higher cytotoxic CD8 cell counts, and increased ACTH levels [307]. Chronic stress has been linked to elevated cortisol levels, a reduction in pro-inflammatory cytokines and poor wound healing [308, 309]. In contrast, chronic stress has also been linked to low cortisol exposure and elevated pro-inflammatory cytokines, TNFα and IL6 [305]. Overall, findings reveal a complex bidirectional crosstalk to exists between the two physiological systems that allows them to enhance or suppress one another (Fig. 4) [118, 180, 181, 259].

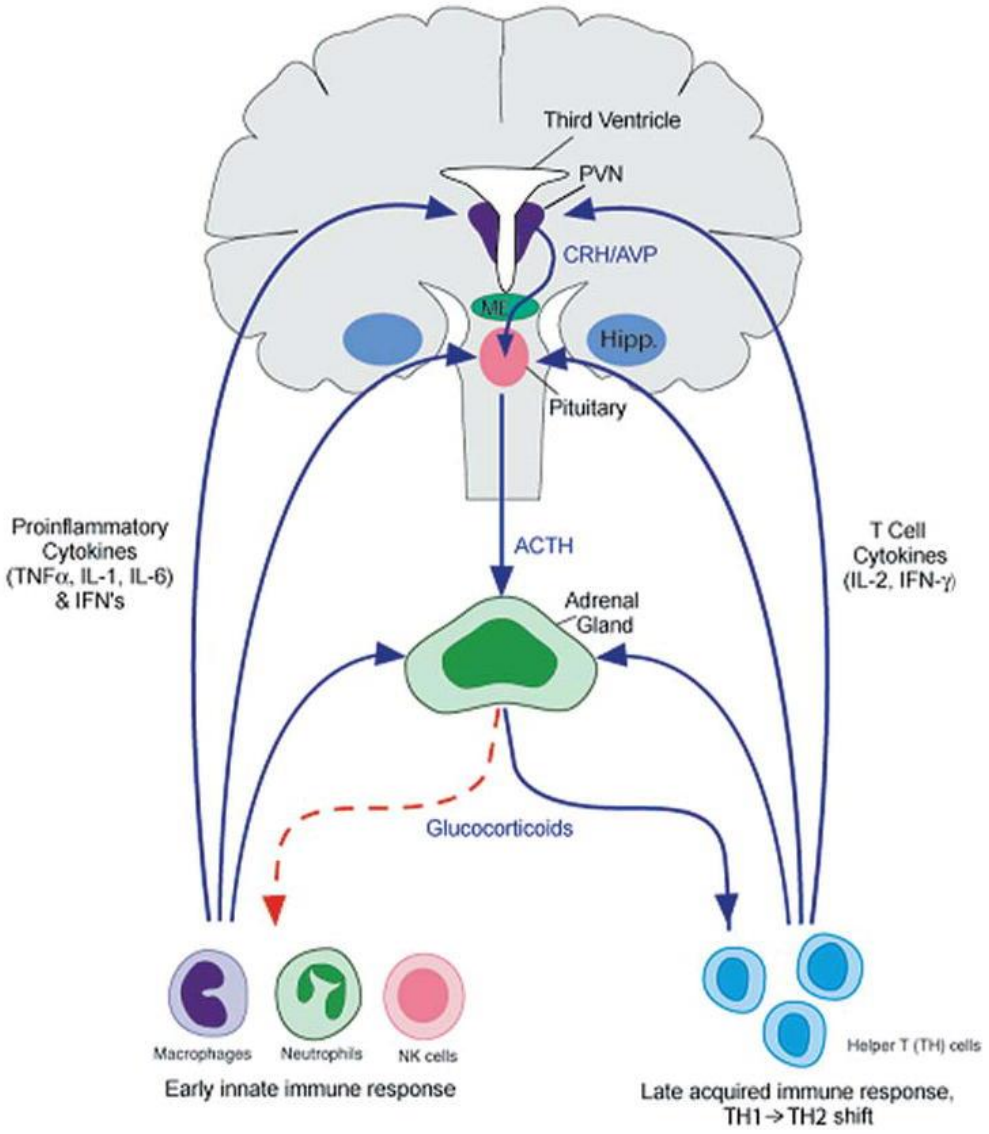


Figure 4. Communication between HPA axis and immune system

The figure above (derived from Silverman [259]) shows the proposed pathway in which bidirectional communication between the brain and body is enabled. During the early innate immune response to acute stress, pro-inflammatory cytokines and interferons are released which stimulate the HPA axis at three different levels to release glucocorticoids. Subsequent cortisol release negatively regulates the inflammatory response and can also shift the phenotype from a Th1 (cellular: pro-inflammatory) towards a Th2 (humoral: anti-inflammatory) immune response. This shift and downregulation of the inflammatory response is believed to protect the body from an overactive and harmful inflammatory response.

Several factors may influence the HPA axis's ability to effectively terminate the physiological stress response potentially leading to excessive inflammation. For instance, a range of studies suggest aging is associated with a reduction in the inhibitory effects of glucocorticoids [182, 310-312], a dampening down of the amplitude of the circadian rhythm [313], an increased variability in diurnal cortisol secretion [299], a downregulation of glucocorticoid receptors in the hippocampus [310, 314], and with increased levels of glucocorticoids [101, 315]. Simultaneously, aging is also accompanied by an up-regulation of the systemic pro-inflammatory response, known as inflammaging [143, 245, 316], and a decrease in the production of anti-inflammatory proteins [277]. Research and theory therefore suggest the aging HPA axis and immune system may fail to efficiently regulate one another leaving older adults more susceptible to both an increased pro-inflammatory phenotype and a vulnerability to disease [245, 299].

Chronic stress is recognised as another potential modulator of HPA axis regulation and subsequent immune functioning. For instance, impaired HPA functioning has been associated with age-related disturbances of cortisol levels in response to psychological stress including reduced cortisol exposure [317]. Cohen and colleagues proposed the Glucocorticoid Receptor Resistance (GCR) model whereby chronic stress leads to the progressive desensitization of glucocorticoid receptors. GCR will result in the failure to effectively regulate the immune system due to the reduced inhibitory influence of cortisol and thus, promoting a subsequent systemic pro-inflammatory phenotype [269, 306]. In a series of studies, Cohen investigated the ability of administered DEX to supress Lipopolysacharide (LPS) stimulated lymphocyte production in healthy adults. Cohen found in those reporting chronic stress a reduced inhibitory effect of cortisol to suppress a pro-inflammatory response. Furthermore, in the same chronic stress group no association was evidenced between

cortisol levels and leukocyte counts in response to inoculation with a virus [269].

In addition or combination with chronic stress, it has been further been proposed that glucocorticoid resistance is a consequence of chronic exposure to pro-inflammatory cytokines [318, 319].

A study involving parents of paediatric cancer patients showed a reduced sensitivity of glucocorticoid receptors on immune cells to anti-inflammatory signals in the stress group, subsequently resulting in the continued production of IL6. This finding was not observed in the control group consisting of parents with healthy children [320]. Likewise, elevated LPS stimulated TNFα levels are shown to be less sensitive to the inhibitory effects of cortisol in a small study of 39 healthy adults after undertaking a social stressor task [279]. GCR may be an initially adaptive mechanism enabling wound healing and other adaptive responses during and immediately following a challenge. However, persisting chemical imbalances including a prolonged inflammatory environment could promote the development of disease.

1.8 Impact of stress on MCI and AD progression

1.8.1 Physical stress

As previously discussed, microglia are partially primed in an AD brain leading to an exaggerated immune response when triggered by secondary stimuli resulting in accelerated neurodegeneration [117-119, 321]. Proposed secondary triggers have emphasised physical stress such as infection, surgery and physical trauma. Thus a large case review study

found a significant association between episodes of infection and increased risk of dementia [28]. Furthermore, specific systemic infections including periodontitis [322] and gut infection with Helicobacter pylori [323] have been associated with the development of AD. In addition, non-infectious chronic inflammatory diseases have been identified as a risk factor for MCI and AD [324]. A few large scale longitudinal studies have yielded interesting findings worth mentioning. For instance, the CAIDE study, following 1,449 participants over 21 years, found joint disorders in midlife were significantly associated with worse cognitive status (MCI, AD, or dementia) later on in life [325]. This association was especially marked for those reporting inflammatory rheumatoid arthritis. Furthermore, chronic inflammatory diseases that develop over a lifetime and typically involve the long-term activation of pro-inflammatory pathways, including obesity [34, 326], diabetes [327], and atherosclerosis [328], are well established risk factors for AD. In addition to acting as risk factors, support for the role of physical stress influencing cognitive decline in AD has been shown by Holmes *et al* who found the presence of a systemic inflammatory event, such as infection, was associated with an increase in the pro-inflammatory cytokine TNFα and an associated two-fold increase in cognitive decline. The effect on cognitive decline was even greater (four-fold) for participants presenting with a history of chronic inflammatory disease at baseline [127]. However, it should be emphasised that it is unlikely that systemic infections and chronic inflammatory disease acting alone would be casual but rather a combined cumulative effect over time influencing disease trajectory [126].

1.8.2 Psychological stress

However, in addition to physical stress factors it is feasible that psychological stress could also act as a secondary trigger and this will now be discussed in more detail.

A wide breadth of research suggests psychological stress triggers a pro-inflammatory immune response in healthy participants [179, 225, 280, 283, 288-290]. Therefore, if the experience of psychological stress led to a systemic inflammatory response and subsequent activation of primed CNS microglia, we would expect to see exacerbated clinical symptoms such as cognitive decline that reflects underlying accelerated AD pathogenesis. As expected in normal cognitively-intact populations, several studies show no relationship between psychological stress and cognitive decline [329, 330] whilst others report mixed or contradictory results [331, 332].

1.8.3 The impact of acute psychological stress

Clinical research investigating the effects of acute psychological stress on the development of MCI and AD is limited. In general, the physiological impact of acute stress is thought to be transitory in nature and reversible. For example, acute administration of glucocorticoids in humans is only related to transient declarative memory impairment [333]. Animal studies show that when rats are placed in acute stress provoking environments, spatial working memory is only temporarily impaired [334] In one study the association between psychological stress and cortical structural changes was solely evidenced in response to repeated rather than single stress paradigms. The authors found that only chronic stress could cause longer term cognitive deficits and hippocampal atrophy [249]. Likewise, suppression of neurogenesis in the dentate gyrus has only been observed in animal models that are subjected to chronic stress [335]. Clearly, more research is needed to clarify the impact of acute stress on neurodegenerative processes. However, findings seem to suggest that long-term cortical structural alterations and memory impairment are more likely to occur in response to chronic stress.

1.8.4 The impact of chronic psychological stress

Clinical studies

In comparison to acute psychological stress, the relationship between chronic psychological stress and cognitive impairment is starting to attract considerably more attention. Chronic psychological stress has recently been exposed to potentially place individuals at heightened risk of cognitive decline [324]. Chronic stress in the form of Post-Traumatic Stress Disorder (PTSD) is consistently linked to a dysregulated HPA axis and increased pro-inflammatory cytokine expression [336-338] including lL6 and TNFα [305]. A key meta-analysis revealed a strong association between PTSD and greater risk of both cognitive impairment and the development of AD [339]. Consistent with these findings, a large cross-sectional study found those exhibiting PTSD symptoms performed worse on all cognitive testing compared to the healthy control group. This relationship became more marked when the authors controlled for depression [340]. In addition, a study following 181,093 war veterans found those with PTSD were at significantly greater risk of developing AD when compared with veterans free of PTSD [341] which has been replicated elsewhere in 3,660 war veterans [342]. A small but interesting study found a significant association between worse explicit memory and PTSD in Holocaust survivors [343]. However, the Holocaust survivors with PTSD held significantly fewer years of education and presented with a lower IQ. Shared characteristics such as these suggest that vulnerability factors for the development of PTSD may share a common pathway with those that predispose individuals to AD. In addition to a pro-inflammatory phenotype, a smaller hippocampal volume was evident prior to the onset of PTSD [333] that is also a known risk factor for AD. Furthermore, it should be noted that increased rates of depression [344] and substance abuse [345] have been observed in PTSD, which could potentially mediate the link

between PTSD and increased risk of AD. Overall, further research is needed to better understand this relationship as the majority of research focuses on Holocaust survivors and war veterans with a limited number of unique samples considered [339].

A recent cross-sectional study of older adults based in Central African Republic and the Republic of Congo found reported chronic stressful life events was associated with an increased likelihood of MCI [346] although this data is limited due to cause or effect cannot be determined. However, the Betula longitudinal cohort study found no association between the occurrence of stressful life events and onset of dementia in 2,462 participants, aged 55 years and older, who were dementia free at the time of study enrolment [330]. Notably however, informant reports were not taken for participants reporting cognitive impairment and thus potentially biasing the results. Those with dementia or cognitive impairment may have under reported stressful events due to failing to remember they had occurred. However, a more recent population based longitudinal study based in Switzerland also found no association between reported lifetime stress and likelihood of dementia in 796 non demented older adults [98]. In direct contrast to these findings, several large scale longitudinal studies observe a significant association between life stress and cognitive decline in later life [347-350] and chronic stress independently acting as a risk factor for MCI and dementia [214, 351, 352]. Similarly, a study following 800 women over a 37 year period, 153 of whom developed dementia, showed those who experienced significant midlife stress assessed in 1968 were at increased risk of AD in 2005 [353]. The authors found in the same cohort that midlife distress was further associated with moderate to severe temporal lobe atrophy and increased white matter lesions later in life [354]. In another longitudinal study tracking 4,108 participants, parental death during childhood or adolescence was significantly associated with an increased risk of AD. Interestingly, this association was independent of

ApoE status [63]. Likewise, a study following 1,000 participants over a 12 year period found those who reported greater chronic distress at baseline were at increased risk of MCI [214] and dementia, a relationship which was not altered by depression [355]. Finally, early reports of perceived stress have been found to predict hippocampal grey matter shrinkage 20 years later [356], which remain significant after controlling for age, education, and depression. Taken together, longitudinal studies suggest that stress may act as a risk factor or exacerbate AD pathogenesis years prior to clinical symptoms emerging.

In a small but important study, the relationship between psychological stress and longitudinal memory loss was examined in 25 cognitively intact participants and 27 aMCI participants for a mean follow-up period of 2 years [61]. Level of stress was measured at baseline and every six months thereafter by the use of an in-depth interview based assessment. In keeping with earlier studies, the results indicated higher stress ratings over the follow-up period was associated with faster decline only in the aMCI group. Likewise, Peavy et al found in 91 non-demented participants, higher stress ratings was associated with worse memory performance on a range of scales but only in the presence of at least one ApoE ε4 allele. Thus, the authors concluded there was a gene-environment interaction between stress and a well-established risk gene for AD [357]. These studies lend support that psychological stress may contribute to the progression of AD but only in those who are predisposed.

Animal studies

Animal models generally support clinical findings, revealing a link between psychological stress and potential neurodegenerative changes. For instance, when animals are placed under chronic stressful conditions the suppression of neurogenesis is observed in the dentate gyrus [335, 358],

hippocampal Cornu Ammonis 3 (CA3) pyramidal neuron dendritic atrophy [359], glucocorticoid resistance in peripheral immune cells [360], and a reduction in the total length and branch numbers of apical dendrites in the medial prefrontal cortex [361]. However, in healthy adult animal models reduced neurogenesis and structural remodelling has often been seen to reverse after the chronic stressor is removed. Nonetheless, with this in mind animal research does suggest that exposure to psychological stress during critical stages of development may produce changes in morphology that seem to persist into adulthood [264-266, 362-365] including hyperactivity of the HPA axis [263, 366-368].

In addition, placing animals in a chronic stress paradigm has been shown to induce memory impairment in rats observed up to four weeks after removal of the stressor [369]. A similar association between chronic stress and cognitive deficits is documented in transgenic mice models of AD [370]. In these studies, exposure of mice to immobilization stress for 8 months is related to severe learning and memory impairments. In association, increased extracellular amyloid plaque deposition in the hippocampus and cortex and neurodegenerative markers was evidenced. In another study, exposing animals to chronic adverse conditions resulted in dendritic atrophy in the hippocampus and medial prefrontal cortex [250, 361, 371] that was accompanied by spatial and memory deficits [249].

A key animal study in the literature examined the effects of psychosocial stress on memory and long-term potentiation in an *in vivo* rat model of AD induced by chronic intracerebroventricular infusion of Aβ (Aβ1-40 and Aβ1-42) [372]. Rats were exposed to “rat intruders” for six weeks, a widely used model of stress known to result in increased corticosterone plasma levels. Rats that experienced both stress and exposure to Aβ accumulation exhibited significantly greater memory impairment on the radial arm water

maze task than compared to rats which experienced stress or Aβ treatment alone. The Aβ/stress rats also demonstrated severely diminished early-phase long term potentiation in the hippocampal CA1 region. These experiments have since been repeated in a paradigm intended to represent normal individuals with a predisposition to AD. Here, rats that demonstrated normal cognitive performance were exposed to a sub-threshold dose of Aβ1-42 (subAβ). In keeping with earlier findings, chronically stressed subAβ rats showed worse cognitive performance and early-phase long term potentiation than that caused by subAβor stress alone [373]. Overall, animal models suggest chronic stress is associated with structural and neurochemical changes integral to memory. However, this data should be treated with caution due to the complexity of comparing animal research to human experiences and physiology.

1.9 Introduction summary

The long-term physiological consequences of psychological stress remain poorly understood, particularly in the context of aMCI. Overall, findings suggest chronic stress is related to HPA dysregulation and potentially, structural and neurochemical changes in the brain. In those with existing AD pathology, psychological stress may further serve as a trigger leading to an exaggerated and harmful CNS pro-inflammatory immune response, which cannot be sufficiently dampened down by cortisol. Thus, the experience of psychological stress may render individuals with aMCI susceptible to further neurodegenerative changes and subsequent accelerated cognitive decline. However, the impact of stress, in particular psychological stress, on cognitive outcomes in aMCI has been underexplored. Furthermore, there is little is known about the role of inflammation and its modulation by cortisol in individuals with aMCI.

1.10 Study hypotheses

We propose that in aMCI participants psychological stress will serve as a secondary trigger activating the primed central microglia inflammatory state and leading to an exaggerated and neurotoxic immune response. Therefore, our primary hypothesis will be that psychological stress will be associated with worsened cognitive decline, a clinical marker of advancing neurodegeneration, from baseline visit 1 to visit 4 over an 18 month period in aMCI participants compared to cognitively intact control participants. The null hypothesis will be that in participants with aMCI the presence of psychological stress will not be associated with worsened cognitive decline over an 18 month period.

Our second hypothesis will be that in aMCI (but not control) participants chronic stress is associated with a pro-inflammatory phenotype and an associated increase in cortisol in a failed attempt to dampen down this exaggerated immune response. The null hypothesis will be that chronic stress will not be significantly associated with a pro-inflammatory phenotype or changes in cortisol levels.

Our third hypothesis will be that a pro-inflammatory phenotype is associated with cognitive decline in the aMCI (but not control) participants. The null hypothesis will be that a pro-inflammatory phenotype is not associated with cognitive decline in the aMCI participants.

Our final hypothesis is that psychosocial modulators of the stress response will influence rates of cognitive decline in aMCI participants through modulation of the physiological stress response (inflammation and cortisol

measures). The null hypothesis will be that psychosocial modulators of the stress response will not influence rates of cognitive decline in aMCI participants and there will be no modulation of the physiological stress (inflammation and cortisol measures) response.

Chapter 2: Methods

The study was coordinated at the Memory Assessment and Research Centre (MARC) at Moorgreen Hospital based in Southampton, Hampshire, UK. The study was funded by the Alzheimer's Society and was sponsored by the University of Southampton. A lay group based at MARC, consisting of dementia caregivers and patients diagnosed with MCI and AD, contributed to the initial study design before ethical approval was obtained. The Alzheimer's Society lay panel further reviewed the study design and potential clinical implications of the study before approving funding. A subsequent Alzheimer's Society lay group reviewed the study progress annually at MARC. As the sponsor, the University of Southampton's Insurance applied along with Indemnity and Insurance provided under the NHS Clinical Negligence Scheme for Trusts. The study was approved by an independent Ethics Committee (NRES Committee South Central – Portsmouth 12/SC/0115) in accordance with local regulations.

The study was conducted over three NHS sites: MARC based at Moorgreen Hospital in Southampton; the Dorset Healthcare University Foundation Trust based at Yeatman Hospital in Sherborne, and the Solent NHS Trust based at St James Hospital in Portsmouth. Each of the three sites held a principal investigator and research coordinator. Site initiation meetings and training were delivered at all sites to ensure compliance with the study protocol and ensure good quality data collection.

2.1 Study sample

Two participant groups were studied with 68 cognitively intact controls enrolled and 135 aMCI participants enrolled. A total of 203 participants

completed visit 1, 188 completed visit 2, 167 completed visit 3, and 155 completed visit 4 across all three research sites. Of the 48 participants who did not complete there were 18 early withdrawals mainly due to moving out of the area or finding the experience of testing too stressful.

There were 30 conversions to dementia over the 18 month study follow-up period (2 conversions completed visit 4). Research personnel interviewed aMCI participants at each study visit to assess whether the participant had potentially converted from aMCI to dementia. For this process, the participant's overall level of comprehension was assessed in addition to the participant's clinical history, neuropsychometric test results, and reports of everyday independent functioning. For participants suspected to have converted to dementia, a referral was made to the site specific Principal Investigator who subsequently reviewed the participant within their memory service. Likewise, a referral was also made to the Principal Investigator for participants reporting a significant cognitive and/or functional deterioration between study visits. An Alzheimer's diagnosis was made using NINCDS-ADRDA criteria [374]. The NINCDS-ADRDA outlines that a diagnosis should not be made in the presence of a substantial concomitant cerebrovascular disease, other dementias, active neurological disease, non-neurological medical comorbidity or use of medication that could affect cognition.

We observed key differences in age and cognition for those completing the follow-up period compared to those who withdrew earlier than the 18 month final study visit. Participants completing all four study visits (n=155) were younger than those withdrawing before visit 4 (n=48) (72.9 years vs 79.7 years, mean difference 6.7 years, 95% CI 3.9 to 9.6, p=<0.0001), and performed better on cognitive testing at baseline, scoring 25.3 points on the MoCA compared with the non-completers who scored 22.6 points

(mean difference 2.7 points, 95% CI 1.7 to 3.7, p=<0.0001). There was no difference in gender, with 79 (51%) of the completers being male compared with 25 (52%) of the non-completers (chi square 0.92 p=0.9). There was also no difference in education, with those completing the study follow-up period holding an average of 13.2 years of education compared with those withdrawing early holding 12.2 years (mean difference 0.6, 95% CI -2.2 to 0.1, p=0.07).

All aMCI participants prior to study enrolment had received a formal MCI diagnosis from a NHS clinician, primarily through a memory service in Older Persons Mental Health. MCI participants were then seen by a research clinician, primarily a medical doctor, to assess if participants met the Petersen aMCI diagnostic criteria [2]. Aiding this diagnostic distinction, the clinical history of the participant, current and past neuropsychological test scores, and observations from those close to the participant were considered. Uncertainty of diagnosis resulted in either participants being re-assessed at a later date or a case discussion with the Chief Investigator. Cognitively intact control participants were recruited from several sources including the University of 3rd Age, carer groups, word of mouth, dementia carergivers known to the research site, and through advertising materials placed in local libraries and GP surgeries. At study enrolment, control participants who reported subjective memory loss or demonstrated a clinical history of memory impairment were excluded.

2.2 Study design

This was a longitudinal population-based cohort observation study comparing cognitive decline to the degree of life stress in 135 participants with aMCI and 68 control participants who were cognitively intact. Study duration was 18 months. Those enrolled on the study and diagnosed with aMCI were termed as the 'participant', whilst those who were cognitively intact were termed as the 'control participant'. The study partner was typically a spouse or an adult child of the aMCI participant, and gave consent to take part in the study. Study partners either lived in the same household or interacted with the participant for at least 10 hours per week. Study partners were asked to provide information about the participant's exposure to recent stressful life events, physical and behavioural symptoms associated with their aMCI diagnosis, and any changes in cognition during the course of the study follow-up period. The study partner and participant, or control participant alone, were asked to attend a minimum of four home or clinic based visits (Visit 1, 2, 3, and 4). The participant and study partner, or control participant alone, were asked to allow an Early Withdrawal visit if participation ended in the study before visit 4.

We administered cognitive assessments at each study visit following guidance from the National Institute on Aging-Alzheimer's Association workgroups, which advises serial neuropsychological assessments documented over time is preferable to track cognitive decline in MCI persons and provides evidence of MCI due to AD [1]. Due to short-term memory being a prominent feature in aMCI [1] we administered the FCSRT-IR (a measure of episodic memory loss) at each study visit. It is common for persons with aMCI to show impairment in other cognitive domains in addition to short-term memory. The National Institute on Aging-Alzheimer's Association workgroups therefore recommend clinical

evaluation of language, executive function, visuospatial skills and attention [1]. This recommendation led to the selection of the MoCA and TMT neuropsychological tests to also be administered at each study visit.

Baseline (Visit 1)

After participants gave informed consent, research personnel recorded a detailed medical history and obtained an additional medical summary of history and medications from the General Practitioner (GP). Medical history included infections, acute systemic inflammatory events, and chronic inflammatory health conditions, with a focus on key conditions including hypertension, hypercholesterolemia and diabetes, which have been identified as prominent risk factors for cognitive impairment and dementia [375].

A blood test was carried out followed by assessments of cognitive function, mood, coping style, personality, perceived social support, perceived health, and the level of psychological stress experienced. The study partners were asked to assist aMCI participants when required. The assessments were administered in the following order where possible:

- Assess participant capacity and willingness to provide informed consent
- Previous and current medications
- Weight and height
- Medical history
- Blood collection for cytokines and DNA
- Participant: VAS, Geriatric Depression Scale (GDS), The Montreal Cognitive Assessment (MoCA), Free and Cued Selective Reminding Test with Immediate Recall (FCSRT-IR), Trail Making Test (TMT) Trial A & B, Recent Life Change Questionnaire (RLCQ), Perceived Stress

Scale (PSS), The Medical Outcomes Study - Social Support Survey (MOSS – SSS), The Neuroticism scale of the NEO Five Factor inventory (NEO FFI), The Coping Inventory of Stressful Situations (CISS)

- Study partner of aMCI participant: RLCQ (Informant version

Participants were asked to complete 6 saliva swabs within 7 days following the study visit and store samples in the fridge until collection. Study partners assisted aMCI participants with this process to ensure compliance with the procedures.

Visit 2 (6 month follow up visit +/- 2 weeks)

After participants gave informed consent to continue in the study, research personnel recorded a detailed medical history including a history and medications since visit 1. Medical history included acute systemic inflammatory events including infections. A blood test was carried out followed by assessments of cognitive function, mood, perceived social support, perceived health, and the level of psychological stress experienced. The study partners were asked to assist aMCI participants when required. The assessments were administered in the following order where possible:

- Assess participant capacity and willingness to provide continued informed consent
- Previous and concomitant medication
- Weight
- Medical history
- Serious Adverse Event (SAE) inquiry
- Blood collection for cytokines
- Participant: VAS, GDS, MoCA, FCSRT-IR, TMT Part A&B, RLCQ, PSS, MOSS – SSS
- Study partner of aMCI participant: RLCQ (Informant version)

Participants were asked to complete 6 saliva swabs within 7 days prior to or following the study visit and store samples in the fridge until collection. Study partners assisted aMCI participants with this process to ensure compliance with the procedures.

Visit 3 (12month follow up visit +/- 2 weeks)

After participants gave informed consent to continue in the study, research personnel recorded a detailed medical history including a history and medications since visit 2. Medical history included acute systemic inflammatory events including infections. A blood test was carried out followed by assessments of cognitive function, mood, perceived social support, perceived health, and the level of psychological stress experienced. The study partners were asked to assist aMCI participants when required. The assessments were administered in the following order where possible:

- Assess participant capacity and willingness to provide continued informed consent
- Previous and concomitant medication
- Weight
- Medical history
- SAE inquiry
- Blood collection for cytokines
- Participant: VAS, GDS, MoCA, FCSRT-IR, Verbal Fluency, TMT Part A&B, RLCQ, PSS, MOSS – SSS
- Study partner of aMCI participant: RLCQ (Informant version)

Participants were asked to complete 6 saliva swabs within 7 days prior to or following the study visit and store samples in the fridge until collection.

Study partners assisted aMCI participants with this process to ensure compliance with the procedures.

Visit 4 (18 month follow up visit +/- 2 weeks)

After participants gave informed consent to continue in the study research personnel recorded a detailed medical history including a history and medications since visit 3. Medical history included acute systemic inflammatory events including infections. A blood test was carried out followed by assessments of cognitive function, mood, perceived social support, perceived health, and the level of psychological stress experienced. The study partners were asked to assist aMCI participants when required. The assessments were administered in the following order where possible:

- Assess participant capacity and willingness to provide continued informed consent
- Previous and concomitant medication
- Weight
- Medical history
- SAE inquiry
- Blood collection for cytokines
- Participant: VAS, GDS, MoCA, FCSRT-IR, Verbal Fluency, TMT Part A&B, RLCQ, PSS, MOSS – SSS
- Study partner of aMCI participant: RLCQ (Informant version)

Participants were asked to complete 6 saliva swabs within 7 days prior to or following the study visit and store samples in the fridge until collection. Study partners assisted aMCI participants with this process to ensure compliance with the procedures.

Visit 4 was the last study visit. Therefore, an appropriate follow-up plan was formed with the participant. The participant's General Practitioner and other key health professionals were notified in writing that the participant had completed their participation in the study and of the follow-up plan if relevant.

Table 1 shows a summary of the assessments at each study visit

	Visit 1	Visit 2	Visit 3	Visit 4
Weight and height	√	√	√	√
Medications and medical history	√	√	√	√
Peripheral blood collection for DNA	√			
Peripheral blood collection for CRP and cytokines	√	√	√	√
Salivary cortisol collection	√	√	√	√
Self-rated health scale: VAS	√	√	√	√
Assessment of low mood: GDS	√	√	√	√
Cognitive tests: MoCA, FCSRT – IR, and TMT	√	√	√	√
Objective psychological stress questionnaire: Participant RLCQ and Informant RLCQ	√	√	√	√
Perceived stress scale: PSS	√	√	√	√
Social support questionnaire: MOSS-SSS	√	√	√	√
Coping style questionnaire: CISS	√			
NEO-FFI Neuroticism scale	√			

2.3 Primary objective

To investigate the association between objective life stress, as measured by the RLCQ, and the rate of cognitive decline, as measured by a comparison of the change in the Free and Cued Selective Reminding Test with Immediate Recall (FSCRT-IR total score), in control and aMCI participants from baseline visit 1 to visit 4 and if significant to explore other alternative outcome measures of cognitive decline (i.e. MOCA and TMT Part B) measured over the same study period.

2.4 Secondary objectives

To investigate the association between perceived life stress, as measured by the PSS, and the rate of cognitive decline, as measured by a comparison of the change in the Free and Cued Selective Reminding Test with Immediate Recall (FSCRT-IR total score), in control and aMCI participants from baseline visit 1 to visit 4 and if significant then to examine other alternative outcome measures of cognitive decline (i.e. MOCA and TMT Part B) measured over the same study period.

To investigate the association between acute and chronic physical systemic inflammatory events and the rate of cognitive decline, as measured by a comparison of the change in the Free and Cued Selective Reminding Test with Immediate Recall (FSCRT-IR total score), in control and aMCI participants from baseline visit 1 to visit 4 and if significant then to measure other alternative outcome measures of cognitive decline (i.e. MOCA and TMT Part B) measured over the same study period.

To examine, in the control and aMCI group, the influence of life stress (as measured by the RLCQ and PSS) on serum cytokine and salivary cortisol measures and interactions between them.

To examine, in the control and aMCI group, the relationship between serum cytokine and salivary cortisol measures on rates of cognitive decline as measured by the RLCQ.

To examine, in the control and aMCI group, the relationship between postulated modulators of stress (social support, coping mechanisms and personality profile) on rates of cognitive decline as measured by the RLCQ and if positive to examine relationships with cytokine levels and salivary cortisol measures.

2.5 Consent procedures

Everyone involved with the consent process had to be:

- Familiar with the study
- Knowledgeable of optional health care
- Aware of the need for informed consent
- Having the time for full discussion with the participant
- Understanding of the participant's particular circumstances

Everyone involved in the consent process was trained and competent to perform their specific role. The role was delegated by the Principal Investigator at each site. The informed consent process was documented in the participant's notes, which included the study name, the date, and the participant number. Written, informed consent, in compliance with the Declaration of Helsinki and Good Clinical Practice, was obtained from each participant prior to entering into the study. The Principal Investigator and clinical research worker at site were responsible for ensuring that consent was voluntary and fully informed. In addition, the person acting as the aMCI participant's study partner was required to provide written, informed consent for their own participation in the study.

As part of this procedure, the Principal Investigator or one of his/her associates explained orally and in writing the nature, duration, and purpose of the study in such a manner that the control participant, aMCI participant and study partner were aware of the potential risks, inconveniences, or adverse effects that could have occurred. Participants were informed that they could withdraw from the study at any time. Any questions that the control participant, aMCI participant and study partner had were answered in full and to the satisfaction of the research participant.

A Patient Information Sheet (aMCI participant)/Participant Information Sheet (control participant) was provided to enable potential participants to find out more about the study. The Information Sheet was given before consent was obtained, allowing sufficient time (a minimum of 24 hours) for the information to be assimilated. During the baseline study visit, the Information Sheet and consent form were completed, dated and signed personally by the control participant/aMCI participant and study partner and then by the person responsible for collecting the informed consent. Participants were given a signed copy of the original information and consent form, the original kept by the Principal Investigator at the study site. A copy of the signed information and consent form was filed in the patient notes.

Participants with capacity gave written informed consent prior to entry into the study. Participants who lacked capacity were not eligible for entry into the study. Trained study personnel continued to monitor the participant's capacity to provide informed consent throughout the study. Participants were advised verbally and in the information sheets that they had the right to withdraw from the study at any time without prejudice or loss of benefits to which they were otherwise entitled.

2.6 Assessment of safety

Adverse Event definition

An adverse event or adverse reaction was defined as serious if it:

(a) Resulted in death

(b) Was life-threatening

(c) Required hospitalisation

(d) Prolonged a current hospitalisation

(e) Resulted in persistent or significant disability or incapacity

(f) Consisted of a congenital abnormality or birth defect.

(g) Was otherwise considered medically significant by the Investigator

Reporting

A Serious Adverse Event (SAE) occurring to a participant was reported to the main Research Ethics Committee if in the opinion of the Chief Investigator the event was:

- Related: Resulted from administration of any of the research procedures, and,
- Unexpected: The type of event was not listed in the protocol as an expected occurrence.

All adverse events occurring in the study were recorded in the participant's medical records. Data on adverse events were collected by research personnel from participants and where appropriate, their study partner at the scheduled visits. The research team reviewed the adverse events immediately to ascertain whether they met the criteria for 'serious'. If the event was assessed as being an SAE then the guidance on reporting SAEs was followed. If an event was assessed as 'serious' a designated study doctor completed a Serious Adverse Event form. The SAE report form was signed and dated by research personnel who were delegated to undertake this task. Notifications of SAEs from sites (using a standardised SAE form provided by the Memory Assessment and Research Centre) were faxed to the Chief Investigator within 24 hours.

The Chief Investigator reviewed the event at the earliest opportunity; made changes to the assessments as appropriate, counter signed the form, dated their signature and treated it as a follow-up report. All SAEs defined as above were reported to the sponsor within 15 days of the Chief Investigator becoming aware of the event.

Potential adverse events of study procedures listed in the protocol

- During the study blood was drawn to perform a variety of tests. The potential risks of drawing blood included temporary discomfort from the needle in the arm, bruising, swelling at the needle site, and, in rare instances, infection.
- The experience of nervousness, tiredness or boredom during the mental testing. Participants were encouraged to have rest periods during testing, if needed, and were free to stop any test or procedure at any time. Study staff was fully trained to administer the neuropsychological tests and had testing experience.
- Participants were asked a variety of personal questions about possible stressful or traumatic life events that the participant may have experienced as a child, during their adult life and in the previous 6 months prior to the study visit. Before and during questioning, all participants were therefore informed that they were free to take a break at any point and did not have to answer study questions or provide a reason for refusing to answer a question. If appropriate, study personnel sign posted participants to other services and advised of available services.

2.7 Data handling & record keeping

All study documents and study data were securely stored in accordance to guidelines for Good Clinical Practice, the site's Standard Operational Procedures, the Data Protection Act (1998) and local regulatory requirements.

A case report form was provided to record all the data required by the protocol and collected by research personnel. A case report form was completed for each participant. Some data (questionnaires and tests) completed by the research personnel, the participant and study partner, was recorded directly into the case report form and therefore regarded as source data. The study data management was consistent with Guidelines for Good Clinical Practice in Clinical Trials, the Data Protection Act, 1998, the Health and Social Care Act, 2001, and other relevant regulatory guidelines as appropriate. The Principal Investigator at each site ensured that all research personnel were familiar and complied with the relevant guidelines.

2.8 Eligibility criteria

2.8.1 Inclusion/Exclusion criteria for aMCI participants

Inclusion criteria

- Participant had to be aged between 50 and 100 years
- The participant met the Petersen criteria for amnestic Mild Cognitive Impairment
- The participant had adequate visual and auditory acuity to allow cognitive testing to be performed

- The participant was willing and able to participate for the 18 month study or until the participant developed dementia
- Participant and study partner were fluent in English language
- MoCA score at baseline was between 17 and 25 points (discretion of the Chief Investigator)
- A study partner was available and was either living in the same household or interacted with the participant for at least 10 hours per week to provide information about the participant's recent level of life stress, physical and behavioural symptoms and changes
- Signed informed consent by participant and study partner prior to the initiation of any study-specific procedure

Exclusion criteria

- Refusal to provide informed consent
- Loss of capacity to provide informed consent during the study
- Absence of a suitable study partner
- Unlikely to have cooperated in the study, not able to be present at all scheduled visits, or not be able to follow study instructions
- Participation in another research study with administration of any investigational drug at time of enrolment
- Any previous or current medical conditions during the study may have impacted upon cognitive performance, left to the Principal Investigator's judgment
- The participant's health was not adequate to comply with study procedures, as ascertained by review of their screening medical history
- Alcohol intake >35 units per week for men, or > 28 units per week for women, or drug abuse
- Participants taking cholinesterase inhibitor medication

- Any psychiatric diagnosis that may had interfered with the participant's ability to perform study assessments
- Participants who took major modifiers of the immune system including corticosteroids and TNFα inhibitors, left to the Chief Investigator's judgment

We only excluded major immunomodulators, corticosteroids and cholinesterase inhibitor medication in order to recruit a representational sample of the target study population.

2.8.2 Inclusion/Exclusion criteria for cognitively intact control participants

Inclusion criteria

- Participant had to be aged between 50 and 100 years
- The participant had adequate visual and auditory acuity to allow cognitive testing to be performed
- The participant was willing and able to participate for the 18 month study or until the participant developed dementia
- Participant was fluent in English language
- MoCA score at baseline was equal to or greater than 26 points (discretion of the Chief Investigator)
- Signed informed consent by participant prior to the initiation of any study-specific procedure

Exclusion criteria

- Refusal to provide informed consent
- Loss of capacity to provide informed consent during the study

- Unlikely to have cooperated in the study, not able to be present at all scheduled visits, or not be able to follow study instructions
- Participation in another research study with administration of any investigational drug at time of enrolment
- Any previous or current medical conditions during the study that may have impacted on cognitive performance, left to the Principal Investigator's judgment
- The participant's health was not adequate to comply with study procedures, as ascertained by review of their screening medical history
- Alcohol intake >35 units per week for men, or > 28 units per week for women, or drug abuse
- Participants taking cholinesterase inhibitor medication
- Any psychiatric diagnosis that may have interfered with the participant's ability to perform study assessments
- Participants taking major modifiers of the immune system including corticosteroids and TNF α inhibitors, left to the Chief Investigator's judgment

2.9 Early withdrawal criteria

Each participant was free to withdraw from the study at any time. Investigators also had the right to withdraw participants from the study. The reason and exact date of withdrawal was noted in the participant's notes. Criteria for premature discontinuation were:

- Consent withdrawal
- Participant conversion to dementia
- Protocol deviation including lack of informed consent
- Any event or circumstance unrelated to the study justifying the discontinuation of study participation in the research clinician's

opinion such as reasons concerning the health or well-being of the participant

2.10 Study procedures

2.10.1 Neuropsychological measures

It was required in this study to objectively measure cognitive decline in participants with aMCI and in the control group. The National Institute on Aging-Alzheimer's Association workgroups suggests serial neuropsychological assessments documented over time is preferable to track cognitive decline in MCI patients and provides evidence of MCI due to AD [1]. Due to short-term memory being a prominent feature in aMCI [1] it was measured at every study visit. It is common for individuals with aMCI to show impairment in other cognitive domains in addition to memory. The National Institute on Aging-Alzheimer's Association workgroups therefore recommends clinical evaluation of language, executive function, visuospatial skills and attention [1]. This recommendation led to the selection of tests administered at each visit in addition to episodic memory assessment. The chosen neuropsychological measures assessing these domains are outlined below.

Cognition for both participant groups was assessed at baseline and at each 6 month subsequent visits during the 18 month follow-up period. Through the course of the study, participants who converted to dementia were withdrawn due to ethical considerations. These participants were most likely to have a more rapid cognitive decline. The other major reason for withdrawal was due to participants experiencing psychological stress. Therefore, a Last Observation Carried Forward (LOCF) analysis was applied when investigating rate of cognitive change over time to avoid excluding

those who significantly declined prior to conversion to dementia or withdrew due to increased psychological stress.

Free and Cued Recall Selective Reminding Test (FCSRT-IR) [376]

Our primary measure of cognition was the FCSRT-IR which measures impairment of episodic memory. The FCSRT-IR is a well-known word list learning test that has been recommended to aid diagnosis of MCI by the National Institute on Aging-Alzheimer's Association workgroups [1]. A professional working group was formed to debate which cognitive measures to incorporate into the study design. Based on the evidence, it was decided to use the FCSRT as the primary outcome measure. The FCSRT-IR measures episodic memory under conditions that control for attention and cognitive processing. The test has been successfully used in a number of longitudinal aging studies and possesses good psychometric properties [377-380]. Further validation of the measure is derived from FCSRT-IR scores significantly correlating with abnormalities observed in imaging studies [381, 382], CSF biomarkers in MCI and AD [383], and neurofibrillary lesions evidenced in parahippocampal regions [384]. Predictive validity has been shown in one important study which suggested a significant cut-off point (40/48 for total recall score) identifying those who went on to convert from aMCI to AD [378].

The FCSRT-IR begins with a study phase in which participants are asked to search a card containing four words (e.g. apple) for an item that goes with a unique category cue (e.g. fruit). This controlled learning encourages semantic processing for effective encoding. After all four items are identified, immediate recall of just those four items is tested. The search procedure is continued until all 16 words are identified and retrieved in immediate recall. This study procedure is followed by three trials of recall each consisting of free recall followed by cued recall for items not

retrieved by free recall. There is 20 seconds of interference between each trial counting down in 3s from 95, 69 and 44. Items not retrieved by cued recall are re-presented during Trial 1 and 2. The overall sum of free and cued recall over the 3 memory trials is called the total recall score.

The Montreal Cognitive Assessment (MoCA) [385]

We administered the MoCA to assess global cognition, which is a well validated and widely used measure assessing domains of impairment commonly encountered in MCI. This test is commonly used in both a NHS clinic setting and in clinical memory research. The MoCA demonstrates good psychometric properties and is an easy-to-administer cognitive test which surpasses the limitations of the MMSE [385-388]. Furthermore, the MoCA shows a high sensitivity to tracking cognitive decline in longitudinal monitoring for both MCI and AD [386]. The MoCA measures 8 cognitive domains within a series of 13 tests: visuospatial/executive function, memory, language, abstraction, delayed recall and orientation. The highest possible score is 30. A score above 26 represents normal cognitive function whereas a score of 25 and below suggest a cognitive impairment. An additional point is given for individuals who report less than 12 years of education.

Trail Making Test – Part A & B (TMT) [389]

The National Institute on Aging-Alzheimer's Association workgroups suggests using the TMT as a measure of executive function [1]. The TMT is among the most commonly used neuropsychological tests that measure executive function in MCI and AD. Participants are required to draw lines to connect alphanumeric stimuli in ascending order that are randomly placed on a page. The time it takes for the participant to complete the task expressed in seconds is used as the score. In trial A the participant is

asked to connect the numbers arrayed randomly across the page in order. In the more complex Part B condition, the participant is required to connect dots containing numbers and letters in alternating sequence (1-A-2-B-3-C etc). The cognitive alternation required by Part B reflects executive functioning, although other cognitive abilities, such as psychomotor speed and visual scanning, are also required for the successful completion of the test. The TMT's sensitivity consistently achieves discrimination between healthy controls and patients diagnosed with MCI [388] and has long been regarded as a reliable, valid and sensitive measure [389-391]

2.10.2 Assessment of stress

2.10.2.1 Psychological stress

Participants were asked to rate their psychological life event(s) and perceived psychological stress at each study visit with the assistance of a researcher if necessary. To overcome limitations of stress research regarding the degree in which individual differences and perception of stress influence the experience of events, we administered both subjective and objective measures of stress. In addition, due to the nature of aMCI (marked by short-term memory loss) the study partner was required to complete an informant version of the Recent Life Changes Questionnaire (RLCQ).

Recent Life Changes Questionnaire (RLCQ) [392]

Our primary measure of stress assesses objective ratings of stress 6 months prior to each study visit. The RLCQ was developed from the Schedule of Recent Experience (SRE) [393] and has been in use and developed since 1975. The RLCQ assesses stressful, neutral, and positive life changes. Each life event item has further been given a weighting based

on previous research called a Life Change Unit (LCU). These weightings were determined in 1965 and then rescaled in 1977 and 1995 [392] with LCU scores ranging from 18 to 123. Therefore, the questionnaire can be scored in several ways including the number of events endorsed and the LCU total for each participant. The RLCQ covers five domains: health, home/family, financial, personal/social, and work. Questions include whether the participant has moved home, experienced the death of a spouse, had financial problems, or have been hospitalised. Participants were asked to indicate if the event had occurred in the previous 6 months by placing a tick in the applicable box provided.

The RLCQ has been used as a gold standard in stress research and is widely used across clinical and research settings. The questionnaire correlates with a number of health outcomes including psychological distress, depression, diabetic control, mortality after surgery, and myocardial infarction [394]. The RLCQ's reliability and validity has also been demonstrated and shows good predictive validity [392, 394-399].

Perceived Stress Scale (PSS) [400]

The PSS is a recognised and widely used measure of global perceived stress. The 10-item version, which originated from the previous 14-item scale, was used in the study in an effort to reduce burden on participants. Validity and reliability for the 10-item version is consistent with the original scale [401] and has been shown to predict numerous adverse health outcomes [184, 401]. Items of the scale assessing stress domains include unpredictability, lack of control, burden overload, and stressful life circumstances. Participants are required to subjectively rate how often they have experienced certain feelings or thoughts over the previous month on a 0 to 4 Likert scale (never, almost never, sometimes, fairly often, very often). The PSS has been shown to demonstrate good psychometric

properties in a range of study populations [400-402] and correlates with biological outcomes [356].

2.10.2.2 Physical stress

Both the control and aMCI participants were assessed, via clinical interview, every 6 months over the 18 month follow-up period to assess frequency of acute systemic inflammatory events and chronic inflammatory conditions. We followed previous methodology used by our academic group [403] to group all acute systemic inflammatory events (acute infections and acute physical events e.g trauma) together. Acute systemic events included upper respiratory tract infections, lower respiratory tract infections, genitourinary infections, gastrointestinal infections, other infections, accidental trauma, surgical intervention and myocardial infarction. Chronic inflammatory events (principally high blood pressure; high cholesterol and diabetes) were documented as present at baseline and present for greater than 6 months in both participant groups. GP surgery medical summaries were sourced by research personnel at visit 1 to inform the recording of physical stress. Within the limitations of retrospective reporting, the combination of sourcing medical summaries alongside research personnel interviewing aMCI participants and their study partners at each visit enabled a broad coverage of systemic inflammatory events to be recorded at baseline and throughout the study follow-up period.

2.10.3 Measurement of resilience factors

The Medical Outcomes Study - Social Support Survey (MOS-SSS) [404]

To assess social support the brief, self-administered, and multidimensional questionnaire called the MOS-SSS was given to participants at each visit.

This questionnaire contains items assessing overall social support as well as the following dimensions of support: emotional (the expression of positive affect, empathetic understanding, and the encouragement of expression of feelings), informational (offer of advice, information, guidance and feedback), tangible (provision of material aid or behavioural assistance), positive social interaction (the availability of other persons to do fun things with), and affection (involving expressions of love and affection) [404]. The measure was developed for the use in a chronically ill patient population however the authors propose the items are universally applicable. The measure uses a five-point scale ranging from none of the time, a little of the time, some of the time, most of the time, and all of the time. Higher scores reflect greater perceived social support. The lowest obtainable score is 19 and the highest is 35. The MOS-SSS has been used in many patient groups and demonstrates good psychometric properties [404-406].

Coping Inventory for Stressful Situations (CISS) [407]

To assess coping styles we administered the CISS at baseline. The CISS is a self-report 48-item inventory consisting of three scales, with 16 items per scale:

- Task-orientated coping: making efforts aimed at solving problems through cognitive restructuring or attempts to alter the situation and solve the problem.
- Emotion-oriented coping: responding to stress with self-oriented emotional reactions, with a focus on reducing own emotional tension caused by the stressor.
- Avoidance-oriented coping: a preference to avoid stressful situations in one of two ways: avoidance by social diversion and avoidance by distraction.

Participants were asked to rate how much they engaged in a range of coping strategies using a Likert scale of 1 to 5. The questionnaire provides an overall mark indicating which coping style is preferred. The highest score indicates which of the three coping styles is used. Research indicates that the CISS is a valid, reliable, and extensively used measure of coping strategies [407-409]

The Neuroticism scale of the NEO Five Factor inventory (NEO-FFI) [410]

To assess personality, specifically neuroticism, we administered the 12-item Neuroticism scale, which has been taken from the original 60-item NEO-FFI. The short form and the standard NEO FFI scale highly correlate with one another. This is a widely used measure with good reliability and validity that is well established [411-413]. The Neuroticism scale consists of the following traits: Anxiety, Hostility, Depression, Self-Consciousness, Impulsiveness, and Vulnerability to Stress. Participants were asked to rate their level of agreement on a scale of 0 (strongly disagree) to 4 (strongly agree) in response to 12 statements. The total score can fall between 0-48 with those scoring higher presenting with a more neurotic personality and subsequent proneness to distress.

2.10.4 Other variables that may influence cognition

We assessed other factors that may afford alternative explanations for significant interactions identified between variables. We recorded a range of demographics at each study visit including age, gender, education and body mass index. Medications and medical conditions were recorded including diabetes, cardiovascular disease, hypercholesterolemia, and infections which were supported by a medical summary obtained from the participant's General Practitioner. Other variables measured include mood and perceived health and well-being.

VAS scale (EQ-5D) [414]

To assess perceived health we administered the VAS scale which is part of the well-known EQ-5D. This is a simple and easy to use instrument that asks participants to rate their perceived health by placing a mark on a visual analogue thermometer-like scale of 0-100 (100= Best health state). The scale allows a generic measure of perceived overall health. The EQ-5D shows reasonable to good psychometric properties and has been widely used across health research including cognitive impairment and dementia [414-417].

Geriatric Depression Scale (GDS) [418]

The GDS was administered by a researcher to assess depression at baseline. This is a well-established and widely used scale developed to rate depression specifically in an older population. The GDS was formed to overcome the limitations of other depression scales that were predominantly developed in healthy younger adults. The maximum score obtainable is 15 with a score over 5 indicating possible depression. The scale covers a range of symptoms relevant to depression in this population including lowered mood, poor self-image, and a lack of motivation.

This scale is simple to administer with each question having a yes/no answer. Participants are asked to only think about how they have felt in the previous 7 days when answering the questions. In this study the shorter 15-item version scale was used in an effort to decrease burden on participants. The shortened version has been used in a variety of settings and demonstrates good validity and reliability including with those diagnosed with cognitive impairment [418-422]. The shortened version has been shown to highly correlate with the original 30 item GDS [418].

2.10.5 Biological measures

To determine if cognitive decline and stress were associated with biological parameters, blood and saliva samples were collected longitudinally across all 4 visits. Collection of samples helped determine whether the immune system; the HPA axis and ApoE ε4 were involved in the progression from aMCI to a more severe stage of aMCI over the study's 18 month follow-up period.

2.10.5.1 Blood collection and analyses

A serum blood sample for inflammatory markers and for genetic status was obtained by venepuncture of a peripheral vein. Samples were transferred at regular time points from sites to store at the University of Southampton laboratory located in Southampton General Hospital. Samples were only identifiable by a unique study number. No other personal data was used for the purpose of labelling stored samples. The Southampton General Hospital and Moorgreen Hospital laboratory was locked at all times. Access was strictly limited to authorised personnel. Only the Chief Investigator and designated research personnel had full access to the stored samples.

Venous blood samples were collected at the inclusion visit and then at every 6 month follow-up visit.

- **DNA analysis:** Collection rates for control participant (91%) and aMCI participant (93%) were good. The study focused on the possession of an ApoE-ε4 allele which is a well-established risk factor for cognitive decline in older adults and has been implicated as a modulator of systemic inflammation. Venous blood samples

were collected and stored in accordance with routine protocols at minus 80˚C. Blood for DNA analysis was primarily intended for ApoE ε4 analysis but samples were also banked for further analysis.

- **Cytokine and CRP analysis:** At baseline collection rates for control participants (92%) and aMCI participants (94%) were good. Blood assays for Th1 and Th2 cytokine profiles and C-reactive protein were quantified from blood using Mesoscale multiplex ELISA plates. The study focused on pro-inflammatory cytokines TNFα; IL6; IFNγ and the anti-inflammatory cytokines IL10 and TGFβ. However, additional samples were banked in a minus 80˚C freezer so that the role of other cytokines can be further explored.

All blood samples were analysed in the same batch as previously described to reduce the chances of measurement error [403] and were analysed blind to the clinical data.

2.10.5.2 Saliva collection and analyses

Cortisol is a highly used biomarker of HPA axis activity and is sensitive to psychological stress [423]. Measuring cortisol in saliva is considered a non-intrusive sampling method in this population. Cortisol was assessed from saliva to determine HPA axis activation and potential HPA dysregulation. Cortisol levels were assayed using sensitive commercial assays at Trier University.

Collection rates for salivary cortisol sufficiently powered analyses: control participants (99%) and aMCI participants (81%). Participants were asked to provide six saliva samples over one day within one week following/prior to each study visit. Saliva was collected using the Salivette device (Sarstedt, Germany). Saliva swabs and instructions were provided to participants at

visit 1 with an opportunity for participants and their informants to ask questions. Materials were subsequently sent to each participant at their home prior to the study follow-up visit. Participants were instructed of the times of collection which included one sample immediately upon awakening (by 8:30am), one sample 30 minutes after the first sample, and then a sample taken at 11am, 3pm, 6pm and 9pm. To prevent contamination, participants were asked to refrain from the following activities during the first 30 minutes after awakening and also 30 minutes before each subsequent saliva sample taken during the rest of the day:

- Eating
- Drinking (water was allowed)
- Exercise
- Smoking
- Brushing teeth

Participants were asked to record the date and time that samples were taken on a study log provided for each visit. Study partners assisted aMCI participants with this process to ensure compliance with the procedures. Samples were refrigerated at the participant's home until collected by study personnel or returned by the participant within one week (preferably a few days) of the samples being taken. Once at site, samples were immediately stored at minus 80˚C.

There are three cortisol measures as follows:

Sample 1: First sample immediately upon the participant awakening.

CAR: The cortisol awakening response is indicated by the difference between the measure 30 minutes after awakening (S2) and the awakening measure (S1). Besides the CAR (which reflect the cortisol increase after awakening), the S1 measure was used for analysis (Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010; Stalder et al., 2016). Participant-reported

timing of assessment was used to compute a time-difference variable that should be used to exclude those CARs with less than 5 min or more than 45 min difference. CAR is missing if S1 or S2 were missing (due to either not being provided, or out-of-time, or cortisol concentration being extremely high).

AUC: The area under the daytime cortisol curve indicates average cortisol output over the day, and is therefore a measurement of individual 'exposure' to cortisol. Computation did not include S2 to avoid confounding with the CAR. The AUC is missing if any of S1, S3, S4, S5, or S6 was missing (due to either not being provided, or out-of-time, or cortisol concentration being extremely high).

2.11 Conversion from aMCI to dementia

At each study visit aMCI participants were seen by research personnel to assess whether the participant had potentially converted from aMCI to dementia. For this process, the participant's overall level of comprehension was assessed in addition to the participant's clinical history, neuropsychometric test results, and everyday independent functioning. Case by case discussions resulted in a final decision being made by a medical doctor. For participants suspected to have converted to dementia, a referral was made to the Principal Investigator of each study site who subsequently reviewed the participant within their memory service. Likewise, a referral was also made to the Principal Investigator for participants between study visits reporting a significant cognitive and/or functional deterioration. AD diagnosis was made using the NINCDS-ADRDA criteria [374]. The NINCDS-ADRDA outlines that a diagnosis should not be made in the presence of a substantial concomitant cerebrovascular disease, other dementias, active neurological disease, non-neurological medical comorbidity or use of medication that could affect cognition.

Chapter 3: Statistical methods and planned analyses

3.1 Statistical analyses

The analyses follows our hypotheses and associated objectives. All significant analyses were assessed and if necessary corrected for the key potential confounders age, gender, education and BMI. The potential confounding effect of the key genetic variable ApoE ε4 status, a known key variable associated with increased cognitive decline, was also systematically assessed.

Data distribution was assessed using Q-Q plots. Parametric data was assessed initially with independent t tests or Pearson correlation with linear regression analysis to adjust for possible confounders. Non-parametric data was, where possible, transformed to a parametric distribution e.g. cortisol samples were transformed using Box-Plot analyses. Non transformed non-parametric data was assessed by a combination of Mann Whitney U or Spearman correlation or was dichotomised. Cytokine data and stress data were also dichotomised following previous methods that have suggested a threshold effect rather than a linear response. Dichotomised data was assessed using chi squared analysis or logistic regression analysis for assessment of confounders.

Statistical advice was obtained from David Culliford (Research design services, Southampton General Hospital) on whether to apply MMRM analyses to the data set. However, MMRM assumes missing data points are missing at random, whereas in this study, early withdrawals were predominantly due to participants converting to dementia or reporting high psychological stress levels. Thus, it was important to capture the effects of these early withdrawal reasons on the study end points.

Furthermore, MMRM analyses rule out the longer term effects of events whereas in this study, we were interested in the influence of chronic and accumulative stress on cognition. Therefore, overall, Last Observation Carried Forward (LOCF) analyses was considered the most appropriate tool.

We followed a systematic approach to the data analyses but have restricted our data presentation to the positive or important negative findings related to our study hypotheses.

3.2 Determination of sample size

Power of the study was based on our primary hypothesis and objective with statistical advice being obtained from David Culliford (Research design services, Southampton General Hospital). Power calculations of 134 participants were based on the assumption found in a previous study [61] that approximately 50% of aMCI participants will experience a negative life event over an 18 month follow-up period. 100 participants will give 80% power to detect a significant (alpha = 0.05) increase of 0.5 s.d. point in the FCSRT-IR in the group with negative life events compared to the group without negative life events. Allowing for a 25% dropout rate over the 18-month study follow-up period would require 134 participants. The smaller size in the control group is because we were not planning, or expecting to see longitudinal change in cognition in the control group but it is powered to detect differences in 4 biological/life event parameters cross-sectionally at one time point (134 aMCI group and 67 in the control group gives 80% power to detect 0.5 standard deviation differences in life events and cortisol levels α = 0.0125).

Chapter 4: Results

Table 2, 3 and 4 provide a summary of key findings observed between the primary outcome for the study, FCSRT total score, and psychological stress, physical stress, and the biological parameters (salivary cortisol and serum inflammatory markers).

Table 2. Change in FCSRT total score LOCF in participants by psychological stress

		RLCQ stress measure		Significance	PSS stress measure		Significance
Study group	**Control**	**BASELINE stress** M: 160.2 pts SD: 133.4		P=0.061 Mean diff: 35.0 pts 95% CI -1.7 to 71.7	**BASELINE stress** M: 12.1 pts SD: 7.0		**P=0.027** Mean diff: 2.4 pts 95% CI 0.3 to 4.4
	aMCI	**BASELINE stress** M: 125.1 pts SD: 104.2			**BASELINE stress** M: 14.5 pts SD: 7.1		
aMCI group: dFCSRT-IR rate of decline		**Visit 2-4** RLCQ low stress	N:39 M:-1.6 SD: 5.4	**P=0.012** Mean diff: 3.1 95% CI 0.7 to 5.4	**Visit 2-4** PSS low stress	N: 42 M: -3.8 SD: 7.4	P= 0.86 Mean diff: -0.2 95% CI -2.8 to 2.4
		Visit 2-4 RLCQ high Stress	N:79 M:-4.6 SD:7.2		**Visit 2-4** PSS high stress	N: 79 M: -3.6 SD: 6.5	

N = number of participants **M** = mean **SD** = Standard Deviation **pts** = points **Mean diff** = Mean difference **CI** = Confidence Interval $\mathbf{X^2}$ = Chi Square **Cortisol Sample 1** = cortisol measure immediately upon awakening **dFCSRT-IR** = change in cognitive decline as measured by the Free and Cued Recall Selective Reminding Test – Immediate Recall *Significance at p <0.05

Table 3 Change in FCSRT total score LOCF in participants by physical stress

		RLCQ stress measure		Significance	PSS stress measure		Significance
Study group	**Control**	**BASELINE stress** M: 160.2 pts SD: 133.4		P=0.061 Mean diff: 35.0 pts 95% CI -1.7 to 71.7	**BASELINE stress** M: 12.1 pts SD: 7.0		**P=0.027** Mean diff: 2.4 pts 95% CI 0.3 to 4.4
	aMCI	**BASELINE stress** M: 125.1 pts SD: 104.2			**BASELINE stress** M: 14.5 pts SD: 7.1		
aMCI group: dFCSRT-IR rate of decline		**Visit 2-4** RLCQ low stress	N:39 M:-1.6 SD: 5.4	**P=0.012** Mean diff: 3.1 95% CI 0.7 to 5.4	**Visit 2-4** PSS low stress	N: 42 M: -3.8 SD: 7.4	P= 0.86 Mean diff: -0.2 95% CI -2.8 to 2.4
		Visit 2-4 RLCQ high Stress	N:79 M:-4.6 SD:7.2		**Visit 2-4** PSS high stress	N: 79 M: -3.6 SD: 6.5	

N = number of participants **M** = mean **SD** = Standard Deviation **pts** = points **Mean diff** = Mean difference **CI** = Confidence Interval $\mathbf{X^2}$ = Chi Square **ASIEs** = Acute Systemic Inflammatory Events **dFCSRT-IR** = change in cognitive decline as measured by the Free and Cued Recall Selective Reminding Test – Immediate Recall
*Significance at p <0.05

Table 4 Change in FCSRT total score LOCF in participants by biological parameters

		RLCQ stress measure		Significance	PSS stress measure		Significance
Study group	**Control**	**BASELINE stress** M: 160.2 pts SD: 133.4		P=0.061 Mean diff: 35.0 pts 95% CI -1.7 to 71.7	**BASELINE stress** M: 12.1 pts SD: 7.0		**P=0.027** Mean diff: 2.4 pts 95% CI 0.3 to 4.4
	aMCI	**BASELINE stress** M: 125.1 pts SD: 104.2			**BASELINE stress** M: 14.5 pts SD: 7.1		
aMCI group: dFCSRT-IR rate of decline		**Visit 2-4** RLCQ low stress	N:39 M:-1.6 SD: 5.4	**P=0.012** Mean diff: 3.1 95% CI 0.7 to 5.4	**Visit 2-4** PSS low stress	N: 42 M: -3.8 SD: 7.4	P= 0.86 Mean diff: -0.2 95% CI -2.8 to 2.4
		Visit 2-4 RLCQ high Stress	N:79 M:-4.6 SD:7.2		**Visit 2-4** PSS high stress	N: 79 M: -3.6 SD: 6.5	

n = number of participants **m** = mean **SD** = Standard Deviation **pts** = points **M diff** = Mean difference **CI** = Confidence Interval **Cortisol Sample 1** = cortisol measure immediately upon awakening **AUC** = Area Under the Curve **dFCSRT-IR** = change in cognitive decline as measured by the Free and Cued Recall Selective Reminding Test – Immediate Recall *Significance at p <0.05

4.1 Core demographics

4.1.1 Baseline core demographics

A total of 203 participants were recruited (68 control participants and 135 aMCI participants). The key demographic variables are listed in Table 5. Age and years of education were normally distributed as determined by Q-Q plots for both participant groups. The controls were younger, more likely to be female, and better educated.

Table 5. Comparison of the key baseline demographic variables between the control and aMCI group

	Control (n=68)	**aMCI** (n=135)	**Statistical significance**
Mean age	68.4 years SD = 9.4	77.6 years SD = 7.4	**P <0.0001*** Mean difference: 9.2 yrs 95% CI -11.6 to -6.8
Gender	47 (F) 69.1%	52 (F) 38.5%	**P <0.0001*** χ^2 16.9 (*df* =1)
	21 (M) 30.9%	83 (M) 61.5%	
Mean years of education	13.9 years SD = 3.4 years	12.5 years SD = 3.4 years	**P =0.007*** Mean difference: 1.4 yrs 95% CI 0.4 to 2.4

n = number of participants SD = Standard deviation X^2 = Chi Square yrs = years *Significance at p <0.05

The key baseline medical variables are listed in Table 6. BMI was also normally distributed as determined by Q-Q plots for both participant groups. A total of 15 participants (5 controls and 10 aMCI participants) were not genotyped for ApoE ε4 status due to insufficient blood sampling or a failure of the assay.

Table 6. Comparison of the key baseline medical variables between the control and aMCI group

	Control	aMCI	Statistical significance
Mean BMI**	n= 68 27.5 units SD = 5.2	n = 135 27.2 units SD = 4.7	P = 0.7 Mean difference: 0.3 units 95% CI -1.1 to 1.8
ε4 status Negative	n = 46 (40.0%)	n = 69 (60.0%)	**P= 0.018*** X^2= 5.598
ε4 status Positive	n = 17 (23.3%)	n = 56 (76.7%)	

*Significance at p <0.05 **BMI calculated by dividing weight in kilograms by height in meters and then dividing the answer by height.
There is no statistical difference between groups in BMI however, aMCI participants were more likely to be an ε 4 carrier.

A total of 203 participants completed visit 1, 188 completed visit 2, 167 completed visit 3, and 155 completed visit 4 across all three research sites. Of the 48 participants who did not complete the study there were 18 early withdrawals (reasons: 18 moving out of the area or the experience of testing too stressful) and 30 conversions to dementia.

Summary: The aMCI group were older, more likely to be male, have less years of education and be ε 4 positive than the control group.

4.1.2 Psychological stress

Psychological stress was measured at baseline and at each subsequent visit for both participant groups. The objective psychological stress scale, RLCQ, was the primary measure of interest whilst the subjective stress

scale, PSS, served as a secondary measure. Both the RLCQ and PSS scores were normally distributed as determined by Q-Q plots for both participant groups.

Table 5. Baseline stress scores.

	Control (n=68)	**aMCI (n=135)**	**Statistical significance**	**Adjusted statistical significance****
RLCQ	Mean: 160.2 pts SD= 133.4	Mean: 125.1 pts SD= 104.2	P=0.061 Mean difference: 35.0 pts 95% CI: -1.7 to 71.7	P=0.66 Mean difference: 8.8 pts 95% CI: -48.5 to 30.9
PSS	Mean: 12.1 pts SD = 7.0	Mean: 14.5 pts SD = 7.1	**P=0.027*** Mean difference: 2.4 pts 95% CI: 0.3 to 4.4	**P=0.012*** Mean difference: 3.2 pts 95% CI: 0.8 to 5.7

*Significant at p <0.05. ** Adjusted for age, gender and education.

Life event stress: RLCQ

As shown in Table 7 there was a suggestion ($p < 0.1$) of slightly increased life events in the control group compared to the aMCI group (mean difference 35.0 pts p=0.061) at baseline. However, when we apply linear regression analysis and corrected for baseline demographics (age, gender and education) the statistical difference in objective stress was no longer significant (p=0.66).

During the course of the study from visits 2 to 4 we also saw no significant difference in life event scores between the aMCI and control group (visit 2 mean difference 4.1pts p =0.8; visit 3 mean difference 21.7 pts p =0.2, visit 4 mean difference -3.5 pts p = 0.9) (see appendix 1.0).

Perceived stress: PSS

There was significantly increased perceived stress scores in the aMCI group compared with the control group (mean difference 2.4 pts p=0.027). This difference remained significant after adjusting for baseline demographics (age, gender and education) (mean adjusted difference 3.2 pts p = 0.012).

During the course of the study from visits 2 to 4 we also saw an increase in PSS scores in the aMCI group compared to the control group (visit 2 adjusted mean difference 3.3pts p =0.01; visit 3 adjusted mean difference 3.3 pts p =0.01, visit 4 adjusted mean difference 3.6 pts p =0.008).

In summary: After adjustment for the key demographic confounders, the aMCI group appear to perceive more stress than the control group even though there was no statistical difference in objective life event stress between the participant groups. During the study follow-up period the aMCI group continued to experience greater perceived stress than compared to the control group.

4.1.3 Physical stressors

Acute systemic inflammatory events were dichotomised into present or absent if no event took place. A mixture of analysis methods was used to examine whether core demographics (age, BMI, gender, ε4 status, education) influenced the frequency of reported physical stressors (acute and chronic stress) in both participant groups at baseline and during the study follow-up period. Independent Samples T Test was applied to the mean years of education, age and BMI whilst Chi Square analysis was applied to ε4 status and gender. In 3 cases we were unable to identify a

more detailed breakdown of their medical records and thus these cases were excluded from analysis.

Firstly, we examined the prevalence of acute systemic inflammatory events (ASIE's) in the control and aMCI group in the 6 months preceding or present at baseline and then throughout the 18 month follow-up period (Table 8). In the 6 month period prior to baseline we found that acute systemic inflammatory events were more common in the aMCI group compared with the control group (21.6% of the aMCI group compared with 9.1% of the control group p=0.03). Acute systemic inflammatory events remained significant after correcting for baseline demographic variables age, gender and education (p = 0.01).

Table 6. Baseline acute systemic inflammatory events.

		Control	aMCI	Total	Statistical significance
Baseline acute systemic inflammatory Events	none in previous 6 months	60 (90.9%)	105 (78.4%)	165	X^2= 4.825 **P=0.03***
	one or more in previous 6 months	6 (9.1%)	29 (21.6%)	35	Correction for age; gender; education **P=0.01***

*Significance at p <0.05

However, there were no significant differences in the number of acute systemic inflammatory events reported between the control and aMCI groups during the 18 month follow-up period (Control: 38 ASIE's [19.0%] c.f. aMCI 71 ASIE's [35.5%] X^2= 0.376 p=0.54).

Chronic inflammatory events

We then examined the presence of 3 chronic inflammatory events (high blood pressure; high cholesterol and diabetes) documented as present at baseline and present for greater than 6 months in both participant groups. We found that high blood pressure was reported with more frequency in the aMCI group than the control group (aMCI 72 (53%) participants c.f control 24 (35%) X^2=5.9 p=0.015). However, this relationship was not significant once the basic demographics, age, gender and education had been corrected for (adjusted p = 0.3). High cholesterol was not reported as more frequent in the aMCI group compared with the control group (aMCI 69 (51%) participants c.f control 26 (38%) X^2=3.0 p=0.08). Likewise, diabetes was reported with more frequency in the aMCI group than the control group (aMCI 21 (16%) participants c.f control 3 (4%) X^2=5.3 p=0.02) but was not significant once the basic demographics, age, gender and education had been corrected for (adjusted p = 0.11). The demographic, ε4 status, was unrelated to chronic physical stress in both the control and aMCI group (appendix 1.1).

In summary, in the 6 months prior to baseline, acute systemic inflammatory events occur more regularly in the aMCI group than the control group but this was not replicated throughout the course of the study. Chronic inflammatory events were not found to occur more frequently in the aMCI group compared with the control group.

4.1.4 Cognitive demographics

The FCSRT total score was the primary outcome for the study with the MoCA and TMT Part B acting as secondary outcomes. The FCSRT total score at baseline was not normally distributed as determined by the Q-Q

plot in the control group but was normally distributed in the aMCI group. The MoCA data at baseline was normally distributed as determined by the Q-Q plot in both participant groups. The TMT data at baseline was not normally distributed as determined by the Q-Q plot in the aMCI group but was normally distributed in the control group.

Baseline

As expected at baseline there were highly significant differences observed in the FCSRT total, the MOCA and TMT Part B between the control and aMCI group (Table 9) with better performance on all measures in the control group.

Table 7. Key baseline cognitive variables

	Control (n=68)	**aMCI (n=135)**	**Statistical significance**	**Adjusted** significance**
FCSRT total	Median = 48 pts IQR [48.0 to 48.0]	Median = 42 pts IQR [34.0 to 47.0]	**P<0.0001***	**P<0.0001***
MoCA	Mean = 27.9 pts SD = 1.5	Mean = 23.0 pts SD = 2.7	**P<0.0001*** Mean difference = 4.9 95% CI 4.4 to 5.6	**P<0.0001*** Adjusted mean difference = 16.2 95% CI 14.0 to 18.3
TMT Part B	Median = 78.5 secs IQR [65.3 to 96.8]	Median = 161.0 secs IQR [111.0 to 240.0]	**P<0.0001***	**p<0.0001***

*Significant at p <0.05. **adjusted for age, gender and education

FCSRT total score

The unadjusted MWU test showed a significant difference between groups. We were unable to perform linear regression, due to the data distribution

not being normal. We therefore took statistical advice from David Culliford at the University of Southampton who advised to categorise data using the median score. We transformed the FCSRT total into a binary variable based on the median score of 47 for the entire group. Those scoring less than 47 were designated as a low score whereas those scoring 47 and above were designated as a high score. This allows participants to be dichotomized into 2 groups. Using the Chi square test, 56 participants (82%) in the control group score greater than the median compared with 23 of 135 participants (17%) in the aMCI group (χ^2 = 81.2 p<0.0001). The distribution remained significant after adjusting for age, gender and years of education using logistic regression (p<0.0001).

MoCA

The unadjusted t test showed a significant lower MoCA score in the aMCI group compared to the control group (P<0.0001 Mean difference 4.9pts 95% CI = 4.4 to 5.6). Adjustment for gender, age or mean education did not substantially alter this relationship (P<0.0001 adjusted mean difference 16.2 pts 95% CI 14.0 to 18.3).

TMT Part B

The MWU test shows a statistical difference between groups. We were unable to perform linear regression, due to the data distribution not being normal. As above, we took statistical advice on managing non-parametric data and categorised data using the median score. We therefore transformed the Part B score into a binary variable based on the median score of 122 for the entire group. Thus those scoring less than 122 were designated as a low speed score whereas those scoring 122 and above were designated as a high speed score. This allowed participants to be dichotomized into 2 groups. Using the Chi square test, 56 of 68

participants (82%) in the control group performed the task in less than the median of 122 seconds compared with 46 of 135 participants (34.1%) in the aMCI group (χ^2= 42.2 p<0.0001). The distribution remained significant after adjusting for age, gender and years of education using logistic regression (p<0.0001).

Rate of cognitive decline over course of study

All cognitive data during the course of the study follow-up period was normally distributed as determined by the Q-Q plot in both participant groups. Table 10 shows rates of cognitive decline (LOCF analysis) during the course of the study in both participant groups. As anticipated, highly significant associations were evidenced with aMCI participants performing worse on all cognitive measures. These associations were not significantly altered when adjusting for age, gender and education.

No significant relationships were found between baseline cognitive scores with the rate of cognitive decline on any of these cognitive measures.

Table 8. Change in cognitive score from baseline to Month 18, including LOCF .

	Control (n=68)	**aMCI (n=123)**	**Statistical significance**	**Adjusted statistical significance****
dFCSRT total LOCF	0.06 pts SD = 1.3	-3.7 pts SD= 6.8	**P<0.0001*** Mean difference: 3.8 pts 95% CI 2.1 to 5.5	**P<0.0001*** Mean difference: 4.2 pts 95% CI 2.2 to 6.1
dMOCA LOCF	0.24 pts SD = 1.8	-1.7 pts SD = 2.8	**P<0.0001*** Mean difference: 1.9 pts 95% CI 1.2 to 2.7	**P<0.0001*** Mean difference: 1.9 pts 95% CI 1.0 to 2.8
dTMTb	-5.2 secs SD 30.1	40.0 secs SD 125.6	**P= 0.005*** Mean difference: 45.2 secs 95% CI 14 to 76	**P= 0.01*** Mean difference: 48.0 secs 95% CI 11 to 84

*Significance at p <0.05 **adjusted for age, gender and education

In summary: The aMCI group show significant cognitive impairment compared with the control group at baseline across all the key cognitive tasks. Moreover, significantly greater rates of cognitive decline on all cognitive outcomes were evidenced in the aMCI group over the 18 month follow-up period compared to the control group.

4.1.5 Comparison between core demographics and medical variables and psychological stress

Core demographics and medical variables and psychological stress in control participants

No relationship was found between demographic variables age, gender and education with objective (RLCQ) or subjective (PSS) measures of stress at baseline (appendix 1.2). No relationship was found between BMI and objective measures of stress (RLCQ) at baseline in control participants (Pearson 0.15 p=0.225) but control participants with a greater BMI were more likely to report increased levels of subjective stress (Pearson 0.25 p = 0.041). The relationship between greater BMI and increased subjective stress was not, however consistently present throughout the study follow-up period (visit 2 Pearson 0.34 p = 0.006; visit 3 Pearson 0.14 p = 0.26; visit 4 Pearson 0.13 p = 0.31). The presence of ApoE ε4 was not associated with alterations in levels of objective or subjective stress measures at baseline (appendix 1.2).

Core demographics and medical variables and psychological stress in aMCI participants

Younger participants reported increased levels of objective stress on the RLCQ (Pearson -0.290 p=0.001) but no relationship was found between age and the PSS (Pearson -0.128 p=0.147). The relationship between young

age and increased objective (RLCQ) stress was not, however consistently present throughout the study follow-up period (visit 2 Pearson -0.025 p = 0.78; visit 3 Pearson 0.17 p = 0.09; visit 4 Pearson -0.015 p = 0.89). No relationship was found between the other demographic variables, gender and education, with objective (RLCQ) or subjective (PSS) measures of stress (appendix 1.2). No relationship was found between BMI and objective measures of stress (RLCQ) or subjective (PSS) measures of stress. The presence of ApoE ε4 was not associated with alterations in levels of objective or subjective stress measures (appendix 1.2).

In summary: Analysis identified age and BMI to influence psychological stress at baseline but this wasn't a consistent finding across the study follow up period. The remaining core demographic and medical variables were not associated with measures of psychological stress.

4.1.6 Comparison between the core demographics and physical stress

Independent Samples T Test was applied to the mean years of education, age and BMI whilst Chi Square analysis was applied to ε4 status and gender. Due to an increased likelihood of short-term memory impairment in those diagnosed with aMCI, research personnel interviewed the study partner in addition to the participant with aMCI at each visit to increase accuracy of retrospective reporting. Moreover, medical summaries were also obtained from the aMCI participant's GP Surgery at baseline.

<u>Baseline core demographics and physical stressors in control participants</u>

Table 11 shows the relationship between the demographic variables age and gender with the presence of acute inflammatory events six months prior to baseline and chronic inflammatory events in control participants. Younger participants were more likely to report the presence of acute systemic inflammatory events prior to baseline. However, this finding was not consistent throughout the study and at later visits no relationship was found between age and reported acute systemic inflammatory events ($p > 0.1$ all cases not shown). Younger participants were less likely to report high blood pressure but no relationship was found between age and reported high cholesterol and diabetes. Gender was not related to reported systemic inflammatory events prior to or during the study period. However, male participants had a higher reported frequency of hypertension, high cholesterol and diabetes. Education years was not related to reported acute or chronic inflammatory events.

BMI was not related to acute systemic inflammatory events prior to baseline or during the course of the study. Increased BMI was associated with an increase reporting of diabetes (diabetes present n=6 mean BMI 34.7 units c.f diabetes absent n = 65 mean BMI 27.2 units mean difference 7.5 95% CI 1.7 to 13.4 $p = 0.012$). No relationships were found between BMI with hypertension or high cholesterol ($p > 0.1$ all cases not shown). The presence of ApoE ε4 was not associated with the presence of acute systemic inflammatory events at baseline or throughout the study period, neither was it associated with chronic physical stress ($p > 0.1$ all cases not shown).

Table 9. Core demographics and physical stress in control participants at baseline

		Age		Male	Female		BMI		
High Blood pressure	No	N=44 Mean= 66.7 SD= 8.7	Independent samples T-test P= 0.048* Mean diff= -4.7 95% CI= -9.34 to -0.04	10 23%	34 77%	X^2= 3.884 P= 0.049*	N=44 Mean=14.2 SD= 3.9	N=44 Mean=27.0 SD= 5.1	Independent samples T-test P= 0.273 Mean diff= -1.4 95% CI= -4.068 to 1.168
	Yes	N=24 Mean= 71.4 SD= 10.0		11 46%	13 54%		N=24 Mean=13.4 SD= 2.6	N=24 Mean=28.4 SD= 5.3	
High Cholesterol	No	N=42 Mean= 67.1 SD=9.2	Independent samples T-test P = 0.152 Mean diff= -3.4 95% CI= -8.01 to 1.27	9 21%	33 79%	X^2= 4.599 P= 0.032*	N=42 Mean=14.2 SD= 3.6	N=42 Mean=27.1 SD= 5.4	Independent samples T-test P= 0.411 Mean diff= -1.1 95% CI= -3.655 to 1.514
	Yes	N=26 Mean=70.5 SD= 9.5		12 46%	14 54%		N=26 Mean=13.4 SD= 3.1	N=26 Mean=28.2 SD= 4.9	
Diabetes	No	N=65 Mean= 68.5 SD=9.5	Independent samples T-test P=0.527 Mean diff= 3.5 95% CI=-7.58 to 14.65	18 28%	47 72%	X^2= 7.024 P= 0.008*	N=65 Mean=13.9 SD= 3.5	N=65 Mean=27.2 SD= 4.9	Independent samples T-test P= 0.012* Mean diff= -7.6 95% CI=-13.419 to -1.697
	Yes	N= 3 Mean= 65.0 SD= 4.6		3 100%	0 0%		N=3 Mean= 13.3 SD= 3.5	N=3 Mean= 34.7 SD= 6.5	
Acute systemic inflammatory events	No	N=60 Mean= 68.8 SD= 9.2	Independent samples T-test P= 0.046* Mean diff= 8.0 95% CI= 0.16 to 15.84	19 32%	41 68%	X^2= 0.581 P= 0.446	N= 60 Mean=13.7 SD= 3.1	N=60 Mean=27.5 SD= 5.3	Independent samples T-test P= 0.681 Mean diff= -0.9 95% CI= -5.424 to 3.567
	Yes	N=6 Mean= 60.8 SD= 8.7		1 17%	5 83%		N=6 Mean= 16.0 SD= 5.8	N=6 Mean=28.4 SD= 4.5	
Infections	No	N= 64 Mean= 68.6 SD=9.1	Independent samples T-test P= 0.020* Mean diff= 15.6 95% CI=2.574 to 28.583	20 31%	44 69%	X^2= 0.897 P= 0.344	N=64 Mean=14.0 SD=3.5	N=64 Mean=27.5 SD= 5.2	Independent samples T-test P= 0.540 Mean diff= -2.3 95% CI=-9.846 to 5.207
	Yes	N= 2 Mean= 53.0 SD=4.2		0 0%	2 100%		N= 2 Mean= 13.0 SD=0.0	N= 2 Mean= 29.8 SD=6.2	

Baseline core demographics and physical stressors in aMCI participants

Age was unrelated to the presence of acute systemic inflammatory events prior to or during the course of the study. Age was also unrelated to reported hypertension or diabetes ($p > 0.1$ all cases not shown). However, aMCI participants who were older (mean age 66 yrs vs 69 yrs mean difference 4.7 yrs 95% CI: 2.320 to 7.129 p=<0.0001) had a significantly increased likelihood of reporting high cholesterol. Gender was also unrelated to the presence of acute systemic inflammatory events prior to or during the course of the study and was also unrelated to reported hypertension or diabetes ($p > 0.1$ all cases not shown). However, men had a significantly increased likelihood of reporting high cholesterol (male: 48 [35.6%] vs female: 21 [15.6%] $X^2= 3.894$ p=0.048). Years of education was unrelated to acute or chronic physical stress ($p > 0.1$ all cases not shown).

However, a high BMI was consistently associated with increased rates of chronic physical stress including high blood pressure, high cholesterol, and diabetes (Table 12).

Table 10. Baseline demographics and BMI in aMCI participants

		BMI	
High Blood pressure	No	N= 63 Mean= 25.3 SD= 3.4	Independent samples T-test **P= <0.0001*** Mean diff= -3.4 95% CI=-4.9229 to -1.9675
	Yes	N= 72 Mean= 28.8 SD= 5.2	
High Cholesterol	No	N= 66 Mean= 25.5 SD= 3.7	Independent samples T-test **P= <0.0001*** Mean diff= -3.3 95% CI= -4.8153 to -1.7712
	Yes	N= 69 Mean= 28.8 SD= 5.1	
Diabetes	No	N= 114 Mean= 26.7 SD= 4.6	Independent samples T-test **P= 0.003*** Mean diff= -3.3 95% CI= -5.4372 to -1.1017
	Yes	N= 21 Mean= 29.9 SD= 4.9	

*Significance at $p < 0.05$

The presence of ApoE ε4 was not associated with the presence of acute systemic inflammatory events prior to baseline (ApoE ε4 positive n = 8 [15%] participants had prior acute systemic events c.f. ApoE ε4 negative n = 19 [27%] X^2= 3.03 p=0.08). However, the presence of ApoE ε4 was associated with a reduced presence of acute systemic inflammatory events throughout the study (ApoE ε4 positive n = 23 [34%] participants had acute systemic events c.f ApoE ε4 negative n = 43 [65%] X^2= 5.2 p=0.023). Further examination showed that this relationship was stronger when examining the presence of infections alone (rather than all acute systemic inflammatory events) (ApoE ε4 positive n = 13 [27%] participants had acute systemic infections c.f ApoE ε4 negative n = 35 [73%] X^2= 9.5 p=0.002). The presence of ApoE ε4 was not associated with the reported high blood

pressure and high cholesterol. However, those who were ApoE ε4 positive in the aMCI group were less likely to report diabetes (p=0.049).

Summary: Male participants were more likely to report a history of high cholesterol across both participant groups with aMCI males also reporting a higher likelihood of having high blood pressure and diabetes. A high BMI was associated with an increased risk of having diabetes in both participant groups with controls with a high BMI also reporting a higher likelihood of having high blood pressure and diabetes. aMCI Carriers of ApoE ε4 had a reduced risk of reporting diabetes and developing acute systemic inflammatory events, particularly infections but this was not found in the control group.

4.1.7 Comparison between core demographics and cognition

The relationship between the core demographics (age, gender and years of education) and core medical variables (BMI and ε4) and cognition was examined. The primary cognitive outcome for the study was the FCSRT total score. The MoCA and TMT Part B acted as secondary measures and these cognitive measures were only explored for relationships if the FCSRT total score was significant.

<u>Relationship between demographics and core medical variables and cognition in the control group</u>

Pearson correlation showed baseline cognition, as determined by the FCSRT total, was unrelated to age (Spearman -0.047 p=0.704); gender (MWU p = 0.337); years of education (Spearman-0.007 p=0.954); BMI (Spearman 0.101 p=0.411) or ApoE ε4 (MWU p = 0.53). We saw the same pattern for rate of decline, as shown by the change in FCSRT LOCF with

age (Pearson -0.108 p=0.378), gender (males 0.38 pts v.s. female -0.09 pts mean diff 0.46 pts (95% CI -0.2 to 1.2) p= 0.16); years of education (Pearson 0.114 p=0.356); BMI (Pearson -0.036 p=0.770) and ApoE ε4 (ε4 negative: 0.15 pts vs ε4 positive: -0.2 pts mean diff 0.3pts 95% CI -0.4 to 1.1 p= 0.4).

Relationship between demographics and core medical variables and cognition in the aMCI group

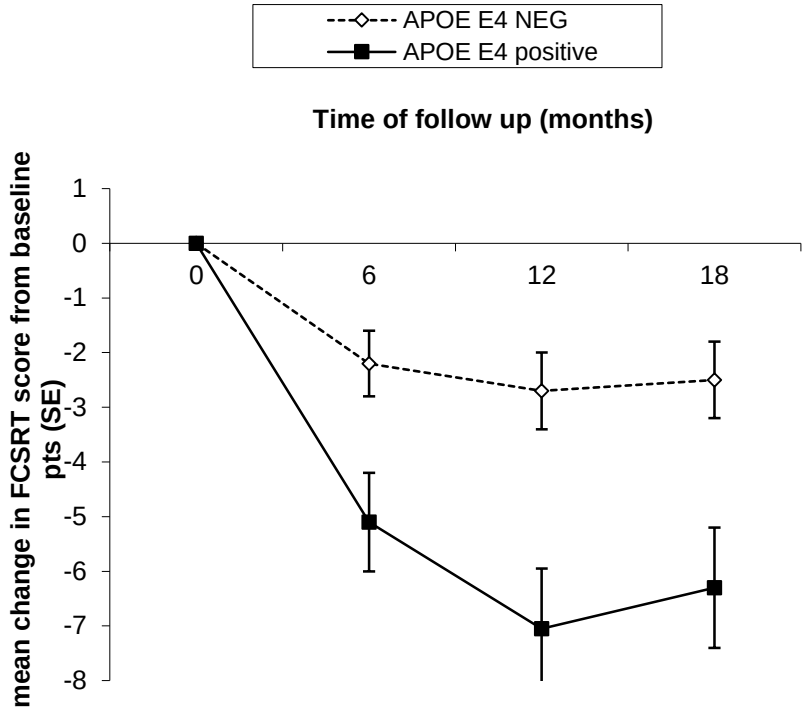
Pearson correlation showed baseline cognition, as determined by the FCSRT total, was unrelated to age (Spearman -0.14 p = 0.11). However males performed better on the FCSRT total (p=<0.0001 Male 46 pts IQR [39 TO 47] vs female 35 pts [28 to 45 pts]) which was also found on the MoCA (Male: -3.3pts vs female: -4.5 pts p= <0.0001 95% CI 1.445 to 3.151) and TMT Part B (MWU=0.052, male: 155.0secs IQR: 107.0 to 227.0 vs female 201.0 secs IQR: 120.0 to 288.8). Years of education was not correlated with FCSRT total score (Spearman -0.12 p = 0.18).

aMCI participants with a high BMI scored better on the baseline FCSRT total score (Spearman 0.18 p=0.03). ApoEε4 positive participants had a lower score on the FCSRT total (ε4 negative: 46 pts IQR [38 to 48] v.s. ε4 positive: 38 pts IQR [29 to 46] p < 0.0001). This was also found for the TMT Part B (MWU p=0.027 ε4 negative: 182 secs [IQR 118.0 to 295.0] ε4 positive: 140.0 secs [IQR: 108.5 to 216.0]) but not for the MOCA (ε4 negative: 23.2 pts vs ε4 positive: 22.5 pts p= 0.15 95% CI -0.25 to 1.6).

For rate of cognitive decline, we found no relationship between change in FCRST LOCF with age (Pearson 0,012 p = 0.90); gender (Male: -3.3pts vs female: -4.5 pts mean diff 1.2pts 95% CI 1.3 to 3.7 p= 0.35) or years of education (Pearson -0.038 p=0.681). Likewise we found no significant

relationship between FCRST LOCF with BMI (Pearson 0.019 p= 0.835). However, there was a marked association between the presence of ApoE ε4 and a greater rate of cognitive decline, as shown by figure 5 (ε4 negative: -2.2 pts vs ε4 positive: -5.7 pts mean diff 3.4pts 95% CI 0.9 to 5.9 p= 0.008). This relationship was not found on the secondary cognitive change test scores. Change in MoCA LOCF (ε4 negative: -1.2 pts vs ε4 positive: -2.1 pts mean diff 0.9pts 95% CI -0.2 to 1.9 p= 0.1) or by change in the TMTb LOCF (ε4 negative: 37 secs vs ε4 positive: 48 secs mean diff 11 secs 95% CI -60 to 38 p= 0.7).

Figure 5. Change in FCSRT LOCF score from baseline to Month 18 in aMCI participants by ApoE status



Summary: No relationships were found between demographics or core medical variables and baseline or changes in cognitive scores in control participants. However, in aMCI participants it was found that

male participants and those with a higher BMI did better in baseline cognitive scores. Participants who carried the ApoE ε4 allele performed worse on the baseline primary cognitive outcome and had a greater rate of cognitive decline over the study period but this effect was not as marked using the other secondary cognitive measures.

4.1.8 Comparison between psychological stress and cognition

Initially we examined rates of cognitive decline, as determined by the FCSRT total score LOCF, in the control group and aMCI group using previously published cut-off points for high stress (defined as >= 300 points on the RLCQ and >=20 pts on the PSS at baseline i.e. stress preceding the first cognitive measure and at any time [v2 to v4] for stress occurring between the first and final cognitive measure). There were no significant findings in control participants between psychological stress and rate of cognitive decline both at baseline or visit 2 to 4. We have therefore not shown the data here however have summarised the data in appendix 1.3. A summary is provided in Table 13 for the aMCI group. As shown previously no relationship was found between demographics and core medical variables in aMCI participants and so no correction was applied. The dMoCA and dTMT Part B were only performed if primary dFCSRT was significant.

Table 11. LOCF for aMCI participants and rate of cognitive decline

		dFCSRT Total recall LOCF	Statistical significance	dMoCA LOCF	Statistical significance	dTMT Part B LOCF	Statistical significance
RLCQ V2 TO V4	High (300+)	N=15 Mean= -8.3 SD= 7.6	Independent samples T-test **P= 0.006*** Mean difference= 5.2 95% CI= 1.520 to 8.789	N= 15 Mean= -1.7 SD= 2.6	Independent samples T-test P= 0.98 Mean difference= -0.02 95% CI= -1.592 to 1.555	N= 15 Mean= 34.3 SD= 79.4	Independent samples T-test P= 0.85 Mean difference= 6.5 95% CI= -62.371 to 75.441
	Low	N= 107 Mean= -3.1 SD= 6.5		N= 108 Mean= -1.7 SD= 2.9		N= 106 Mean= 40.8 SD= 131.1	

*Significance at p <0.05

Delta change data for the aMCI group is normally distributed. Established cut off points for the RLCQ questionnaire were determined by the authors who recommend 300 or more points.

The aMCI group who reported increased stressful life events through the course of the study deteriorated at a faster rate on the FCSRT total score LOCF. This relationship was not seen with baseline stressful life events reports.

Stress scores relationship with rate of cognitive change using the median

Using the previously published cut off points for high stress (defined as > 300 points) on the RLCQ [392], objective stress ratings were unrelated to cognitive decline in the control group (see figure 6) though conversely, were shown to significantly influence cognitive decline in the aMCI group (see figure 8). However, only a very small number of cases (n = 8 to 15 pts) were observed in the very high stress group for the RLCQ and small numbers for the PSS (high PSS stress defined as >20 points, n = 36 to 37pts). We therefore took statistical advice from David Culliford at the University of Southampton who advised to dichotomise each participant group into a binary variable of low and high stress using the median score of each participant group to increase power (RLCQ: control group 160.2pts vs aMCI group 125.2pts; PSS control group 12.1pts vs aMCI group 14.5pts).

Using this method, like before we found no significant findings between moderate stress, on the RLCQ, and cognitive rate of change using the median as a cut off for the control group (see figure 7). However, in the aMCI group moderate stress ratings demonstrated a significant relationship with increased rates of cognitive decline for the primary cognitive measure, FCSRT total score LOCF and also the secondary measures, the MOCA LOCF and TMT Part B LOCF (Table 14 and figure 9) through the course of the study. Notably, no significant interactions were observed between cognitive rate of change and perceived stress. Appendix 1.3 provides a summary of results for both the PSS and RLCQ and rates of cognitive decline.

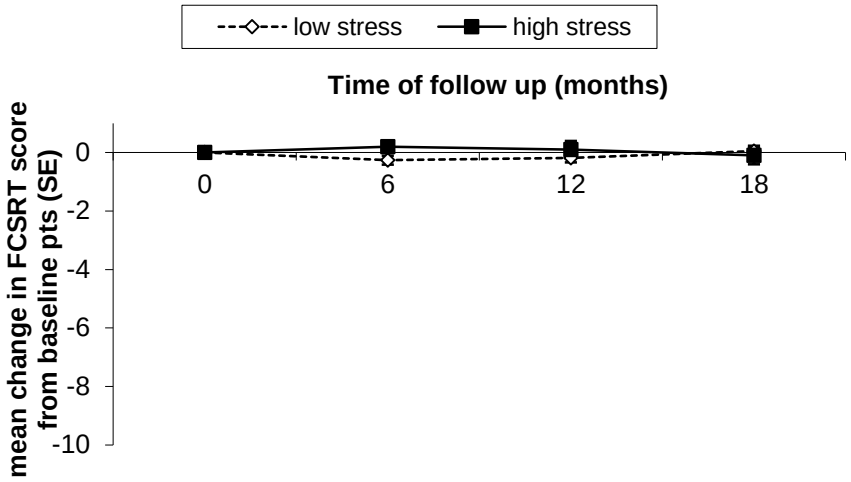
Table 12. Stress and cognitive rate of change using the median for aMCI participants (median cut off point RLCQ 113)

		dFCSRT LOCF	Statistical significance	dMoCA LOCF	Statistical significance	dTMT Part B LOCF	Statistical significance
RLCQ V2 to V4	High (above 113)	N= 79 Mean= -4.6 SD= 7.2	Independent samples T-test **P= 0.012*** Mean difference= 3.1 95% CI= 0.693 to 5.419	N= 79 Mean= -2.1 SD= 2.7	Independent samples T-test **P= 0.014*** Mean difference= 1.4 95% CI= 0.286 to 2.467	N= 77 Mean= 61.0 SD= 128.3	Independent samples T-test **P= 0.012*** Mean difference= -69.9 95% CI= -109.778 to -14.048
	Low	N= 39 Mean= -1.6 SD= 5.4		N= 40 Mean= -0.8 SD= 3.1		N= 40 Mean= -0.9 SD= 115.0	

*Significance at p <0.05

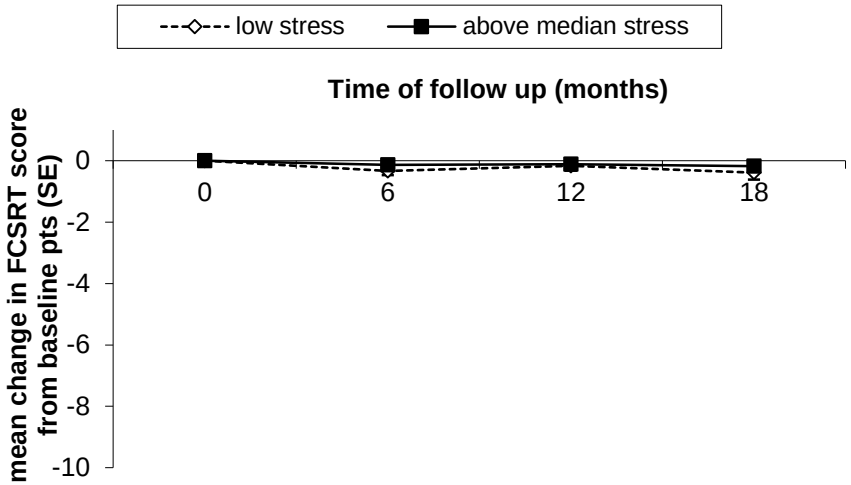
Figure 6 and 7 show objective psychological stress, using both the median and high stress thresholds, is not related to the rate of cognitive decline on the FCSRT total score LOCF over the 18 month follow-up period in the control group.

Figure 6 Change in FCSRT total score LOCF from baseline to Month 18 in the control group by high objective stress



RLCQ >300 pts equals high stress n= 10, ≤ 300 pts equals low stress n= 58.

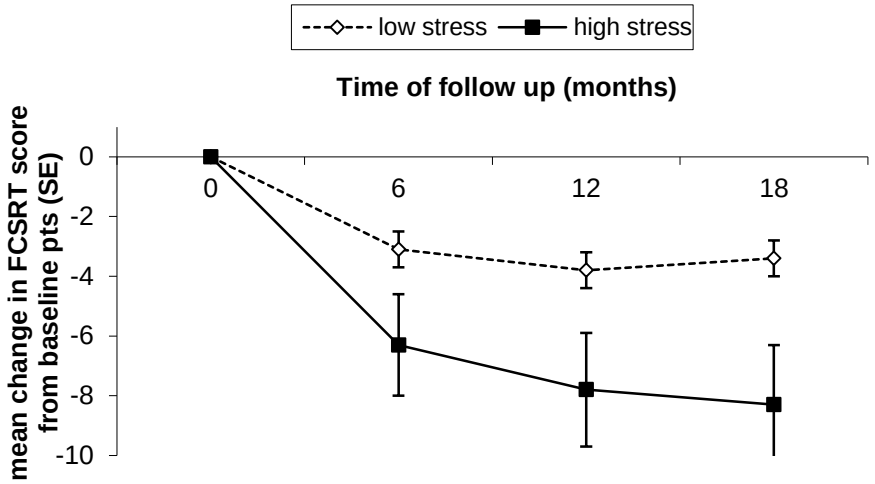
Figure 7 Change in FCSRT total score LOCF from baseline to Month 18 in the control group by objective stress using the median



RLCQ >113 pts equals above median stress n= 50, ≤ 113 pts equals low stress n= 18.

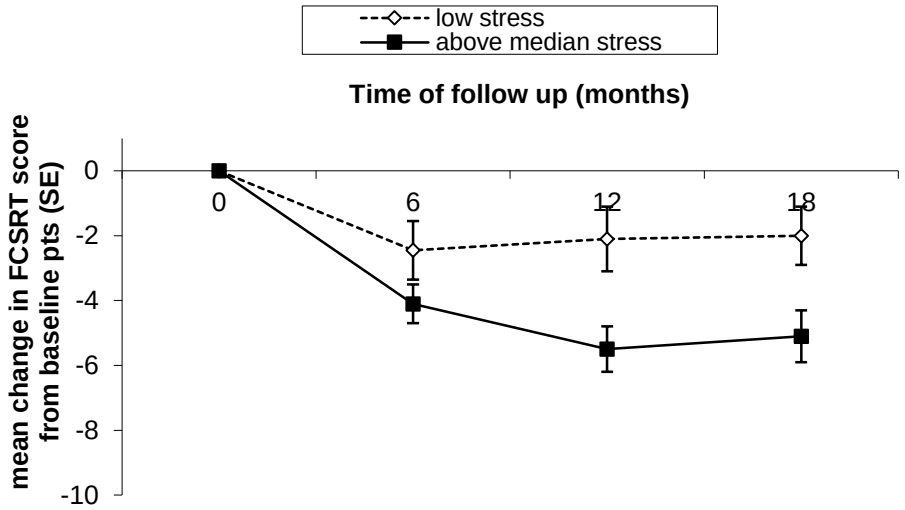
Figure 8 and 9 show those in the aMCI group reporting moderate and high stress on the RLCQ accelerated at a faster rate on the FCSRT over the course of the study follow-up period than compared to aMCI participants reporting less psychological stress.

Figure 8 Change in FCSRT total score LOCF from baseline to Month 18 in the aMCI group by high objective stress



RLCQ >300 pts equals high stress n= 15, ≤ 300 pts equals low stress n= 104

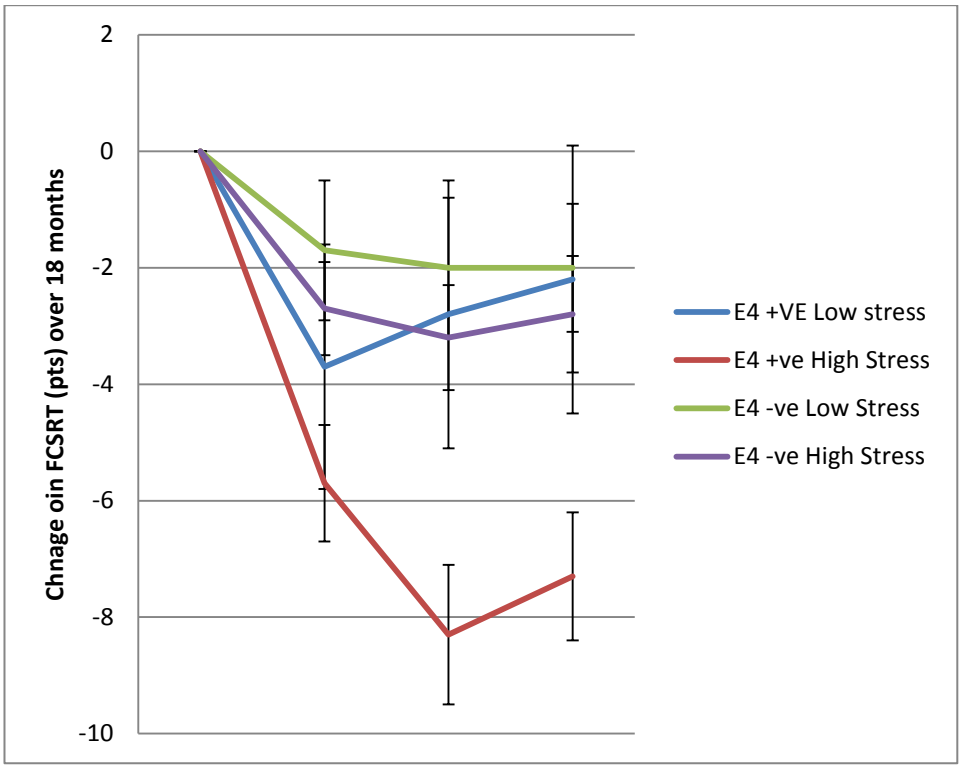
Figure 9 Change in FCSRT total score LOCF from baseline to Month 18 in the aMCI group by objective stress using the median



RLCQ >113 pts equals above median stress, n= 79, ≤ 113 pts equals low stress n= 40

Finally, figure 10 shows those aMCI participants identified as ε4 carriers who reported low levels of objective psychological stress (RLCQ) declined at a slower rate on the FCSRT, than compared to aMCI ε4 carriers reporting increased objective psychological stress.

Figure 10. Change in FCSRT LOCF score from baseline to Month 18 in the aMCI group by objective stress and ApoE status



>113 pts equals above median stress. n= 79 ≤ 113 pts equals low stress n= 40

Summary: Stress measures at any time (baseline or throughout the course of the study) were not associated with a change in cognitive decline in the control group, which was not influenced by ApoE status. However, in the aMCI group we find moderate (and high) levels of objective life stress events occurring during the course of the study were associated with increased rates of cognitive decline across a range of measures. Those in the aMCI group who were ε4 carriers

declined at a faster rate when exposed to greater objective life stress than compared to aMCI ε4 negative participants.

4.1.9 Comparison between physical stressors and cognition

We examined the relationship between physical stress (acute and chronic stress) and rate of cognitive change. Cognition was measured by the primary measure of interest, FCSRT total score or change in FCSRT LOCF with secondary cognitive measures, the MoCA and TMT Part B examined if FRSRT findings were significant.

Acute systemic inflammatory events and cognitive decline

In the control group the presence of reported acute systemic inflammatory events (ASIE's) six months prior to baseline did not influence rate of cognitive decline as measured by the FCSRT (ASIE negative (n=60): -0.03 pts vs ASIE positive (n=6): 0 pts mean diff 0.03pts 95% CI -1.05 to 1.12 p= 0.95). Likewise the presence of reported acute systemic inflammatory events (ASIE's) during the study did not influence rate of cognitive decline as measured by the FCSRT (ASIE negative (n=28): -0.07 pts vs ASIE positive (n=38): 0.1 pts mean diff 0.17pts 95% CI -0.45 to 0.81 p= 0.6).

In the aMCI group the presence of reported acute systemic inflammatory events six months prior to baseline did not influence rate of cognitive decline as measured by the FCSRT (ASIE negative (n=95): -3.4 pts vs ASIE positive (n=26): -4.4 pts mean diff 1.0pts 95% CI -2.0 to 4.0 p= 0. 5). Likewise the presence of reported acute systemic inflammatory events during the study did not influence rate of cognitive decline as measured by the FCSRT (ASIE negative (n=57): -3.5 pts vs ASIE positive (n=64): -3.8 pts mean diff 0.3pts 95% CI -2.2 to 2.7 p= 0.9).

Chronic inflammatory conditions and rate of cognitive decline

In the control group the presence of hypertension did not influence rate of cognitive decline as measured by the FCSRT (hypertension negative: -0.46 pts vs hypertension positive: -0.16 pts mean diff 0.62pts 95% CI -0.01 to 1.25 p= 0.054). Likewise the presence of high cholesterol did not influence rate of cognitive decline as measured by the FCSRT (high cholesterol negative: 0.02 pts vs high cholesterol positive: 0.11 pts mean diff 0.09pts 95% CI 0.7 to -0.5 p= 0.8). Likewise the presence of diabetes did not influence rate of cognitive decline as measured by the FCSRT (diabetes negative: 0.06 pts vs diabetes positive: 0 pts mean diff 0.06pts 95% CI -1.4 to 1.5 p= 0.9).

In the aMCI group the presence of hypertension did not influence rate of cognitive decline as measured by the FCSRT (hypertension negative: -3.2 pts vs hypertension positive: -4.2 pts mean diff 1.0pts 95% CI -1.5 to 3.4 p= 0.5). Likewise the presence of high cholesterol did not influence rate of cognitive decline as measured by the FCSRT (high cholesterol negative: -3.6 pts vs high cholesterol positive: -3.9 pts mean diff 0.3pts 95% CI -2.1 to 2.8 p= 0.8). Likewise the presence of diabetes did not influence rate of cognitive decline as measured by the FCSRT (diabetes negative: -3.7 pts vs diabetes positive: -3.9 pts mean diff 0.2pts 95% CI -3.3 to 3.6 p= 0.9).

ε 4 status

In the aMCI group, systemic inflammatory events did not influence rate of cognitive decline over the 18 month follow-up period in those who were ε4 carriers (no events -6.8 [1.3] pts c.f. events -5.1 [1.7] mean dif -1.7 [-6.1 to 2.7] pts p = -0.40). However, we found in aMCI participants categorised as ε4 negative the presence of systemic inflammatory events was associated with an increased rate of cognitive decline (no events -0.9 [0.6] pts c.f.

events -3.4 [1.1] mean dif 2.5 [0.08 to 5.0) pts p = 0.04). This effect was not observed in the control group.

In summary: Physical stress, reported as acute or chronic inflammatory events, was not related to cognitive decline in either the control and aMCI participants. However, we found ApoE status played an important role, with aMCI participants categorised as ε4 negative presenting with a faster rate of cognitive decline in the presence of physical stress over the study follow-up period. Thus, physical stress may act as a significant contributor to disease progression but only in those who are not ε4 carriers.

4.1.10 Interaction between physical stress and psychological stress

Interaction between stressors at baseline

Control Group

In the control group there was no significant interaction between objective or subjective psychological stress (as measured by the RLCQ and PSS) and physical stress (acute systemic inflammatory events, diabetes; high cholesterol; high blood pressure) either at baseline or during the course of the study ($p > 0.1$ all cases not shown).

aMCI group

In the aMCI group we found the RLCQ was not related to acute systemic inflammatory events and chronic physical stressors at baseline or through the study follow-up period ($p > 0.1$ all cases not shown). However, aMCI participants experiencing greater perceived stress during the course of the study (using cut off point 14+ at any time point to denote higher perceived

stress) were at increased risk of chronic inflammatory conditions including high blood pressure (low stress: 16 [13.2%] vs high stress 48 [39.7%] X^2= 5.653 p=0.017), high cholesterol (low stress: 17 [14.0%] vs high stress: 47 [38.8%] X^2=3.980 p=0.046) and a potential trend for diabetes (low stress: 3 [2.5%] vs high stress: 15 [12.4%] X^2= 3.038 p=0.081) although no relationship was found with acute systemic inflammatory events (low stress: 3 [7.1%] vs high stress: 10 [12.6%] X^2= 0.14 p=0.70).

In summary: Psychological stress (both RLCQ and PSS) was unrelated to all physical stress measures in the control group. In the aMCI group we found a relationship between perceived stress (PSS) and chronic physical stress, with those reporting increased perceived stress more likely to report a chronic physical stressor. However, the RLCQ did not influence physical stress in the aMCI group at baseline or during the study follow-up period.

4.2 Biological data

Inflammatory markers (CRP; anti-inflammatory cytokines; pro-inflammatory cytokines) and cortisol measures (Sample 1; CAR; AUC) were measured at baseline and throughout the study in both participant groups. We examined how these biological parameters were influenced by the core demographics as well as how they interact with one another, stress and with cognition.

4.2.1 CRP and cytokine demographics

In addition to CRP the following 8 cytokines were analysed; 4 pro-inflammatory cytokines TNFα; IL6; IFNγ, IL12; and 4 anti-inflammatory cytokines IL10; IL4; IL13; TGFβ (Transforming Growth Factor Beta). At baseline the pro-inflammatory cytokine IL12 was detectable in less than 5% of the assays in both the control and aMCI groups (control group undetectable in 21 [33%] participants; aMCI group undetectable in 32 [26%] participants) (X^2 1.0 p = 0.3). Likewise, at baseline the anti-inflammatory cytokine IL13was detectable in less than 5% of the assays (control group undetectable in 24 [38%] participants; aMCI group undetectable in 60 [48%] participants)(X^2 2.0 p = 0.2). Notably the anti-inflammatory cytokine IL4 was detectable in less than 5% of the assays in both groups and was significantly less detectable in the aMCI group compared with the control group (control group undetectable in 25 [39%] participants; aMCI group undetectable in 69 [56%] participants. X^2 4.6 p = 0.03). No further analysis of these undetectable cytokines was undertaken. The distribution of the remaining cytokines and CRP was as follows: IFNγ, IL10; IL6 and CRP were non parametric whilst TNFα and TGFβ were parametric.

Since we considered 6 cytokines in each participant group to reduce our false positive rate we corrected our statistical significance level to $p < 0.05/6$ i.e $p < 0.008$ in keeping with Bonferonni correction for multiple comparisons.

Comparison of mean or medians serum levels across both participant groups showed no gender differences for the distribution of all measured cytokines or CRP. There was no significant (at $p < 0.008$) relationship between age and cytokines levels for IFNγ, IL10, CRP, TGFβ and TNFα but there was a positive correlation between IL6 and age (IL6 spearman 0.36 p <0.0001).

Comparison between participant groups for CRP and cytokine levels at baseline

At baseline there were no significant differences for CRP or the pro-inflammatory cytokines TNFα; IFNγ or IL6 or anti-inflammatory cytokine IL10 between the control and aMCI groups. However, there was a significant reduction of the anti-inflammatory cytokine TGFβ compared with the control group (Table 15).

Table 13. Baseline inflammatory markers

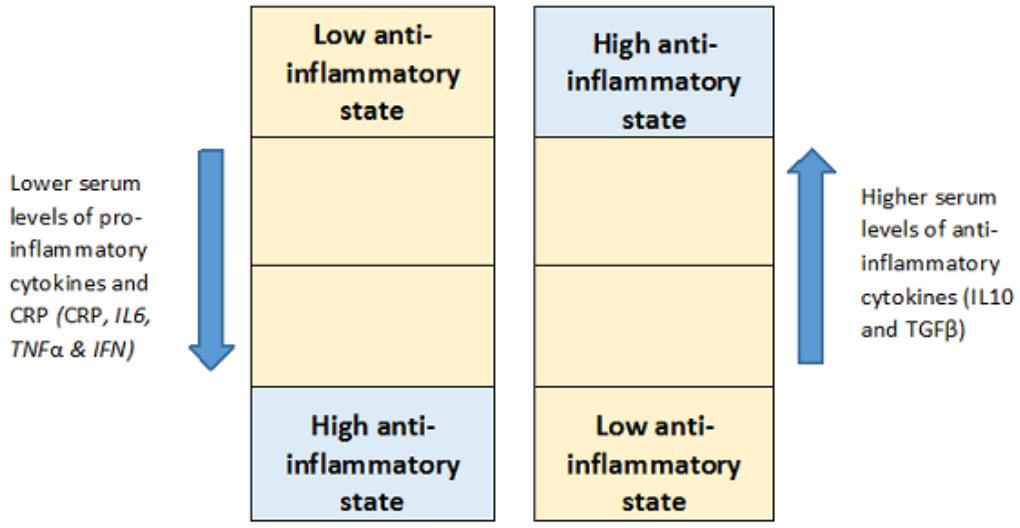
*Significance at p <0.008

	CONTROL	MCI	MWU p value
IFNγ	3.5 [IQR 1.8 TO 5.9]	3.2 [IQR 2.0 TO 5.6]	P = 0.9
IL10	0.23 [IQR 0.11 to 0.37]	0.16 [IQR 0.09 to 0.30]	P = 0.10
IL6	0.45 [IQR 0.32 to 0.71]	0.57 [IQR 0.42 to 0.97]	P = 0.009
CRP	521 [IQR 288 to 1377]	518 [IQR 222 to 1037]	P = 0.57
TNFα	2.7 SD: 0.9	2.8 SD: 0.9	P = 0.5
TGFβ	22195 SD: 7508	19145 SD: 5130	**P = 0.004***

Visit 2 to 4 (Study duration)

For the purpose of study analysis, dichotomisation of CRP or cytokines, based on previous published methodology [403], suggests a CRP of less than 1mg/ml should be considered low. Serum levels of pro-inflammatory cytokines were considered low if recorded in the lowest quartile at baseline (i.e for TNFα low = equal to or less than 2.19 pg/ml; IL6 low = equal to or less than 0.42 pg/ml; IFNγ low = equal to or less than 1.97 pg/ml). For anti-inflammatory cytokines dichotomisation was based on the highest quartile i.e TGFβ was considered low/mod if fell in the lowest 3 quartiles (i.e. TGFβ low/moderate = equal to or less than 21867 pg/ml; value falls in the highest quartile = greater than 21867 pg/ml; IL10 low/moderate = equal to or less than 0.3036 pg/ml; value falls in the highest quartile = greater than 0.3036 pg/ml) (see figure 11).

Figure 11 Dichotomisation of CRP and cytokines levels



Chi Square was used to examine differences for CRP or cytokines between the control and aMCI groups. No significant differences were shown ($p > 0.1$ all cases not shown).

4.2.2 Core demographics and inflammatory markers

The relationship between the core demographics (age, gender and education years) and core medical variables (BMI andε4) and serum inflammatory marker levels (TNFα, TGFβ, CRP, IFNγ, IL6 and IL10) was examined at baseline and through the course of the study.

Core demographics and inflammatory markers at baseline in control participants

Age

The pro-inflammatory cytokine, IL6 (Spearman correlation: 0.307 p=0.013), and CRP (Spearman correlation: 0.387 p=0.002) positively correlated with age showing those who were older presented with increased peripheral levels of CRP and IL6. The remaining cytokines, TGFβ (Pearson correlation -0.090 p=0.480), TNFα (Pearson correlation -0.028 p=0.823), IL10 (Spearman correlation 0.126 p=0.320) and IFNγ (Spearman 0.047 p=0.712) were unrelated to age in the control group.

Gender

IFNγ was not significantly related to gender at the $p< 0.008$ level (MWU p=0.035, male median: 2.7 [IQR: 1.3 to 3.9] vs female: 3.9 [IQR: 2.2 to 7.0]). Likewise, the remaining inflammatory markers TNFα (male 20 vs female 44 p= 0.529 95% CI: -0.3188 to 0.6139), TGFβ (male 20 vs female 44 p= 0.866 95% CI: -4424.0241 to 3734.9332), IL6 (MWU p=0.612 male median: 0.4 [IQR: 0.3 to 0.6] vs female median:0.5 [IQR: 0.3 to 0.7]), CRP

(MWU p= 0.653 male: 695.8 [IQR: 343.6 to 1196.5] female: median 511.8 [IQR: 276.0 to 1474.8]) and IL10 (MWU p= 0.328 Male: median 0.3 [IQR: 0.1 to 0.4] vs female: median 0.2 [IQR: 0.1 to 0.4]) were unrelated to gender in the control group.

Years of Education

The interaction effect between years of education and the inflammatory markers TGFβ (Pearson correlation -0.008 p=0.949), TNFα (Pearson correlation 0.071 p=0.579), IL10 (Spearman correlation 0.126 p=0.320), CRP (Spearman correlation -0.177 p=0.161), IL6 (Spearman correlation -0.129 p=0.311) and IFNγ (Spearman 0.047 p=0.712) was not significant.

BMI

A raised CRP (Spearman correlation: 0.387 p=0.002) was observed in those presenting with a greater BMI. The interaction effect for the remaining cytokines, TGFβ (Pearson correlation 0.076 p=0.552), TNFα (Pearson correlation 0.079 p=0.535), IL6 (Spearman correlation 0.307 p=0.013), IL10 (Spearman correlation 0.038 p=0.764) and IFNγ (Spearman -0.187 p=0.139) and BMI in the control group was not significant.

ε 4 status

There were no significant interaction effect between ε4 status and measured cytokines or CRP and thus we have not presented the results (p > 0.1 all cases not shown).

Core demographics and inflammatory markers at baseline in aMCI participants

TGFβ did not significantly correlate with any demographic variable, however, we do see significant interactions between the core demographics and cytokines or CRP shown in Table 17 in the aMCI group.

Table 14. Core demographics and inflammatory markers in aMCI participants at baseline

		Age at baseline	Years of education	BMI
IL6	Spearman Correlation	0.268	-0.122	0.187
	Significance	**0.003***	0.177	**0.038**
	N	124	124	124
IL10	Spearman Correlation	0.201	-0.060	0.001
	Significance	**0.025**	0.510	0.989
	N	124	124	124
CRP	Spearman Correlation	0.113	-0.176	0.266
	Significance	0.212	**0.050**	**0.003***
	N	124	124	124
IFNγ	Spearman Correlation	0.007	-0.113	-0.012
	Significance	0.936	0.212	0.895
	N	124	124	124
TNFα	Pearson Correlation	0.174	-0.194	0.176
	Significance	**0.053**	**0.031**	**0.050**
	N	124	124	124
TGFβ	Pearson Correlation	-0.006	-0.016	-0.004
	Significance	0.948	0.863	0.962
	N	124	124	124

*Significance at p <0.008

Age

We see IL6 positively correlate positively with age showing that both anti-inflammatory and pro-inflammatory cytokines increase with age in aMCI participants (See Table 17).

Gender

Comparison of serum levels showed no gender differences for the distribution of all measured cytokines or CRP levels (all $p > 0.05$) and thus have not presented the results ($p > 0.1$ all cases not shown).

Education

Comparison of serum levels showed no gender differences for the distribution of all measured cytokines or CRP levels (See Table 17).

BMI

Increased serum CRP (0.266 p=0.0003) was observed in aMCI participants who presented with a greater BMI (See Table 17).

ε 4 status

Lower serum CRP levels were observed in the ε4 positive aMCI group at baseline (ε4 negative median 788.8 µg/ml [IQR: 262.2 to 1593.0] vs ε4 positive median 389.3 µg/ml [IQR 145.4 to 780.0] MWU p=0.001). No significant differences were observed for TNFα, TGFβ, IL6, IL10 and IFNγ (p > 0.1 all cases not shown).

Core demographics and inflammatory markers during the course of the study in control participants

Through the course of the study follow-up period we find gender and ε 4 status were not related to cytokine or CRP serum levels in the control group (p > 0.1 all cases not shown). Table 18 summarises the remaining findings identified in the control group between visits 2 to 4.

Age

Age did not influence CRP or any of the remaining cytokines in the control group (See Table 18).

Gender

Gender was consistently not related to cytokines or CRP and thus have not presented the results (p > 0.1 all cases not shown).

Education

Higher education years were related to higher serum IL10 levels but not related to other serum cytokines or CRP serum levels. Notably, there were only 4 cases included in the group reporting more years of education.

BMI

BMI was not related to serum cytokine levels but was positively related to CRP levels with increased CRP levels associated with a higher BMI. See Table 18.

ε 4 status

No relationships were found between ε4 carriers and elevated or depressed levels of CRP or cytokines through the course of the study, all X^2 $p>0.1$ ($p > 0.1$ all cases not shown).

Table 15. Relationship between inflammatory markers and core demographics in the control group from visit 2 to 4

		Age	Statistical significance	BMI	Statistical significance	Education	Statistical significance
CRP	Low	N= 34 Mean= 68.3 SD= 9.2	Independent samples T-test P= 0.704 Mean difference= -0.910 95% CI= -5.670 to 3.850	N= 34 Mean= 25.8 SD= 3.5	Independent samples T-test **P= 0.004*** Mean difference= -3.9 95% CI= -6.4272 to -1.2885	N= 34 Median= 14.5 IQR: 11 to 17	MWU= 0.119
	High	N= 30 Mean= 69.2 SD= 9.8		N= 30 Mean= 29.7 SD= 6.2		N= 30 Median= 12.5 IQR: 11 to 15	
IL6	Low	N= 11 Mean= 64.9 SD= 9.0	Independent samples T-test P = 0.139 Mean difference= -4.6 95% CI= -10.831 to 1.554	N= 11 Mean= 24.5 SD= 3.1	Independent samples T-test **P = 0.028** Mean difference= -3.8 95% CI= -7.2029 to -0.4319	N= 11 Median= 15.0 IQR: 11 to 17	MWU= 0.474
	High	N= 53 Mean= 69.6 SD= 9.4		N= 53 Mean= 28.3 SD= 5.4		N= 53 Median= 13 IQR: 11 to 16	
TNFα	Low	N= 9 Mean= 66.2 SD= 7.8	Independent samples T-test P= 0.466 Mean difference= -2.4 95% CI= -9.022 to 4.181	N= 9 Mean= 26.0 SD=2.0	Independent samples T-test P=0.091 Mean difference= -1.7 95% CI= -3.7876 to 0.2944	N= 9 Median = 16 IQR: 11.5 to 16.0	MWM= 0.528
	High	N= 56 Mean= 68.6 SD= 9.4		N= 56 Mean= 27.8 SD= 5.6		N= 56 Median= 13 IQR: 11 to 16	
IFNγ	Low	N= 4 Mean= 64.5 SD= 9.4	Independent samples T-test P= 0.311 Mean difference= -4.9 95% CI= -14.346 to 4.646	N= 4 Mean= 28.1 SD= 3.2	Independent samples T-test P= 0.783 Mean difference= 0.7 95% CI= -4.6477 to 6.1415	N= 4 Median= 14.5 IQR: 11.5 to 18.25	MWU= 0.494
	High	N= 60 Mean= 69.4 SD= 9.2		N= 60 Mean= 27.4 SD= 5.3		N= 60 Median= 13 IQR: 11 to 16	
IL10	Low	N= 62 Mean= 68.2 SD= 9.1	Independent samples T-test P= 0.888 Mean difference= 0.7 95% CI= -8.876 to 10.231	N= 62 Mean= 27.6 SD= 5.3	Independent samples T-test P= 0.431 Mean difference= 2.1 95% CI=-3.2698 to 7.5629	N= 62 Median= 13 IQR: 11 to 16	**MWU= 0.008***
	High	N= 4 Mean= 67.5 SD= 12.9		N= 4 Mean= 25.5 SD= 4.3		N= 4 Median= 22 IQR: 15 to 25.5	
TGFβ	Low	N= 55 Mean= 69.4 SD= 9.0	Independent samples T-test **P= 0.045*** Mean difference= 6.4 95% CI= 0.137 to 12.736	N= 55 Mean= 27.1 SD= 5.1	Independent samples T-test P= 0.072 Mean difference= -3.2418 95% CI= -6.7757 to 0.2921	N= 55 Median= 13 IQR: 11 to 16	MWU= 0.564
	High	N= 10 Mean= 63.0 SD= 10.0		N= 10 Mean= 30.3 SD= 5.3		N= 10 Median= 13 IQR: 11.75 to 15.0	

Significance at p <0.008

Core demographics and inflammatory markers during the course of the study in aMCI participants

The core demographics (age, gender, education, BMI, and ε4 status) did not significantly interact with serum levels of cytokines or CRP and thus, we have not presented the results ($p > 0.1$ all cases not shown).

In summary: At baseline age was associated with an increased serum level of IL6 and BMI was associated with an increased CRP level in both the control and aMCI participant groups. In the aMCI group participants carrying the ApoE ε4 allele had a lower CRP level that was not found in the control group. The relationship between BMI and CRP remained significant throughout the course of the study in the control group but not the aMCI group.

4.2.3 Comparison between psychological stress and inflammatory markers

Correlational analysis was applied to examine the relation between baseline measures of stress (RLCQ and PSS) and baseline CRP and cytokine serum levels in both participant groups. In addition, dichotomisation of baseline psychological stress, based on median scores, was examined to further explore the relation between inflammatory markers at baseline and through the course of the study follow-up period. No significant interactions were observed for the RLCQ or PSS and CRP, TNFα, IL6, TGFβ, or IL10 in either participant group and thus we have not presented the data ($p > 0.1$ all cases not shown). However, at baseline in the control group, psychological stress (RLCQ) was related to lower levels of the pro-inflammatory cytokine IFNγ (Spearman -0.33 P=0.008). This was also found on dichotomisation of the data using the median (19 of 31 [61%] participants had high levels of IFNγ in the high stress group compared with

28 of 33 [85%] in the low stress group. X^2 4.5 p = 0.033). Notably this relationship was not found in the aMCI group (Spearman 0.04 P=0.6) and no other significant relationships were found with RLCQ and IFNγ during the course of the study or with PSS and IFNγ at baseline or during the study (p > 0.1 all cases not shown).

In summary: at baseline the presence of psychological stressful life events was associated with a decrease in the pro-inflammatory cytokine IFNγ in the control group but no relationship was found in aMCI participants.

4.2.4 Comparison between physical stress and inflammatory markers

Acute stress and pro-inflammatory markers

No significant interaction between acute systemic inflammatory events and serum inflammatory markers was evidenced in the control or aMCI group at baseline. However, the presence of acute systemic inflammatory events throughout the study were significantly related to raised IL6 serum levels in the control group (IL6 raised in n =17 (63%) with no ASIE v.s. n = 34 (97%) in those with a reported ASIE X^2 12.2 p<0.0001). This was not found in the aMCI group (IL6 raised in n =47 (90%) with no ASIE v.s. n = 34 (89%) in those with a reported ASIE X^2 0.025 p=0.9). No other significant findings were observed in the control or aMCI group for the remaining cytokines or CRP during the duration of the study (p > 0.1 all cases not shown).

Chronic Inflammatory conditions and inflammatory markers

At baseline and through the course of the study in both participant groups no consistent relationship was shown between serum cytokines or CRP

levels with chronic inflammatory stress events ($p > 0.1$ all cases not shown).

Summary: The control group shows an association with the presence of acute systemic inflammatory events and increases in serum IL6 levels not seen in the aMCI group. No relationships were found between chronic inflammatory events and serum CRP or cytokines levels.

4.2.5 Comparison between cognition and inflammatory markers

CRP or cytokines and baseline cognitive scores and change in cognitive scores in the control group

No significant correlations (at $p < 0.008$) were found between baseline serum CRP or cytokines and baseline FRSCT total score or change in FCSRT over the course of the study. Dichotomisation of the data made no appreciable differences to these findings ($p > 0.1$ all cases not shown).

CRP or cytokines and baseline cognitive scores and change in cognitive scores in the aMCI group

No significant correlations (at $p < 0.008$) were found between baseline serum CRP or cytokines and baseline FRSCT total score. There was a significant positive correlation between baseline serum TGFβ levels and change in FCSRT over the course of the study (Pearson 0.25 $p = 0.006$) (See figure 12 and 13) but no significant relationships with other serum cytokines at baseline and change in cognition over the course of the study. Thus, increased TGFβ serum levels at baseline was related to a slower rate of cognitive decline through the course of the study follow-up period.

Figure 12. Baseline TGF β and change in FCSRT total score LOCF from baseline to Month 18 in aMCI participants.

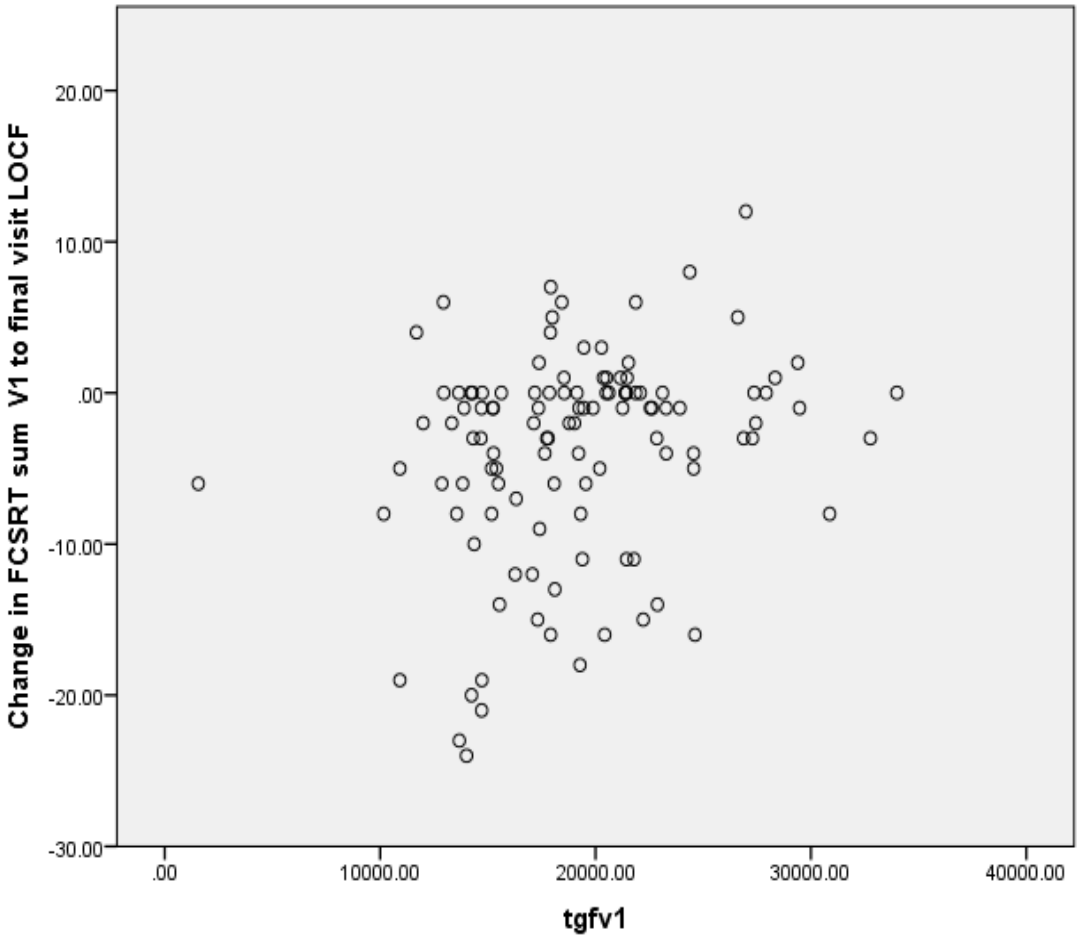
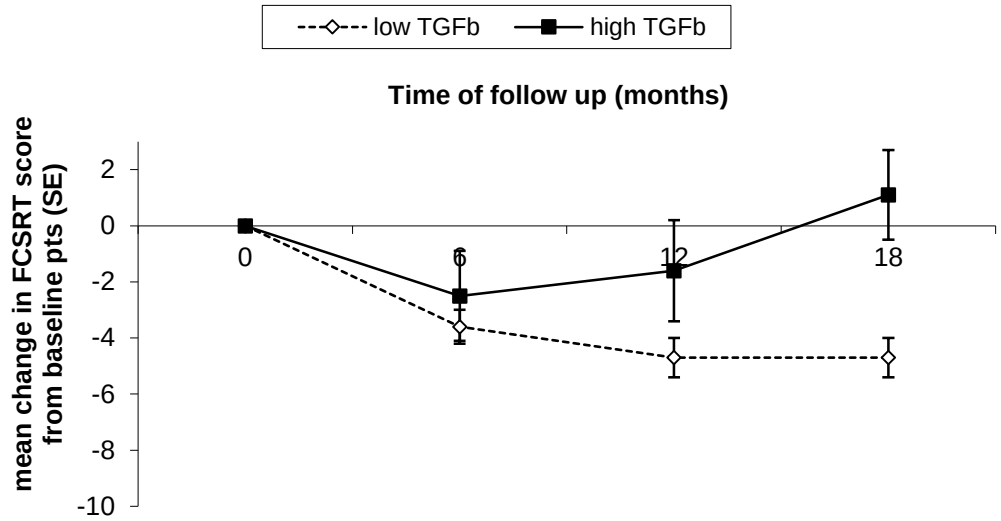


Figure 13. Change in FCSRT total score LOCF in the aMCI group by serum TGFβ levels



Low TGFβ n= 102 High TGFβ n= 8

Dichotomised data showing the relationship between change in FCSRT is for baseline CRP and cytokines and for the duration of the study are shown in Tables 19 and 20 respectively. No significant relationships at $p < 0.008$ were found.

Table 16. Baseline dichotomised CRP and cytokine levels and cognitive decline through the follow-up period in aMCI participants

		dFCSRT sum LOCF
CRP	Low (n=88)	-4.2 (7.2)
	Mod/high (n=26)	-2.6 (5.4)
Mean difference		-1.6
95% CI		-4.6 to 1.5
P value		0.31
TNFα	Low (n=24)	-3.2 (6.6)
	Mod/high (n=90)	-4.0 (6.9)
Mean difference		0.8
95% CI		-2.3 to 4.0
P		0.6
IL6	Low (n=30)	-4.8 (7.5)
	Mod/high (n=84)	-3.5 (6.6)
Mean difference		-1.4
95% CI		-4.3 to 1.5
P		0.4
IFNγ	Low (n=29)	-3.9 (6.3)
	Mod/high (n=85)	-3.8 (7.1)
Mean difference		-0.1
95% CI		-3.0 to 2.9
P		1.0
TGFβ	Mod/Low (n=86)	-4.5 (6.9)
	High (n=28)	-1.8 (6.2)
Mean difference		-2.7
95% CI		-5.6 to 0.3
P		0.07
IL10	Mod/Low (n=84)	-3.8 (6.6)
	High (n=30)	-3.9 (7.7)
Mean difference		0.1
95% CI		-2.8 to 3.0
P		1.0

*Significant at p <0.008. Mean used for normal distributions and independent samples T-test applied.

Table 17. Visit 2 to 4 dichotomised CRP and cytokine levels found through the course of the study and cognitive decline for the follow-up period (LOCF) in aMCI participants

		dFCSRT LOCF sum
CRP	Low (n=44)	-3.4 (7.1)
	Mod/high (n=44)	-3.1 (5.4)
Mean difference		-0.3
95% CI		-2.9 to 2.4
P		0.8
TNFα	Low (n=8)	1.2 (4.6)
	Mod/high (n=102)	-4.1 (6.8)
Mean difference		5.3
95% CI		0.4 to 10.2
P		0.03
IL6	Low (n=11)	-4.5 (8.5)
	Mod/high (n=97)	-3.4 (6.5)
Mean difference		-1.1
95% CI		-5.2 to 3.3
P		0.6
IFNγ	Low (n=8)	-5.5 (8.1)
	Mod/high (n=104)	-3.5 (6.7)
Mean difference		-2.0
95% CI		-6.9 to 3.0
P		0.4
TGFβ	Low/Mod (n=97)	-4.3 (7.0)
	High (n=8)	1.1 (4.5)
Mean difference		-5.0
95% CI		-10.3 to – 0.4
P		0.03
IL10	Mod/Low (n=97)	-3.5 (6.7)
	High (n=8)	-2.3 (2.5)
Mean difference		-1.2
95% CI		-6.0 to 3.7
P		0.6

*Significant at p <0.008. Mean used for normal distributions and independent samples T-test applied. All data treated as normally distributed.

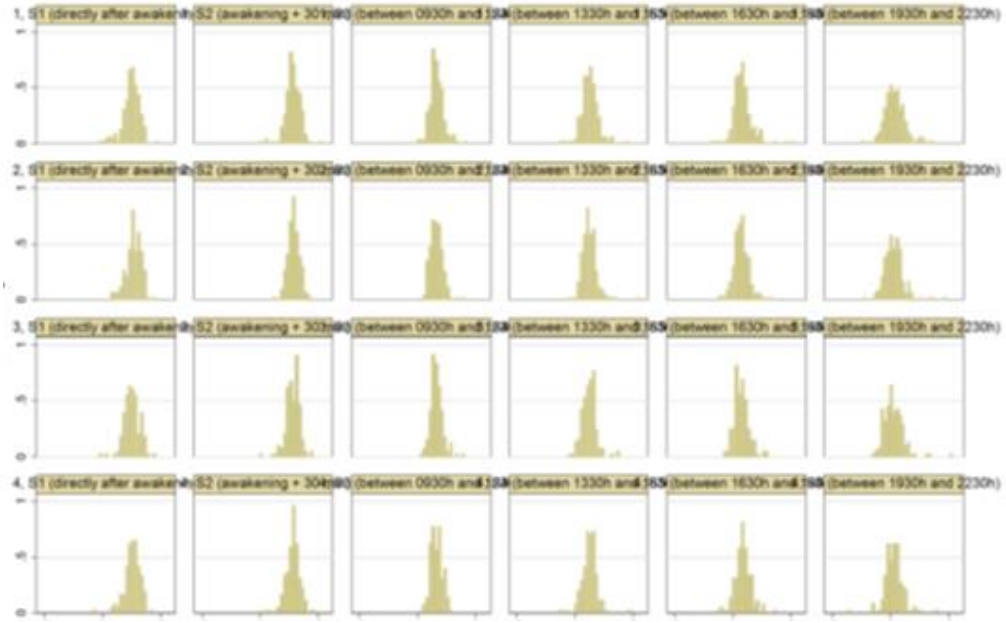
Summary: The anti-inflammatory cytokine TGFβ is associated with a slower rate of cognitive decline as measured by the FCSRT in the aMCI group. Although not significant at the p<0.008 level this finding is consistent with trends (p<0.05) of an increased rate of decline in participants with high TNFα.

4.2.6 Cortisol demographics

Trier Biochemical Lab provided data for 4181 salivary observations. The cortisol data was processed before samples were used for analyses. Of the 3976 usable samples 204 (5 %) were out of time (i.e. not on schedule based on criteria noted in the variable), resulting in 3772 valid samples, which gives an 'on-time compliance' of 3772 / 4800 = 79 %. Therefore, the out-of-time cortisol samples were not used for calculating aggregates (CAR and AUC). A couple of further samples were removed from analysis, as they likely did not reflect cortisol concentrations in saliva: m_e=28 (0.7 %) were extremely high (> 100 nmol/L), suggesting a substance interfering with the cortisol assay. After removing these measures, the final number of samples were 3746. The mean assay CV of those samples was 5.8% indicating a precise measurement. Extremely high cortisol samples were not used when calculating the aggregates measures (CAR and AUC) as they likely did not reflect cortisol concentrations.

The distribution of cortisol values was heavily skewed with the original scale (nmol/L). The best transformation was found using a Box Cox transformation command (cortisol measure x 0.0686185 -1 / 0.0686185), which resulted in approximately normally distributed transformed cortisol values.

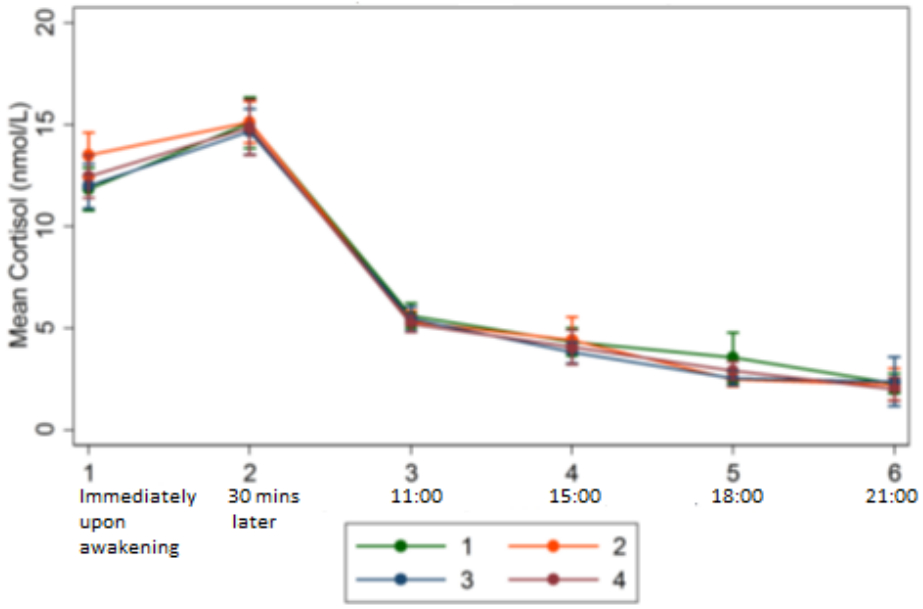
Figure 14. Distribution of cortisol after Box Cox transformation



The graphs show cortisol measure over a 24 hour period for each of the 4 visits

Figure 14 and 15 show mean cortisol level over a 24 hour period for all 6 time points (immediately upon awakening, 30 minutes later, 11:00, 15:00, 18:00, 21:00) measured at each of the 4 study visits. Findings show the classical early morning rise in cortisol immediately upon awakening followed by a drop in cortisol during the course of the day.

Figure 15. Mean cortisol for all 6 time points over 24 hours



Computation of cortisol measures

The above plots suggest a very good measurement quality for cortisol samples, with the only exception of the relatively high value immediately upon awakening at visit 2 (reason unknown). After transformation, we can see that data was normally distributed. Therefore, we decided to use the mean values for all analysis (Table 21).

Table 18. Mean cortisol values at baseline for both participant groups

	Sample 1 visit 1	CAR visit 1	AUC visit 1
Control	2.5	0.4	122.8
aMCI	2.7	0.3	134.6

Cortisol levels in both participant groups

Sample 1, CAR and the AUC measures were analysed at baseline and through the course of the study. We averaged the cortisol measures from visit 2 to visit 4 to determine aggregate scores. Cortisol levels between the control and aMCI group were then compared (Table 22).

Table 19. Comparison of cortisol values in the control and aMCI group at baseline and through the study follow-up period

	Participant group	N	Mean	SD	Statistical significance	Adjusted Statistical significance**
Sample 1 visit 1	Control	45	2.4	0.8	Independent Samples T-test P=0.547	
	aMCI	56	2.5	0.7	Mean difference=-0.09 95% CI=-0.39760 to 0.21187	
Sample 1 visit 2 to 4	Control	62	2.5	0.5	Independent Samples T-test **P=0.050***	P=0.11 95% CI 0.9 to 3.4
	aMCI	98	2.7	0.6	Mean difference=-0.2 95% CI=-0.3710 to -0.0003	
CAR visit 1	Control	44	0.4	0.7	Independent Samples T-test P=0.588	
	aMCI	56	0.4	0.6	Mean difference= 0.07324 95% CI=-0.19394 to 0.34042	
CAR visit 2 to 4	Control	61	0.3	0.5	Independent Samples T-test P=0.234	
	aMCI	96	0.2	0.5	Mean difference=0.1 95% CI= -0.0665 to 0.2701	
AUC visit 1	Control	40	131.3	62.2	Independent Samples T-test P=0.273	
	aMCI	51	148.5	81.4	Mean difference=-17.14 95% CI=-48.04706 to 13.75738	
AUC visit 2 to 4	Control	58	132.7	83.4	Independent Samples T-test P=0.186	
	aMCI	88	148.0	56.6	Mean difference=-15.4 95% CI=-38.2536 to 7.5072	

During the course of the study sample 1 was significantly different between the two participant groups. The aMCI group presented with increased levels of cortisol immediately upon awakening compared to the control group. However, after we adjusted for age and gender due to the known influence these variables have on influencing cortisol levels the relationship was no longer significant. The CAR and AUC measures did not differ between participant groups suggesting cortisol levels are not affected by disease state.

There was no significant difference in cortisol levels between the control and aMCI group either at baseline or through the study follow-up period.

4.2.7 Core demographics and cortisol

Independent Samples t test was used to examine the relationship between the core demographic, gender, and the three cortisol measures (Sample 1, CAR, and AUC). Pearson correlation was used to analyse the remaining core demographics (education, BMI and age) and cortisol measures.

Core demographics and cortisol at visit 1 in control participants

In the control group the core demographics gender, education, BMI and ε4 were unrelated to cortisol levels ($p > 0.1$ all cases not shown). Age was the only demographic variable found to influence cortisol at baseline showing those who were older presenting with increased cortisol levels, as determined by the AUC measure (Pearson Correlation 0.293 $p=0.024$).

Core demographics and cortisol at visit 1 in aMCI participants

In the aMCI group the core demographics gender, education, BMI and ε4 were unrelated to cortisol levels ($p > 0.1$ all cases not shown). However, similarly to the control group we also see significant interactions observed between age and sample 1 (Pearson correlation -0.279 p=0.003), the CAR (Pearson correlation 0.219 p= 0.023), and the AUC (Pearson correlation -0.206 p=0.040). The cortisol response immediately upon awakening decreases with age in aMCI participants as did overall cortisol exposure, determined by the AUC measure. Subsequently, we see cortisol levels between samples 1 and 2 (CAR) rise increasingly with age.

Core demographics and cortisol from visit 2 to 4 in control participants

Age, education, gender, BMI and ε4 did not significantly influence any of the cortisol measures (Sample 1, CAR, and the AUC) in the control group. Therefore, these demographic variables were unrelated to cortisol exposure during the course of the study ($p > 0.1$ all cases not shown).

Core demographics and cortisol from visit 2 to 4 in aMCI participants

Education, gender, BMI and ε4 did not significantly influence any of the cortisol measures (Sample 1, CAR, and the AUC) in the aMCI group during the course of the study ($p > 0.1$ all cases not shown). Only the demographic variable age influenced cortisol during the course of the study in the aMCI group. The older aMCI participants presented with lower cortisol levels immediately upon awakening, as determined by sample 1 (Pearson correlation -0.238 p=0.018).

In summary: The demographic, age, consistently influenced cortisol levels in both participant groups at baseline and through the course of the study.

4.2.8 Comparison between psychological stress and cortisol

Pearson correlation was applied to examine the interaction effect between reported psychological stress (objective and subjective stress ratings) at baseline and cortisol levels (Sample 1, CAR, and the AUC) at baseline and during the study follow-up period. In addition, dichotomisation of baseline psychological stress was examined with cortisol levels at baseline and during the study follow-up period. Psychological stress scores were dichotomised into a binary variable of low and high stress using the median for each group as a cut off (RLCQ: control group 160.2 vs aMCI group 125.2; PSS control group 12.1 vs aMCI group 14.5).

Baseline cortisol and psychological stress: control group

Pearson correlation was applied to examine the relationship between the three cortisol measures (awakening sample 1, CAR, and the AUC) and participant subjective and objective stress scores. There was no significant interaction effect between either objective nor perceived stress with cortisol at baseline in the control group and we have therefore, not presented the data (see appendix 1.4).

Dichotomisation of the baseline psychological stress scores showed that the primary psychological stress scale of interest, RLCQ, was related to Sample 1 (low stress 2.6 vs high stress 2.2 p=0.045 mean difference 0.4 95% CI 0.008 to 0.742) and the AUC measure (low stress 138.4 vs high

stress 109.0 p=0.050 mean difference 29.4 95% CI 0.008 to 58.708). Thus in the control group we find above median objective stress ratings is associated with reduced cortisol exposure immediately upon awakening and a reduced exposure to cortisol during the course of the day.

Baseline cortisol and stress: aMCI group

The primary psychological stress scale of interest, RLCQ and cortisol showed a low correlation in the aMCI group (Table 23). We see in participants reporting greater stress show a slower rise in cortisol between samples 1 and 2 (CAR: -0.206 p=0.03). Increased reported stress on the RLCQ (0.198 p=0.05) positively correlates with increased cortisol exposure, as measured by the AUC, at baseline in the aMCI group.

Table 20. Baseline cortisol levels and stress in the aMCI group

		RLCQ informant	PSS
Sample 1 visit 1	Pearson Correlation	0.150	0.187
	Significance	0.122	0.054
	N	108	106
CAR visit 1	Pearson Correlation	-0.206*	-0.096
	Significance	**0.03***	0.330
	N	107	105
AUC visit 1	Pearson Correlation	0.198	0.176
	Significance	**0.05***	0.084
	N	98	97

*Significant at p <0.05

Dichotomisation of baseline objective stress (RLCQ) and perceived stress (PSS), with baseline and follow-up cortisol measures did not appreciably alter these relationships in the aMCI group. At baseline the RLCQ was

related to the Sample 1 and CAR measures with aMCI participants who report above median high stress present with significantly increased cortisol exposure immediately upon awakening (Sample 1: low stress 2.4 vs high stress 2.7 p=0.012 mean difference -0.4 95% CI: -0.626 to -0.080) and with a slower rise in cortisol from sample 1 to sample 2 (CAR: low stress 0.6 vs high stress 0.05 p<0.0001 mean difference 0.4 95% CI 0.199 to 0.614). However, we also found that the PSS was significantly related to sample 1 (low stress 2.4 vs high stress 2.7 [56] p= 0.034 mean difference -0.3 95% CI -0.581 to -0.024) and the AUC measure (low stress 122.3 vs high stress 162.8 p=0.016 mean difference -40.5 95% CI -73.118 to -7.869) with those who report above median high stress present with increased cortisol exposure immediately upon awaking and also over the course of the day, as measured by the AUC.

Dichotomisation of visit 2 to 4 psychological stress and cortisol in control and aMCI group

Independent Samples t test showed no consistent significant interaction effect between reported psychological stress and cortisol exposure through the course of the study in either the control or aMCI group (see appendix 1.4). A lone interaction was seen between the RLCQ and CAR measure (low stress 0.4 vs high stress 0.1 p=0.035 mean difference 0.2 95% CI 0.017 to 0.467) suggesting those who report above median high stress present with a slower rise in cortisol from sample 1 to 2 in the aMCI group. The PSS was not significantly related to any cortisol measure through the course of the study.

Summary: After dichotomising psychological stress we found two measures of cortisol (Sample 1 and AUC) were related to objective psychological stress at baseline in the control group i.e. those

reporting greater psychological stress presented with reduced cortisol exposure immediately upon awakening and during the course of the day. In contrast, in the aMCI group both correlations and dichotomisation supported evidence that aMCI participants who report greater stress (objective and subjective) present with increased cortisol exposure both immediately upon awakening and during the course of the day.

4.2.9 Comparison between physical stress and cortisol

Independent Samples t test was used to examine the relationship between acute physical stress occurring in the 6 months prior to baseline and baseline cortisol levels (sample 1, CAR, AUC) and also recurrent acute physical stresses (i.e. systemic inflammatory events through the course of the study) and chronic physical stress with average cortisol levels (sample 1, CAR, AUC) during the course of the study. We followed previous methodology used by our academic group [403] to group acute systemic inflammatory events together however, if a significant finding was found we then broke the acute systemic events down to include upper respiratory tract infection, lower respiratory tract infection, genitourinary infection, gastrointestinal infection, other infections, accidental trauma, surgical intervention, and Myocardial infarction.

<u>Control group; acute and chronic systemic inflammatory events and cortisol levels</u>

Acute systemic inflammatory events were not related to cortisol exposure in the control group at baseline (Sample 1 No ASIE 2.4 vs ASIE 2.3 95% mean diff 0.1 CI: -0.5 to 0.8 p = 0.7; CAR No ASIE 0.4 vs ASIE 0.5 95% mean diff 0.1 CI: -0.6 to 0.5 p = 0.8; AUC No ASIE 124 vs ASIE 105 mean diff 19 95% CI: -29 to 67 p=0.4).

However, when we examined cortisol levels during the course of the study we find the presence of acute systemic inflammatory events during the course of the study was associated with decreased cortisol exposure immediately upon awakening (Sample 1) and overall levels AUC (Sample 1 No ASIE 2.7 vs ASIE 2.4 95% mean diff 0.3 CI: 0.1 to 0.6 p = 0.008; CAR No ASIE 0.2 vs ASIE 0.2 95% mean diff 0.1 CI: -0.1 to 0.4 p = 0.4; AUC No ASIE 164 vs ASIE 107 Mean diff 57 95% CI: 14 to 101 p=0.01).

Chronic physical stress (diabetes, high blood pressure, and high cholesterol were not related to cortisol levels in the control group through the course of the study (p > 0.1 all cases not shown).

aMCI group; acute and chronic systemic inflammatory events

Acute systemic inflammatory events were not related to cortisol exposure in the aMCI group at baseline (Sample 1 No SIE 2.5 vs SIE 2.5 95% mean diff 0.1 CI: -0.5 to 0.3 p = 0.7; CAR No SIE 0.2 vs SIE 0.3 95% mean diff 0.1 CI: -0.2 to 0.3 p = 0.7; AUC No SIE 143 vs SIE 156 mean diff 12 95% CI: -70 to 45 p=0.7).

Acute systemic inflammatory events were also not related to cortisol exposure in the aMCI group during the course of the study (Sample 1 No SIE 2.7 vs SIE 2.7 95% mean diff 0.01 CI: -0.3 to 0.2 p = 0.9; CAR No SIE 0.2 vs SIE 0.2 95% mean diff 0.01 CI: -0.2 to 0.2 p = 0.9; AUC No SIE 154 vs SIE 142 mean diff 12 95% CI: -12 to 35 p=0.4).

Chronic physical stress (diabetes, high blood pressure, and high cholesterol were not related to cortisol levels in the aMCI group through the course of the study (p > 0.1 all cases not shown).

In summary: Overall, acute systemic inflammatory events asserted greater influence over cortisol levels in the control group during the course of the study than compared to the aMCI group. Acute systemic inflammatory events were significantly related to the sample 1 and AUC measures. This was not replicated in the aMCI group.

4.2.10 Comparison between cognition and cortisol

Correlation analysis was applied to examine the relationship between the three cortisol measures (immediately upon awakening sample 1, CAR, AUC) and cognition at baseline and during the course of the study. Cognition was determined by the primary cognitive outcome of interest, the FCSRT total, followed by secondary cognitive measures (MoCA and TMT Part B) if a significant interaction was evidenced.

Control group

In the control group no significant interaction is observed between cortisol exposure and cognition or cognitive rate of decline, as measured by the primary cognitive outcome of interest, FCSRT total, at baseline (Sample 1 Spearman -0.053 p= 0.672; CAR Spearman 0.083 p=0.508; Spearman AUC 0.004 p= 0.975) or by the FCSRT LOCF total score through the course of the study (Sample 1 Pearson -0.035 p=0.787; CAR Pearson 0.047 p= 0.720; AUC Pearson 0.052 p=0.696).

aMCI group

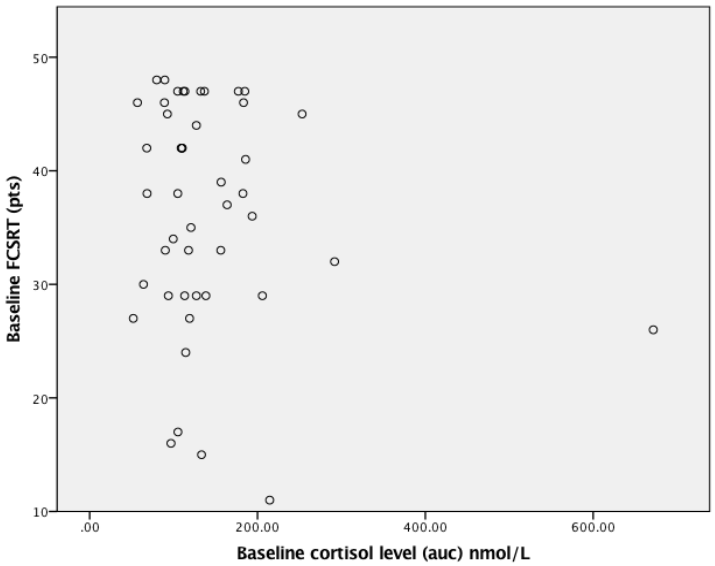
Cortisol levels were not related to cognition as measured by the primary cognitive outcome of interest, FCSRT, in the aMCI group at baseline

(Sample 1 Spearman 0.001 p= 0.991; CAR Spearman -0.025 p=0.796; AUC Spearman -0.080 p=0.429) or the FCSRT LOCF score through the course of the study (Sample 1 Pearson -0.138 p= 0.180; CAR Pearson 0.179 p= 0.085; AUC Pearson -0.180 p=0.097).

The role of $\varepsilon 4$

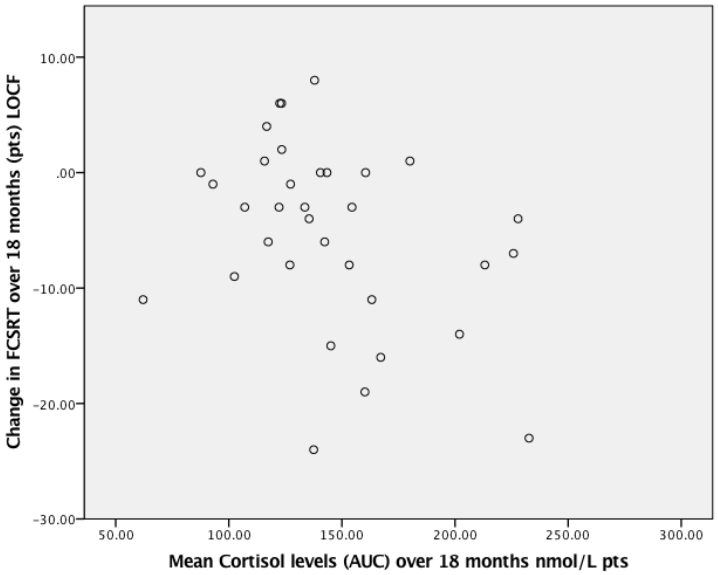
In control participants, ApoE status exerted no significant influence over the relationship between cortisol and cognition, either at baseline or over the 18 month follow-up period. Furthermore, ApoE status did not influence the relationship between cortisol and cognition in the aMCI group at baseline (see Figure 16). However, we find in aMCI participants who are ApoEε4 carriers, higher cortisol levels are associated with an increased rate of cognitive decline (see figure 17). This relationship was observed only on the AUC measure and over the course of the 18 month follow-up period, suggesting the accumulative harmful effect of increased overall cortisol exposure on cognition.

Figure 16. Mean cortisol and baseline FCSRT-IR in aMCI $\varepsilon 4$ carriers



Spearman – 0.13 P = 0.4. The mean cortisol levels (AUC) at baseline and baseline cognitive score are not significantly related.

Figure 17. Mean cortisol and change in FCSRT LOCF score from baseline to Month 18 in aMCI ε 4 carriers



Pearson – 0.35 p = 0.04. The mean cortisol levels (AUC measure) through the study follow-up period and change in cognitive score are significantly related.

In summary: Cortisol was consistently unrelated to cognition at baseline or through the course of the study in both participant groups. However, in aMCI participants categorised as ε4 carriers who present with increased cortisol levels, cognitively decline at an accelerated rate on the FCSRT total score over the study follow-up period.

4.2.11 Interaction between inflammatory markers and cortisol

Correlational analysis was applied to examine the relationship between inflammatory markers (CRP, IFNγ, TNFα, IL6 IL10 and TGFβ) and cortisol measures (Sample 1, CAR, and the AUC) in both participant groups. Dichotomisation of the cytokine data was also performed and results presented below.

<u>Control group</u>

At baseline, cytokine levels and CRP were not related to cortisol exposure as measured by Sample 1, the CAR or the AUC in the control group and therefore have not presented the data ($p > 0.1$ all cases not shown). Independent Samples t test was applied to explore the relationship between cortisol exposure and inflammatory markers from visit 2 to 4 in the aMCI group. Through the course of the study TNFα, IFNγ, IL10, IL6 and TGFβ levels were not related to cortisol exposure ($p > 0.1$ all cases not shown). However, lower serum levels of CRP were significantly associated with an increased CAR i.e. rise in cortisol from sample 1 to 2 (Low CRP: cortisol mean 0.5 vs High CRP: cortisol mean 0.2, p=0.018 mean difference: 0.3 95% CI: 0.050 to 0.525).

<u>aMCI group</u>

IL6 was the only cytokine observed to significantly interact with cortisol exposure at baseline in the aMCI group. Overall, increased cortisol immediately upon awakening and during the course of the day inversely correlated with lower IL6 levels (Sample 1 Spearman -0.266 p=0.007 and the AUC Spearman-0.245 p= 0.018). The remaining cytokines IFNγ, TNFα, IL10 and TGFβ) or CRP did not significantly correlate with cortisol and therefore have not presented the data ($p > 0.1$ all cases not shown).

Independent Samples t test was applied to explore the relationship between cortisol exposure and inflammatory markers from visit 2 to 4 in the aMCI group. We found no relationship between serum levels of TNFα, IFNγ, IL10, and TGFβ to cortisol levels and therefore have not presented the data ($p > 0.1$ all cases not shown). However, as with the baseline sample 1 correlation, we do see an association between low levels of the pro-inflammatory cytokine, IL6, with higher cortisol levels immediately upon

awakening (Low IL6: cortisol mean 3.1 vs High IL6: cortisol mean 2.7, p=0.047 95% CI: 0.005 to 0.849) and an association between low serum levels of IL6 with a reduced rise in cortisol exposure from sample 1 to sample 2 (Low IL6: cortisol mean -0.2 vs High IL6: cortisol mean 0.2, mean diff 0.4 p=0.036 95% CI: 0.0247 to 0.7372). Furthermore, a low serum CRP was also associated with a reduced rise in cortisol from sample 1 to sample 2 (Low CRP: cortisol mean 0.02, vs High CRP: cortisol mean 0.4, p= 0.006 95% CI: 0.096 to 0.565).

In summary: We find no significant associations between cytokine levels and cortisol measures in the control group. However, we do see a higher rise in cortisol in the control group being associated with a drop in CRP levels. In the aMCI group we see a number of associations with cortisol measures and IL6 and CRP suggesting the opposite interaction i.e. a higher rise in cortisol being associated with an increase in CRP and IL6 levels.

4.3 Psychosocial modulators

A number of other mood; personality and social variables were measured at baseline in both the control and aMCI group. We examined how these psychosocial modulators were impacted upon by the core demographics and how they interacted with the biological parameters, reported stress and cognition at baseline and through the course of the study. Psychosocial modulators were normally distributed as determined by Q-Q plots for both participant groups

4.3.1 Psychosocial modulator demographics

Independent Samples t-test was used to examine the psychosocial modulators listed in Table 24 to determine key differences between the participant groups.

Table 21. Key baseline variables between participant groups at visit 1

	Control (n=69)	**aMCI** (n=135)	**Statistical significance**	**Adjusted statistical significance****
Mood (GDS)	1.96 SD=2.1	3.24 SD=2.3	**P=<0.0001*** Mean difference: 1.28 95% CI 0.6 to 1.9	**p=0.001*** Adjusted mean difference: 1.28 95% CI 0.5 to 2.0
VAS (EQ-5D)	83.0 SD=13.0	75.0 SD=15.6	**p<0.0001*** Mean difference: 8.1 95% CI 3.8 to 12.4	**p=0.002*** Adjusted mean difference: 8.4 95% CI 3.2 to 13.6
Social support (MOSS-SSS)	3.9 SD= 0.9	4.1 SD= 0.83	p = 0.2 Mean difference 0.17 95% CI -0.07 to 0.43	
Neuroticism (NEO-FFI)	16.5 SD= 8.5	19.6 SD= 8.1	**P=0.02*** Mean difference: 3.1 95% CI 0.6 to 5.5	**P=0.004*** Adjusted mean difference: 4.3 95% CI 1.4 to 7.3
Coping style (CISS) – Problem solving	51.8 SD=11.6	44.3 SD=10.6	**p<0.0001*** Mean difference: 7.6 95% CI 4.3 to 10.8	**p=0.001*** Adjusted mean difference: 6.6 95% CI 2.7 to 10.6
Coping style (CISS) – Emotion orientated	48.2 SD=9.0	51.2 SD=9.6	**p=0.03*** Mean difference: 3.1 95% CI -5.9 to -0.3	**p=0.047*** Adjusted mean difference: 3.4 95% CI 0.04 to 6.8

*Significant at p <0.05. Mean used for normal distributions and independent samples t-test applied. **Adjusted for age, gender and education years.

Mood - GDS

Data from the GDS scale was normally distributed. The control group had lower depression scores than the aMCI group. Correcting for age, gender or years of education did not substantially alter this relationship.

Visual analogue scale - VAS

Data from the VAS for the aMCI group was normally distributed. A higher VAS scale score means better perceived health. The control group rated themselves as feeling healthier compared with the aMCI group. This relationship was not substantially altered when correcting for age, gender or years of education.

Social Support

Data from the MOSS-SS was normally distributed. The average score for all 18 items of the four subscales plus an additional item was calculated. A higher MOSS-SS score means greater perceived social support. There was no significant difference between the two groups.

Personality

Data from the NEO-FFI neuroticism scale was normally distributed. For the purpose of analysis data was categorized as either male or female and then converted into t-scores. Those with raw scores ranking higher (56+) on the scale are considered to be more neurotic. The aMCI group ranked higher on neuroticism than the control group. After correcting for age, gender and years of education this relationship remained significant.

Coping

Data from the CISS questionnaire was normally distributed. For the purpose of analysis data was categorized as either male or female and then converted into t-scores enabling comparison across the different sub-coping styles.

There were significant differences between groups for task (problem) orientated coping with the control group scoring higher than the aMCI group with an unadjusted mean difference of 7.6. Emotion orientated coping was lower in the control group compared with the aMCI group.

After adjusting for age, gender and years of education the task or emotion orientated coping relationship were not substantially altered. The remaining coping styles including avoidance, distraction and social were not significant.

In summary: Overall, there were a number of significant differences between the two groups. The MCI group had more depressive symptoms; more self-rated health problems; more neuroticism and had a more emotion and less task orientated approach to coping with stress than the control group.

4.3.2 Core demographics and psychosocial modulators

Pearson correlation and Independent Samples t test were applied to examine the relationship between the core demographics (age, gender, education, BMI and ε4) and psychosocial key modulators (VAS, GDS, CISS, NEO-FFI, and the MOSS-SS) at baseline (and at each subsequent visit for depression as measured by the GDS; Self health rating as measured by VAS; social support as measured by the MOSS-SSS).

Control group at baseline

Years of education, gender and ε4 status were not related to any psychosocial modulator in the control group at baseline and we have thus not presented the data (see appendix 1.5). However, those who were older at baseline reported a reduced sense of physical well-being, as measured by the VAS scale, compared to younger participants in the control group (Pearson correlation -0.289 p=0.017). Age further influenced participant's choice of coping style, as measured by the CISS, with those in the control

group who were younger more likely to use a task orientated style (Pearson correlation-0.235 p=0.056). BMI was only related to the GDS (Pearson correlation 0.214 p=0.08) showing those presenting with a higher BMI being more likely to report possible depressive symptoms. The NEO FFI personality questionnaire was not influenced by any of the core demographics and nor was the CISS emotion orientated coping style. The degree of social support, as measured by the MOSS-SS negatively correlated with BMI (Pearson -0.247 p=0.042) showing those who weighed more reported less social support.

Table 22. Psychosocial modulators and age and BMI in control group

		Age at baseline	BMI
VAS SCALE	Pearson Correlation	-0.289	-0.036
	Significance	0.018*	0.769
	N	68	68
NEO-FFI	Pearson Correlation	0.022	0.073
	Significance	0.862	0.551
	N	68	68
CISS task	Pearson Correlation	-0.235	0.034
	Significance	0.056	0.785
	N	67	67
CISS emotion	Pearson Correlation	-0.065	0.069
	Significance	0.600	0.581
	N	67	67
CISS avoidance	Pearson Correlation	0.094	0.131
	Significance	0.448	0.289
	N	67	67
CISS social	Pearson Correlation	0.066	-0.156
	Significance	0.593	0.205
	N	68	68
MOS-SSS	Pearson Correlation	-0.164	-0.247
	Significance	0.181	0.042*
	N	68	68
GDS	Pearson Correlation	-0.098	0.214
	Significance	0.426	0.080
	N	68	68

*Significant at p <0.05.

aMCI group

Table 26 shows a summary of results. Age and ε4 status were not related to psychosocial modulators in the aMCI group and thus we have not presented the data (p > 0.1 all cases not shown).

Gender influenced social support, as measured by the MOSS-SS, with male participants reporting greater support. Moreover, gender was related to personality, as measured by the NEO FFI with males presenting as less neurotic.

aMCI participants who were more educated were more likely to employ a task oriented coping style and report better mood.

BMI was significantly related to two types of coping styles as well as a suggested trend with an emotion orientate coping preference. Overall, aMCI participants who weighed more showed a greater likelihood of preferring coping styles favouring avoidance.

Table 23. Psychosocial modulators and key demographics in aMCI group

		BMI	Years of education	Gender	Statistical significance
VAS SCALE	**Pearson Correlation**	-0.097	0.090	Male:83 Mean: 75.6 SD: 15.4	Independent Samples T Test P= 0.507 Mean diff: 1.8 95% CI: -3.619 to 7.290
	Significance	0.262	0.300	Female:52 Mean:73.7 SD: 15.9	
	N	135	135		
NEO-FFI Neuroticism	**Pearson Correlation**	0.071	-0.131	Male:80 Mean: 18.3 SD: 7.9	Independent Samples T Test **P= 0.023*** Mean diff: -3.4 95% CI: 6.398 to -0.472
	Significance	0.433	0.148	Female:44 Mean: 21.8 SD: 8.1	
	N	124	124		
CISS task	**Pearson Correlation**	-0.074	0.197*	Male:80 Mean: 45.2 SD: 10.3	Independent Samples T Test P=0.166 Mean diff: 2.8 95% CI:-1.166 to 6.711
	Significance	0.419	**0.029***	Female:43 Mean: 42.5 SD: 10.9	
	N	123	123		

CISS Emotion	**Pearson Correlation**	0.161	-0.141	Male:80 Mean: 51.7 SD: 9.5	Independent Samples T Test P= 0.467 Mean diff: 1.3 95% CI: -2.275 to 4.931
	Significance	0.074	0.120	Female:43 Mean:50.4 SD:9.8	
	N	123	123		
CISS avoidance	**Pearson Correlation**	0.254	-0.014	Male:80 Mean: 52.7 SD: 51.4	Independent Samples T Test P=0.483 Mean diff: 1.3 95% CI: -2.342 to 4.927
	Significance	0.005*	0.878	Female:43 Mean:51.4 SD:10.3	
	N	123	123		
CISS distraction	**Pearson Correlation**	0.291	0.117	Male:80 Mean: 50.0 SD: 10.0	Independent Samples T Test P= 0.779 Mean diff: 0.6 95% CI: -3.344 to 4.453
	Significance	0.001*	0.198	Female:43 Mean:49.4 D:11.2	
	N	123	123		
MOS-SSS	**Pearson Correlation**	-0.139	-0.012	Male:77 Mean: 4.2 SD: 0.8	Independent Samples T Test P= 0.049* Mean diff: 0.3 95% CI: 0.001 to 0.602
	Significance	0.123	0.892	Female: 47 Mean: 3.9 SD:0.9	
	N	124	124		
GDS	**Pearson Correlation**	0.043	-0.215*	Male: 83 Mean: 3.2 SD: 2.3	Independent Samples T Test P= 0.671 Mean diff: -0.177 95% CI: -1.001 to 0.464
	Significance	0.622	0.012*	Female: 52 Mean: 3.4 SD: 2.4	
	N	135	135		

*Significant at p <0.05.

In summary: significant interactions between the core study demographics and psychosocial variables were more frequently evidenced in the aMCI group.

4.3.3 Comparison between psychological stress and psychosocial modulators

Pearson correlation was applied to examine the relationship between psychosocial key modulators (GDS; MOSS-SS; NEO FFI; VAS, CISS) recorded at baseline (and at each subsequent visit for depression as measured by the GDS; Self health rating as measured by VAS; social support as measured by the MOSS-SSS) and psychological stress (RLCQ and PSS). In addition, psychological stress scores were dichotomised into a binary variable of low and high stress using the median for each group as a cut off (RLCQ: control group 160.2 vs aMCI group 125.2; PSS control group 12.1 vs aMCI group 14.5).

Control group at visit 1

Greater objective stress (RLCQ) was associated with low mood reported on the GDS and with lower levels of perceived social support, as measured by the MOSS-SSS but no other relationships were found (See table 27). However, a number of relationships were found with perceived stress (PSS). Thus, the PSS stress score was significantly associated with increased depression scores, neuroticism, the CISS emotional orientated coping style, reduced perceived social support as measured by the MOSS-SSS and reduced health well-being as measured by the VAS.

Table 24. Comparisons between psychosocial modulators and stress scores in the control group at baseline

		V1 RLCQ	V1 PSS
GDS – mood	Pearson Correlation	0.28	0.557
	Significance	0.020*	< 0.001*
	N	68	68
NEO-FFI neuroticism	Pearson Correlation	0.151	0.594
	Significance	0.220	< 0.001*
	N	68	68
CISS emotion coping	Pearson Correlation	0.007	0.417
	Significance	0.958	< 0.001*
	N	67	67
MOS-SSS – Social support	Pearson Correlation	-0.245*	-0.435
	Significance	0.044*	< 0.001*
	N	68	68
VAS SCALE- well being	Pearson Correlation	-0.127	-0.343
	Significance	0.302	0.004*
	N	68	68

*Significant at p <0.05.

aMCI group

No significant relationships were found between objective measures of stress (RLCQ) and psychosocial modulators. For psychosocial modulators unrelated to objective stress in the aMCI group see table 28.

However, a number of significant associations were found with perceived stress measures (PSS) and psychosocial modulators (see table 28). Thus in the aMCI group, we see greater perceived psychological stress is highly related to increased neuroticism reported on the NEO personality questionnaire and with a preference for an emotion oriented coping style. Increased perceived stress was further related to lower mood, worse health well-being and reduced social support.

Table 25. Comparisons between psychosocial modulators and stress scores in the aMCI group.

		V1 RLCQ	V1 PSS
V1 GDS total score	Pearson Correlation	0.098	0.401
	Significance	0.260	<0.**0001***
	N	134	0129
V1 NEO-FFI Score	Pearson Correlation	0.082	.0492
	Significance	0.366	**<0.0001***
	N	123	0.121
V1 CISS emo t score	Pearson Correlation	0.013	0.454
	Significance	0.885	**<0.0001***
	N	122	0.121
V1 MOS-SSS overall	Pearson Correlation	0.016	-0.253
	Significance	0.859	**0.005***
	N	124	0.124
V1 VAS SCALE	Pearson Correlation	-0.092	-0.277
	Significance	0.292	**0.001***
	N	134	129

*Significant at p <0.05.

In summary: Objective psychological stress, as measured by the RLCQ, was related to mood, social support and neuroticism in the control group. However, the RLCQ was unrelated to all psychosocial variables in the aMCI group. However, perceived psychological stress, as measured by the PSS, was influenced by multiple psychosocial modulators at baseline in both participant groups. We see consistently in both participant groups that those experiencing greater perceived stress report increased levels of neuroticism, a preference towards an emotional form of coping style, low mood, decreased social support and worse self-rated health.

4.3.4 Comparison between cognition and psychosocial modulators

A mixture of parametric and non-parametric analysis was applied to examine the psychosocial key modulators (GDS; MOSS; NEO; VAS, CISS) recorded at baseline (and at each subsequent visit for depression as measured by the GDS; Self health rating as measured by VAS; social support as measured by the MOSS-SSS) and cognition (FCSRT total) and change in cognition over time. The majority of psychosocial variables were unrelated to baseline cognition and rate of cognitive decline in both control and aMCI participants and thus the data has not been presented.

Control group

At baseline the only psychosocial modulator related to the primary cognitive measure of interest, the FCSRT total, was the neuroticism personality NEO score (Pearson 0.256 p=0.0235). No other interaction between cognition and the remaining psychosocial modulators was observed. Furthermore, no relationship between psychosocial modulators and cognitive decline was found (see appendix 1.6).

aMCI group

Spearman Correlation showed there was no relation between measured psychosocial modulators and the FCSRT total score or the change in the FCSRT LOCF total score through the course of the study follow-up period (see appendix 1.6).

Summary: Neither measures of mood (GDS) or potential modulators of stress (neurotic personality; social support or self-rated health measures) exerted a consistent significant influence over baseline

measures of cognition or rates of cognitive decline in either of the participant groups.

4.3.5 Comparison between physical stress and psychosocial modulators

Independent Samples t test was applied to examine the relationship between psychosocial key modulators (GDS; MOSS-SSS; NEO; VAS, CISS) recorded and physical stress (acute and chronic) at baseline (and at each subsequent visit for depression as measured by the GDS; Self health rating as measured by VAS; social support as measured by the MOSS-SSS). **However, since a relationship between psychosocial modulators and biological outcomes was not formally identified a priori then these analyses should be considered as exploratory only.**

Visit 1 control group

Acute systemic inflammatory events were not related to the majority of psychosocial modulators in the control group at baseline. For chronic stress, the only relationship found showed an interaction between reported high blood pressure and reduced health well-being, as measured by the VAS scale (No Hypertension 86.2 pts vs Hypertension 77.0 pts p=0.005 mean diff 9.2 pts 95% CI = 2.918 to 15.408).

Visit 1 aMCI group

Similar to the control group, acute inflammatory events did not relate to psychosocial modulators. However, we do see a greater number of significant associations evidenced between psychosocial modulators and chronic physical stressors in the aMCI group.

High blood pressure was significantly associated with those favouring an avoidant (No Hypertension 49.6 pts vs 54.5 pts p=0.005 mean difference: 4.9 pts 95% CI: 1.5 to 8.297), social (No Hypertension 50.5 pts vs 54.2 pts p=0.031 mean difference: 3.7 pts 95% CI: 0.34 to 7.1) or distraction (No Hypertension 46.8 pts vs 52.2 pts p=0.004 mean difference 5.4 pts 95% CI: 1.7 to 8.9) coping style. Those with high cholesterol in the aMCI group were more likely to prefer an emotion orientated coping style (low cholesterol 49.2 pts vs 53.3 pts p=0.017 95% CI= 0.3 to 1.5) and be more neurotic (low cholesterol 17.4 pts vs 21.7pts mean difference 4.3 pts: p=0.003 95% CI= 1.5 to 7.04). Diabetes was unrelated to all psychosocial modulators ($p > 0.1$ all cases not shown).

Visit 2 to visit 4 control group

Independent Samples t test was applied to examine the relationship between psychosocial variables and acute physical stress through the course of the study in the control group. Psychosocial modulators were unrelated to acute physical stressors and thus have not presented the data ($p > 0.1$ all cases not shown).

Visit 2 to visit 4 aMCI group

Independent Samples t test was applied to examine the relationship between physical stress and psychosocial variables through the course of the study in the aMCI group.

aMCI participants who rated themselves as more neurotic experienced an increased number of acute systemic inflammatory events between visit 2 and 4 (No ASIE 17.8 pts vs ASIE 21.1pts p=0.026 mean difference: 3.3 pts 95% CI= 0.4 to 6.15). Likewise, those reporting greater neuroticism had an

increased risk of high cholesterol (low cholesterol 17.4 pts vs high cholesterol 21.7 pts p= 0.003 mean difference: 4.2pts 95% CI: 1.4 to 7.04).

Preferred coping style further impacted on the likelihood of experiencing a physical stressor through the course of the study. Those favouring an emotion oriented coping style were at greater risk of high cholesterol (no high cholesterol 49.2 pts vs high cholesterol 53.2 pts p=0.017 mean difference: 4.1 pts 95% CI: 0.7 to 7.5). We further found those favouring an avoidant (no high blood pressure 49.6 pts vs 54.5 pts p=0.005 mean difference 4.9 pts 95% CI 1.5 to 8.3), distraction (no high blood pressure 46.8 pts vs 52.2 pts p=0.004 mean difference 5.4 pts 95% CI: 1.7 to 9.0) and social (no high blood pressure 50.5 pts vs high blood pressure 54.2 pts p=0.031 mean difference 3.7 pts 95% CI: -0.3 to 7.1) coping style were at further risk of high blood pressure.

In summary, a greater number of psychosocial modulators including neuroticism and various coping styles measured at baseline are related to increased chronic physical stressors in the aMCI group at baseline and through the course of the study.

4.3.6 Comparison of cortisol and psychosocial modulators

Pearson correlation was applied to examine the relationship between psychosocial key modulators (GDS; MOSS-SSS; NEO; VAS, CISS) recorded at baseline (and at each subsequent visit for depression as measured by the GDS; Self health rating as measured by VAS; social support as measured by the MOSS-SSS) and salivary cortisol levels (Sample 1, CAR, and the AUC).

Cortisol levels in control participants at baseline and visit 2 to 4

Only social support (MOSS-SS) correlated with cortisol levels (Sample 1 measure) and only in the control group at baseline. Greater perceived social support was associated with increased cortisol levels immediately upon awakening (Pearson 0.323 p=0.008). The remaining psychosocial variables were not related to the cortisol measures and thus have not presented the data (p > 0.1 all cases not shown).

Cortisol levels in aMCI participants at baseline and visit 2 to 4

Similar to the control group we found psychosocial modulators did not influence cortisol levels at baseline in the aMCI group. A preferred emotion orientated coping style as measured by the CISS (Pearson correlation 0.236 p=0.023) was related to increased cortisol exposure (sample 1) through the course of the study. However, the remaining psychosocial variables were not related to the cortisol measures and thus have not presented the data (p > 0.1 all cases not shown).

In summary, we see a minimal influence of psychosocial variables modulating cortisol levels in the control and aMCI group.

4.3.7 Comparison of inflammatory markers and psychosocial modulators

A mixture of Pearson and Spearman correlation was applied to examine the relationship between the psychosocial key variables (GDS; MOSS; NEO; VAS, CISS) recorded at baseline (and at each subsequent visit for depression as measured by the GDS; Self health rating as measured by

VAS; social support as measured by the MOSS-SSS) and peripheral cytokine levels (IFNγ, TNFα, IL6, IL10, TGFβ) and CRP. At baseline the pro-inflammatory cytokines IL-4, IL12, and IL13 were detectable in less than 5% of the assays in both the control and aMCI groups, thus no further analyses of these cytokines was undertaken in relation to psychosocial variables. Furthermore, as previously discussed, to reduce our false positive rate we used a conservative significant p value of < 0.008 in keeping with Bonferonni correction for multiple comparisons.

Visit 1 Control group

Overall in the control group we only see interactions between coping style with serum cytokine and CRP levels. Avoidant coping style was significantly correlated with serum IFNγ levels (Spearman 0.33 $p = 0.008$) and CRP levels (Spearman 0.34 $p = 0.006$), task oriented coping style with serum TGFβ levels (Pearson 0.37 $p = 0.003$). The remaining cytokines and CRP did not relate to any psychosocial modulators at baseline in the control group ($p > 0.1$ all cases not shown).

Visit 1 aMCI group

In the aMCI group we see a significant relationship between mood, self-reported health and coping style with serum cytokine levels. Thus, those reporting lower mood on the GDS were more likely to demonstrate increased IL10 serum levels (Spearman correlation 0.240 $p=0.007$). Examination of other serum inflammatory markers showed that low mood also showed non-significant ($p < 0.05$) trends with a range of pro-inflammatory markers (IL6 Spearman correlation 0.19 $p=0.03$; CRP Spearman correlation 0.22 $p=0.02$). Furthermore, aMCI participants who reported worse health well-being, as measured by the VAS, presented with increased TNFα serum levels (Pearson correlation -0.242 $p=0.007$). Finally

those with a higher emotional coping style had lower serum TGFβ levels (Pearson correlation -0.29 p=0.002).

In summary: At baseline, across both participant groups we see relationships between higher levels of pro-inflammatory states with avoidant or emotional coping behaviours and anti-inflammatory states with task coping behaviours. In addition, in the aMCI group but not the control group, we see low mood associated with an increase in the anti-inflammatory cytokine IL10 and poor reported self-health associated with increased serum TNFα **levels.**

Chapter 5: Discussion

The current study examined the relationship between psychological stress and cognitive decline in a cohort of aMCI participants compared to cognitively intact control participants. The aMCI group were more likely to be male, were older and held less years of education than compared to the control group. These differences are most likely to be largely due to sampling methods e.g. controls were in part derived from volunteer societies e.g. university of the 3rd age and so the effects of age, gender and education were controlled for in all cross group comparison analyses. However, overall, there were still clear differences observed in a number of key variables between the control and aMCI group, which were not altered after correcting for these key demographic confounders.

As expected, the aMCI group also showed significant cognitive impairment compared with the control group across all the key cognitive tasks at baseline and rates of cognitive decline over the 18 month follow-up period. Moreover, the aMCI group reported greater perceived stress at baseline and during the course of the study than compared to the control group. Interestingly, there was no statistical difference in reported objective life event stress (RLCQ) between the two participant groups, suggesting another variable is responsible for the observed increase in perceived stress. This may be a consequence of the aMCI group having concerns over a failing memory or receiving a recent diagnosis for memory problems.

The key study findings in relationship to the study hypotheses will now be discussed.

5.1.1 Psychological stress and rate of cognitive decline

We proposed that in aMCI participants psychological stress would serve as a secondary trigger activating the primed central microglia inflammatory state and leading to an exaggerated and neurotoxic immune response. Therefore, we predicted psychological stress will be associated with worsened cognitive decline, a clinical marker of advancing neurodegeneration, over the 18 month follow-up period only in aMCI participants. As expected, findings support this hypothesis showing that our primary measure of objective stressful life events occurring during the course of the study was associated with increased rates of cognitive decline across a range of measures in the aMCI group including the primary cognitive measure, the FCSRT. This finding was independent of ApoE status. Furthermore, as predicted, objective stressful life events were not associated with a change in the rate of cognitive decline in the control group over the 18 month follow-up period.

Psychological stress and cognitive decline

There are continued discussions to date debating whether the experience of psychological stress can increase risk of cognitive decline, MCI status, and the development of AD [214]. Some suggest the tentative link between stress and worse cognition is due to confounding factors, which ultimately reflect the underlying progression of AD pathology. For instance, symptoms of stress may result from a person's awareness of an already failing memory, or are an early neuropsychiatric symptom, such as anxiety, resulting from accumulating AD pathology. However, a previous key study shows the association between distress and cognitive decline remained significant after controlling for AD neuropathology at baseline [59]. In the current study, we did not see perceived psychological stress, measured by the PSS, influence cognitive decline in either of the participant groups.

Thus, study findings do not support the theory that concerns over a failing memory or the emergence of early neuropsychiatric symptoms are responsible for the relationship observed between increased stress and a faster rate of cognitive decline in an MCI population. Moreover, in addition to perceived stress, we assessed objective stressful events via a checklist measure (RLCQ) in an effort to rule out bias resulting from early neuropsychiatric symptoms. Study findings show that, unlike perceived stress, it was higher objective life event stress that was associated with a faster rate of cognitive decline. In both cases correcting for baseline cognitive state did not alter this relationship.

Our results are consistent with previous findings including several longitudinal cohort studies that demonstrate a link between the experience of psychological stress and cognitive decline later in life [347-350] and independently acting as a risk factor for MCI and dementia [214, 351-353]. In addition, findings from this study confirm those of an earlier, although smaller, study conducted by Peavy *et al* who found a faster rate of cognitive decline in 27 aMCI participants reporting greater psychological stress in the previous 6 months [61].

Interestingly, in later study of non-demented participants Peavy *et al* found that greater stress ratings were associated with worse memory performance but only in the presence of at least one ApoE ε4 allele suggesting a gene-environment interaction [357]. In contrast, this study suggests that both measures of psychological stress and being an ApoE ε4 carrier were independently associated with an accelerated rate of cognitive decline. This finding is in agreement with a previous study tracking 4,108 participants also showing that chronic stress was significantly associated with an increased risk of AD that was independent of ApoE status [63].

Overall, there is mounting support that psychological stress contributes to the development of AD, and this study confirms that objective measures of stress are related to the rate of cognitive decline in an aMCI group independently to ApoE ε4 allele status.

Cortisol and cognitive decline

Current literature shows a relationship between hypersecretion of cortisol with MCI status [99, 100] cognitive decline [99, 107, 108] and AD [101-103]. This relationship becomes stronger in the presence of ApoE ε4, a well-known risk factor for AD [104-106]. In addition, the chronic stress condition, PTSD, is linked to a greater risk of both cognitive impairment and the development of AD [339], with vulnerability factors for the development of PTSD, including a dysregulated HPA axis, being partly similar to those of AD [336-338]. In the current study, initial analyses showed cortisol levels did not differ between the control and aMCI groups, and neither did cortisol influence cognitive trajectory during the course of the study in either of the participant groups.

However, when ApoE ε4 was considered together with cortisol, we observed increased cortisol exposure was indeed associated with an accelerated rate of cognitive decline in the aMCI group. The relationship was only found in aMCI participants presenting as ε4 carriers, and over the course of the study. Thus, findings suggest cortisol exposure exerts an accumulative harmful effect on cognition over time, and only in those presenting with existing AD pathology. Therefore, increased cortisol levels may potentiate MCI and AD progression providing partial support for the glucocorticoid cascade theory [110]. The glucocorticoid cascade theory proposes hippocampal atrophy, initiated by the pathological processes

associated with AD, dysregulates negative feedback control of the HPA axis leading to hypercortisolemia. This environment becomes neurotoxic and subsequently accentuates HPA axis dysregulation and contributes to neurodegenerative processes.

Notably however, Peavy et al found greater cortisol exposure to be associated with a slower rate of cognitive decline in aMCI participants [61], highlighting the complexity of interpreting findings that investigate the effects of cortisol on cognition. Well-known methodological issues reported in salivary cortisol sampling may account for some of these inconsistencies. Although salivary cortisol is a commonly used measure in stress research [424] its reliability as a measure has often been questioned with a number of biological, genetic, health, gender related variables and lifestyle mediators shown able to influence cortisol levels [423-427]. Therefore, other and potentially more reliable methods of cortisol sampling should be considered that may reduce the variability of findings, including sampling via the use of hair [428]. Sampling methods such as these would also help address other methodological problems, including participant non-compliancy with sampling instructions. Non-compliancy would be particularly salient in our study population of aMCI participants who experience memory loss and problems with concentration. Furthermore, measuring cortisol exposure via hair would provide an historical account of HPA axis activation in individuals.

5.1.2 Psychological stress and biological parameters

Data from this study also supports our second hypothesis that in aMCI (but not control) participants chronic stress would be associated with a pro-

inflammatory phenotype and an associated increase in cortisol in a failed attempt to dampen down this exaggerated immune response.

As expected, in the control group, high cortisol levels were related to lower levels of the serum pro-inflammatory marker CRP, and high objective psychological stress was associated with decreased cortisol and serum pro-inflammatory cytokine IFNϒ levels. These findings are in keeping with an intact regulated HPA axis in which stress-induced raised cortisol levels reduce the pro-inflammatory drive that then results in reduced cortisol levels. On the contrary in the aMCI group, high cortisol levels were related to high levels of the serum pro-inflammatory marker CRP and IL6, suggesting a failure of negative feedback between the immune system and HPA axis. Furthermore, high objective psychological stress was associated with a slower CAR, suggesting a less reactive HPA axis under stressed conditions in those with aMCI. Overall, findings indicate in aMCI persons a dysregulated HPA axis, in which psychological stress increases cortisol but cortisol does not then dampen down the pro-inflammatory response that continues to drive cortisol levels upwards. In support of this finding, previous studies suggest potential dysregulation of the HPA axis in individuals with MCI [111], and in AD a hyper-secretion of cortisol has been documented [101-103].

Likewise, although we found a direct relationship between the presence of objective psychological and acute physical stress and increased serum pro-inflammatory levels, including IL6, in the control group as shown previously [280, 287-291], we did not find this relationship in the aMCI group. Similarly, we found chronic physical stress was associated with a decrease in IFNϒ in control participants but not in the aMCI group. The lack of findings in the aMCI group may reflect a lack of sensitivity in

measuring CRP and cytokines in peripheral blood. For instance, at baseline we found the pro-inflammatory cytokine IL12 and IL13 were detectable in less than 5% of the assays in both the control and aMCI groups. Additionally, we used Bonferroni correction, which we recognise may be too conservative to apply in some cases particularly when analysing a small data set such as this study population. This would explain why no relationships were found between chronic inflammatory events and serum CRP or cytokines levels in either of the participant groups. However, it is also possible that the lack of a direct relationship evidenced between increased objective psychological stress and serum pro-inflammatory markers may also reflect the marked dysregulation of the HPA axis found in the aMCI group. Thus in the aMCI group raised cortisol levels as a result of stress are having little impact on the serum inflammatory markers and so any direct relationships between objective measures of stress and markers of inflammation are lost.

5.1.3 Cognitive decline and inflammatory markers

Our third hypothesis predicted that a pro-inflammatory phenotype is associated with cognitive decline in the aMCI (but not control) participants. In this study, findings show increased serum levels of the anti-inflammatory cytokine TGFβ is associated with a slower rate of cognitive decline (FCSRT) over the 18 month follow-up period in the aMCI group.

TGFβ is an anti-inflammatory cytokine typically expressed in the brain in low levels by neuronal and glia cells. In the main, TGFβ is neuroprotective and regulates key events including tissue repair and neuronal survival [429, 430]. Animal studies show a reduction of TGFβ receptors expressed by neurons during the early phase of AD [431] and a reduction in neuronal

TGFβ signaling subsequently promoting excessive Aβ accumulation [429]. Furthermore, a recent animal study using an AD model in rats found the pre-treatment of TGFβ prior to administration of an Aβ1-42 injection suppressed consequent neuroinflammatory and neurodegenerative processes including the prevention of a reduction in neurotrophic factors, anti-inflammatory cytokines and the prevention of Aβ1-42 induced increases of pro-inflammatory mediators and cytokines such as TNFα [432]. Moreover, increased levels of this anti-inflammatory cytokine in the brain has recently been associated with AD pathology and accompanying neuroinflammation [433] suggesting TGFβ is attempting to supress the pro-inflammatory drive observed in AD. Thus, a reduction in this anti-inflammatory mediator could lead to further inflammation and accelerated disease progression.

However, the role of TGFβ in AD pathogenesis remains under researched in clinical studies and even more so in an aMCI population. Findings to date show a significant reduction of TGFβ1 levels in plasma and in cultured circulating peripheral blood mononuclear cells of AD participants compared to healthy controls [434-436]. Likewise, our findings show a significant reduction in serum levels of TGFβ in aMCI participants compared to the control group. Notably however, other studies of AD patients show increased plasma levels of TGFβ [437] and for levels to correlate with disease progression, presenting as increased in the early stages of AD followed by a subsequent reduction as the disease progresses [165].

Fitting within this picture, our findings also show increased serum levels of the pro-inflammatory cytokine TNFα is associated with accelerated cognitive decline in the aMCI group. Although it should be noted that this

was an observed trend and did not reach statistical significance at the Bonferonni cut off. However, as previously mentioned, we recognise applying Bonferroni correction may be too conservative to use in this small study population or when priori evidence show significant findings and thus, important relationships may potentially be lost. We therefore suggest this finding should be tentatively considered as significant as it is in line with a number of previous studies showing elevated levels of TNFα is associated with worse cognitive performance [128-133], MCI status [13, 89-92], and importantly, has predicted conversion from MCI to AD several years before [89, 152, 153].

Overall, findings from this study suggest in an aMCI population that higher levels of the anti-inflammatory cytokine TGFβ is protective against cognitive decline whilst higher levels the pro-inflammatory cytokine TNFα is associated with accelerated cognitive decline.

5.1.4 Psychosocial modulators and rate cognitive decline

Our final hypothesis proposed we would see psychosocial modulators of the stress response influence rates of cognitive decline in aMCI participants through modulation of the physiological stress response (inflammation and cortisol measures).

The aMCI group reported significantly greater neuroticism, lower mood and a preference for an emotion orientated coping style at baseline than compared to the control group. The aMCI group presenting with increased neuroticism and a lower mood may be indicative of early neuropsychiatric symptoms due to mounting AD pathology. However, these findings may

also reflect participant concerns regarding their failing memory, being recently diagnosed with aMCI, and anxiety over the increased likelihood of future conversion to AD. Furthermore, aMCI participants preferring an emotion orientated coping style may stem from a belief that their diagnosis is unchangeable and thus, chose not to adopt a more proactive problem-solving coping style. Indeed, it is generally acknowledge in the literature that people chose, to some extent, different coping styles depending on the circumstances of the stressor [407].

Previous findings from cohort studies indicate neuroticism to be a risk factor for cognitive impairment [213], MCI [214], and the development of AD [216]. Likewise loneliness has been identified as a risk factor for dementia amongst a cohort of 7867 individuals in China [228]. Other studies show neuroticism [215] and lack of social support [226] are also associated with cognitive decline. However, we found that neither measures of mood (depression and perceived well-being) nor potential modulators of stress (neuroticism, coping and social support) exerted a consistent significant influence over baseline measures of cognition or rates of cognitive decline in the aMCI group. These findings were unexpected, in particular regarding the previously documented association between greater neuroticism and reduced social support with accelerated cognitive decline. Exploring these findings further, we dichotomised the baseline FCSRT total score using the median to create two groups. Those scoring less than 42 were designated as a low score whereas those scoring 42 and above were designated as a high score, and thus performing better on the FCSRT. Using this method, we found cognitive status at baseline did not influence the relationship between any of the psychosocial variables and rate of cognitive decline in either of the participant groups.

It is possible that we did not track this cohort of aMCI participants for an adequate period of time, to allow us to fully assess the prolonged cumulative effects of psychosocial mediators on rate of cognitive decline [182, 186, 189, 190]. For example, a pertinent study showing a relationship between neuroticism and cognitive decline tracked participants for a mean of 4.9 years [59]. Whilst other key studies finding increased loneliness to act as a risk factor for conversion to AD tracked participants for 3 years [228] and with cognitive decline for 10 years [149]. Alternatively, it is feasible that psychosocial modulators could serve as risk factors for aMCI prevalence but not exert a large enough effect on disease trajectory once individuals progress to the clinically symptomatic stage.

5.1.5 Other non-hypothesised study findings

ApoE ε 4 allele

As shown in a number of studies, and repeated here, carriers of the ApoE ε4 allele were more frequent in the aMCI group than the control group and aMCI participants carrying this allele had a markedly increased rate of cognitive decline than non-carriers. However, the aMCI participants carrying the ApoE ε4 allele also presented with a lower CRP level that was not found in the control group. This finding agrees with the ADNI cohort study that tracked MCI and AD patients over a year. Similarly, low levels of CRP were evidenced in those who were ε4 carriers [442]. The reason for this finding is not clear but may reflect the lower levels of systemic inflammatory events reported in ε4 carriers. In the 6 month period prior to baseline we found an increased number of acute systemic inflammatory events reported by the aMCI group. However, when we looked at ApoE status we found a reduced number of acute systemic inflammatory events, in particular infections, reported by aMCI participants who were ε4 carriers.

This finding was not observed in the control group. Thus ApoE ε4 may offer some protection against infections for reasons unclear but at the expense of an increased rate of cognitive decline. Overall, more research is needed to better understand how ApoE status drives the differences observed in serum CRP level, and in the occurrence of systemic inflammatory events in an aMCI population.

ApoE ε4 further played an important role in how physical stress influenced cognitive decline in the aMCI group. We found in those who were ε4 carriers, the presence of systemic inflammatory events did not alter the rate of cognitive decline through the study follow-up period. However, the presence of systemic inflammatory events was associated with an increased rate of cognitive decline in aMCI participants who were ε4 negative. Thus, the findings suggest physical stress accelerates disease progression in those who would otherwise potentially deteriorate at a slower rate due to lacking the ε4 risk gene. These findings fall in line with a previous study that found the presence of systemic inflammatory events was associated with an accelerated cognitive decline in patients diagnosed with AD [403]. Notably and as mentioned earlier, we see a reverse effect between psychological stress and the rate of cognitive decline, with increased cortisol exposure associated with an accelerated rate of cognitive decline only in aMCI ε4 carriers.

The influence of psychosocial variables

We saw minimal evidence of psychosocial factors modulating cortisol levels. This did not conform with expectation, in particular for the personality trait neuroticism, which has previously been associated with greater cortisol exposure over the course of the day [207, 438] and lower

cortisol levels in the morning [439]. However, we do find multiple significant interactions between psychosocial modulators and serum inflammatory markers. In the main, we see an increase in neuroticism and a preference for an emotion orientated coping style associated with reduced levels of TGFβ. To our knowledge, this interaction with TGFβ has not been shown in a healthy participant group before nor in those reporting cognitive complaints or diagnosed with MCI. In agreement with previous findings, we did see a relationship between low mood and the increased pro-inflammatory markers CRP, TNFα and IL6 [440, 441] and with low levels of the anti-inflammatory cytokine IL10. We further found poor self-rated health was associated with increased levels of the pro-inflammatory marker TNFα.

Overall, these findings are predominantly observed in the aMCI group suggesting those with mounting AD pathology share a susceptibility to experience low mood, increased neuroticism, altered coping mechanisms, and report worse self-rated health. Thus, it is plausible that like cognitive decline, the increased perceived stress observed in the aMCI group in addition to the proposed psychosocial modulators (depression, poor self-rated health, increased neuroticism, altered coping mechanisms) are the end result of a pro-inflammatory environment previously evidenced in aMCI and AD brains, rather than these behaviours modulating the cortisol/cytokine axis. Further support for this theory comes from the aMCI group in this study, who present with lower levels of TGFβ, and therefore more likely to be exposed to increased inflammation.

Of interest, we note that low mood, high neuroticism, poor self-rated health, reduced social support and altered coping styles was associated with increased levels of perceived stress in both the control and aMCI

group at baseline. Consistent findings such as these across both participant groups warrant further investigation. However, due to these study findings being correlational it is unclear whether the psychosocial factors directly influenced perceived stress or vice versa. More likely, we suggest these factors influence one another through bidirectional communication.

The interaction between physical and psychological stress

In this study we found those aMCI participants reporting greater perceived stress during the 18 month follow-up period were at increased risk of experiencing a chronic inflammatory condition, including high blood pressure and high cholesterol. This relationship was not evidenced in the control group, suggesting an increased susceptibility to the potentially harmful effects of psychological stress in those predisposed to dementia. These findings lend support to the allostatic load theory, whereby chronic perceived stress forces the adaptive physiological stress response to persist thus subjecting the body to prolonged chemical imbalances and elevated physiological states including high blood pressure [182, 188-190]. Consequently, the cumulative costs of prolonged exposure to stress mediators and altered physiological states become harmful to health [182, 186, 189, 190].

5.1.6 Limitations and future direction

Limitations

Several limitations to this study should be considered and are now outlined.

Firstly, MCI participants are recognised as a heterogeneous group that can differ in both clinical presentation and disease pathology. However, we opted for the subtype of amnestic MCI to reduce this heterogeneity in an effort to capture the symptomatic preclinical phase of AD [2, 4]. All aMCI participants prior to study enrolment had received a formal MCI diagnosis from a NHS clinician, primarily through a memory service in Older Persons Mental Health. MCI participants were then seen at baseline by a research clinician, primarily a medical doctor, and underwent a standardised screening process to confirm amnestic MCI whilst ruling out cognitive impairment attributed to other causes including depression, excessive alcohol consumption, and anxiety. Uncertainty over diagnosis resulted in participants either being re-assessed at a later date or a case discussion with the Principal Investigator, a Geriatric Psychiatrist, at each site. A total of 30 aMCI participants converted (17%) to AD over the 18 month follow-up period, falling in line with previous findings for conversion rates from MCI to AD [19].

We cannot exclude the possibility that some participants in the control group may have exhibited early non-symptomatic AD pathology. Without the use of imaging techniques this could not be fully controlled for. However, control participants who reported significant subjective memory loss at any time point during the study follow-up period were excluded from the study. Furthermore, none of the control participants showed a

rapid cognitive decline or converted to MCI during the 18 month follow-up period.

We recognise that the use of a questionnaire check list measuring psychological stressful events is open to reporting bias. A limited number and type of stressful events are typically listed and participants, in particular those with aMCI, retrospectively may forget events occurring in the previous 6 month. However, research personnel encouraged compliance with questionnaire guidelines prior to administration and assisted throughout administration when necessary and appropriate. In addition, due to the nature of aMCI (marked by short-term memory loss) the study partner completed an informant version of the primary stress measure of interest (RLCQ) that was the main outcome used for analysis.

We used salivary cortisol as a measure that has been a popular method used over recent decades. However, the measurement can be affected by a range of factors leading some to suggest other methods of collection may be superior, such as hair samples [427, 443, 444]. In this study, every effort was taken to standardise salivary cortisol collection, storage, and processing. Research personnel allocated considerable time assisting participants comply with protocol requirements. Furthermore, an information sheet was provided for each visit requiring participants to record collection times. In addition, study partners assisted aMCI participants with sample collection and storage. In line with current research practise, we asked participants to collect multiple samples over the course of the day for each of the 4 visits during the 18 month follow-up period. This allowed us to capture changeability of cortisol levels in an adequately powered study population. We believe these methods significantly improved sample quality of the data.

Finally, we acknowledge that genetic analysis was not complete due to some samples not being collected however, there was an above 90% collection rate recorded for both participant groups leaving adequate power for analysis.

Future directions for research

The study findings suggest psychological stress acts as an independent risk factor for cognitive decline in those with aMCI. Therefore, it would be beneficial to track such a population over a longer period of time to better understand the long-term impact of psychological stress on cognitive decline and AD conversion rates. Furthermore, it is unclear whether the stress response mechanisms responsible for this observed cognitive decline involve inflammatory processes, increased cortisol exposure, or a combination of both. The use of imaging data would shed light on how this complex multi-system physiological response accelerates neurodegeneration, and the role of ApoE status.

The study findings show HPA axis activity as an important consideration in aMCI disease progression. The HPA axis has already been implicated in dementia risk by previously named theories, including the Glucocorticoid Cascade Hypothesis. Unfortunately, due to inconsistent findings during recent years, the potential neurotoxic effects of cortisol in an AD model has received less attention. However, our findings highlight the role of cortisol in neurodegeneration warrants further investigation, particularly in reference to those who are ε4 carriers. We further propose the use of alternative more robust cortisol measures, such as hair sampling, would be advantageous in measuring the long-term effects of cortisol concentration, than compared to salivary cortisol used in this study. Future research

should not investigate the HPA axis in isolation, and like in this study, should be examined alongside the immune system. Unfortunately some cytokines were undetectable in this study, and it is possible that we did not measure other key cytokines of interest. Therefore, future research would benefit from using an improved measurement, and include a more extensive range of anti and pro-inflammatory cytokines.

We acknowledge that the use of stress questionnaires and checklists, as used in this study, carry the limitations already mentioned. Therefore, future research should use an in-depth interview assessment of psychological stress, which would provide a more robust and descriptive measure. Furthermore, in addition to the use of a subjective stress measure, for instance the PSS administered in this study, it would be beneficial to include a standardised measure of anxiety. This would enable researchers to better distinguish between the effects of perceived stress, anxiety, and objective life stress on cognitive decline.

Future clinical directions

Reported findings from this study may have important clinical implications in an aMCI population. The biological mechanisms derived from this study lend themselves to biological interventions such as pharmacological management. The lack of responsiveness of the inflammatory signal to cortisol may explain why attempts at reducing inflammation and cognitive decline using steroids has not been successful and instead may point to the use of more specific targeted cytokine agents by dampening down TNF α or augmenting TGFβ.

Psychological stress is also potentially modifiable without the need for costly pharmaceutical intervention. Thus, individuals with cognitive impairment could be advised on life style choices directly aiming to reduce psychological stress and thus act as a cost effective tool to slow disease trajectory. The development of behavioural interventions that involve stress relieving techniques, including mindfulness, in an aMCI population should be explored. Likewise, aMCI persons could benefit from receiving advice on life event choices, including avoiding unnecessary stressful changes such as moving home or agreeing to elective surgery. In addition, reinforcing more adaptive coping strategies and cognitive appraisal processes may potentially reduce the perception of stress and prevent subsequent negative consequences upon health.

5.1.7 Conclusion

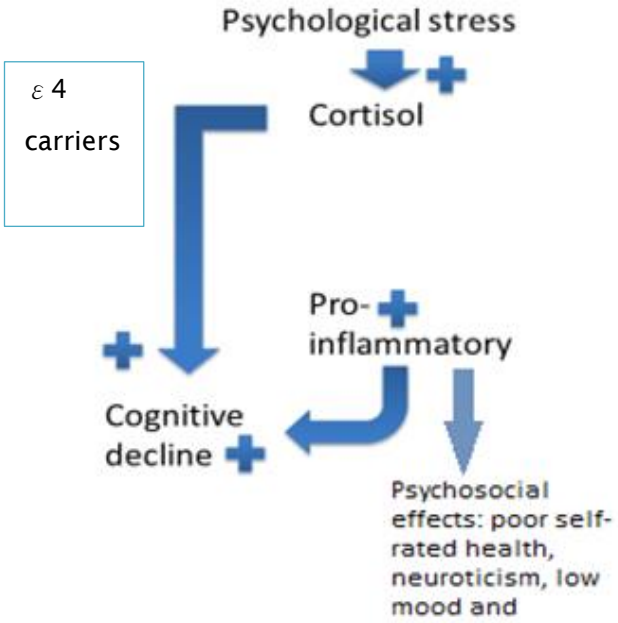
In summary, we found exposure to objective psychological stress accelerated cognitive decline in the aMCI group. This relationship was not observed in the control group. Although this was an observational study design, in which a causal relationship cannot be proved, the findings are consistent with other studies. The other key variables related to accelerated rates of cognitive decline in the aMCI group were ApoE ε4, higher cortisol exposure in ε4 carriers, reduced levels of the anti-inflammatory cytokine TGFβ, increased levels of the pro-inflammatory cytokine TNFα, and in those who were ε4 negative the presence of systemic inflammatory events.

The negative effects of objective psychological stress on cognition in aMCI appear to be mediated by the stress hormone, cortisol, with increased levels of cortisol associated with an accelerated rate of cognitive decline in

ε4 carriers. Furthermore, we found aMCI participants with increased levels of TGFβ at baseline exhibited a slower rate of cognitive decline throughout the study follow-up period, suggesting those with a more pro-inflammatory immune profile deteriorate at a faster rate. Study findings thus implicate both the immune system and HPA axis in the progression of aMCI neurodegenerative changes (Figure 18).

Figure 18. Drivers of cognitive decline in an aMCI population

Figure 18 depicts the presence of objective psychological life stress in an aMCI population results in an increase of cortisol, which we propose leads to subsequent cognitive decline in ApoE ε4 carriers. Additionally, aMCI participants who present with a potentially pro-inflammatory immune profile (reduced TGF β and increased TNFα) cognitively decline at a faster rate. This effect is independent of cortisol and psychological stress.



Study findings also indicate a dysregulation of both the immune system and HPA axis in the aMCI group. For instance, the cortisol response to stress appears to be supressed in control participants but not in the aMCI

group. Previous research implicates numerous factors that may dysregulate bidirectional communication of these stress response systems in MCI persons including aging [245, 299], chronic stress [269, 306], and hypercortisolemia resulting from mounting AD pathology [110]. These factors are pertinent to this study population, which in combination, may accelerate disease progression through exposure to a neurotoxic environment resulting from increased cortisol levels and a pro-inflammatory phenotype. In turn this pro-inflammatory drive potentially leads to the appearance of a number of psychosocial effects including increased distress; lowered mood; poor self-rated health and increased neuroticism (Figure 18).

In conclusion, the present study findings suggest that objective psychological stress is a risk factor for cognitive decline, mediated by cortisol, in an aMCI population. Further research is warranted to understand whether increased cortisol exposure and a reduced anti-inflammatory profile act as independent risk factors for accelerated cognitive decline as suggested by our findings, or in combination with one another, an interaction that was undetected by our study measures.

Appendices

Appendix 1.0

Table 26. RLCQ scores compared between the control and aMCI groups: Visit 2 to 4

			Control	aMCI	Significance
RLCQ	Low	Number	18	40	χ^2 0.910 p=0.340
		%	31.0%	69.0%	
	High	Number	49	79	
		%	38.3%	61.7%	

Appendix 1.1

Table 27. The impact of ApoE ε 4 on chronic physical stress in the control group at baseline

	ε 4	N	Mean	SD	Significance	Mean Diff	95% CI
Diabetes	No	46	0.04	0.2	Independent Samples t test p=0.803	-0.2	-0.138 to 0.107
	Yes	17	0.06	0.2			
High blood pressure	No	46	0.37	0.5	Independent Samples t test p=0.584	0.08	-0.199 to 0.350
	Yes	17	0.29	0.5			
High Cholesterol	No	46	0.46	0.5	Independent Samples t test p=0.097	0.2	-0.042 to 0.485
	Yes	17	0.24	0.4			

Table 28. The impact of ApoE ε 4 on chronic physical stress in the MCI group at baseline

	ε 4	N	Mean	SD	Significance	Mean Diff	95% CI
Diabetes	No	69	0.2	0.4	Independent Samples t test p=0.049	0.1	0.001 to 0.233
	Yes	56	0.1	0.3			
High blood pressure	No	69	0.6	0.5	Independent Samples t test p=0.140	0.1	-0.044 to 0.311
	Yes	56	0.5	0.5			
High Cholesterol	No	69	0.5	0.5	Independent Samples t test p=0.936	0.01	-0.172 to 0.187
	Yes	56	0.5	0.5			

Appendix 1. 2

Table 29. The relationship between core demographics and psychological stress in the control group at visit 1

		Age at baseline	BMI	Years of education
RLCQ	Pearson	-0.087	0.149	-0.010
	Significance	0.480	0.225	0.935
	N	68	68	68
PSS	Pearson	0.075	0.248	-0.021
	Significance	0.543	**0.041**	0.863
	N	68	68	68

Table 30. The relationship between gender and psychological stress in the control group at visit 1

	Gender	N	Mean	SD	Significance	Mean Diff	95% CI
PSS	Male	21	12.4	5.9	Independent Samples t test p=0.8	0.4	-3.289 to 4.051
	Female	47	12.0	7.4			
RLCQ	Male	21	162.1	133.2	Independent Samples t test p=0.9	2.8	-67.612 to 73.302
	Female	47	159.3	135.0			

Table 31. The relationship between the core demographics and psychological stress in the MCI group at visit 1

		Age at baseline	BMI	Years of education
RLCQ	Pearson	-0.290	0.020	-0.009
	Significance	**0.001**	0.821	0.916
	N	134	134	134
PSS	Pearson	-0.128	0.047	-0.019
	Significance	0.147	0.599	0.831
	N	129	129	129

Table 32. The relationship between gender and psychological stress in the aMCI group at visit 1

	Gender	N	Mean	SD	Significance	Mean Diff	95% CI
PSS	Male	81	13.60	6.5	P=0.87	-2.4	-5.054 to 0.348
	Female	48	15.96	8.0			
RLCQ	Male	82	123.35	96.2	P=0.813	-4.6	-43.046 to 33.869
	Female	52	127.94	116. 8			

Table 33. The relationship between the core demographics and the RLCQ in the control group: Visit 2 to 4

	V2-4 RLCQ	N	Mean	SD	Significance Independent Samples t test	Mean Diff	95% CI
BMI	Low	18	27.0	5.1	P=0.652	-0.7	-3.537 to 2.231
	High	49	27.6	5.3			
Years of education	Low	18	12.3	2.3	**P=0.015**	-2.3	-4.125 to -0.462
	High	49	14.6	3.6			
Age at baseline	Low	18	71.1	9.1	P=0.170	3.6	-1.578 to 8.750
	High	49	67.5	9.5			

Table 34. The relationship between the core demographics and the RLCQ in the control group: Visit 2 to 4

	V2-4 PSS	N	Mean	SD	Significance Independent Samples t test	Mean Diff	95% CI
BMI	Low	34	26.6	4.8	P=0.163	-1.8	-4.305 to 0.739
	High	33	28.4	5.5			
Years of education	Low	34	13.6	3.4	P=0.421	-0.7	-2.377 to 1.006
	High	33	14.3	3.6			
Age at baseline	Low	34	68.4	9.2	P=0.994	0.02	-4.629 to 4.663
	High	33	68.4	9.8			

Table 35. The relationship between the RLCQ and gender in the control group: Visit 2 to 4

			Male	Female	Significance
RLCQ	Low	Number	5	13	χ^2 0.145
		%	27.8%	72.2%	P=0.703
	High	Number	16	33	
		%	32.7%	67.3%	

Table 36. The relationship between the RLCQ and gender in the control group: Visit 2 to 4

			Male	Female	Significance
PSS	Low	Number	11	23	χ^2 0.033
		%	32.4%	67.6%	P=0.856
	High	Number	10	23	
		%	30.3%	69.7%	

Table 37. The relationship between the RLCQ and ApoE ε 4 in the control group: visit 2 to 4

			ApoE ε 4 carrier		**Significance**
			No	Yes	
RLCQ v2-v4	Low	Number	13	4	χ^2 0.141
		%	76.5%	23.5%	P=0.707
	High	Number	33	13	
		%	71.7%	28.3%	

Table 38. The relationship between the PSS and APOE ε 4 in the control group: visit 2 to 4

			ApoE ε 4 carrier		
			No	Yes	**Significance**
PSS	Low stress	Count	27	7	χ^2=1.534
		%	79.4%	20.6%	P=0.216
	High stress	Count	19	10	
		%	65.5%	34.5%	

Table 39. Core demographics and the RLCQ visit 2 to 4 aMCI group

	RLCQ	N	Mean	SD	Significance Independent Samples t test	Mean Diff	95% CI
BMI	Low	40	26.3	4.4	P=0.105	-1.4	-3.202 to 0.306
	High	79	27.7	4.6			
Years of education	Low	40	12.6	3.8	P=0.869	-0.1	-1.409 to 1.192
	High	79	12.7	3.2			
Age at baseline	Low	40	78.9	8.1	P=1.117	2.2	-0.553 to 4.911
	High	79	76.7	6.6			

Table 40. The relationship between the core demographics and the PSS visit 2 to 4 aMCI group

	PSS	N	Mean	SD	Significance Independent Samples t test	Mean Diff	95% CI
BMI	Low	42	25.6	3.7	P=0.004	-2.6	-4.379 to -0.857
	High	79	28.2	5.1			
Years of education	Low	42	13.1	3.7	P=0.209	0.8	-0.456 to 2.058
	High	79	12.3	3.1			
Age	Low	42	79.9	6.8	P=0.003	4.2	1.492 to 6.878
	High	79	75.7	7.298			

Table 41. The relationship between gender and the RLCQ visit 2 to 4 in the aMCI group

			Gender		Significance
			Male	Female	
RLCQ	Low Stress	Number	24	16	χ^2=0.046
		%	60.0%	40.0%	P=0.830
	High Stress	Number	49	30	
		%	62.0%	38.0%	

Table 42. The relationship between gender and the PSS visit 2 to 4 in the aMCI group

			Gender		
			Male	**Female**	**Significance**
PSS	Low stress	Number	23	19	χ^2 = 1.424
		%	54.8%	45.2%	P=0.233
	High stress	Number	52	27	
		%	65.8%	34.2%	

Table 43. The impact of ApoE on the RLCQ visit 2 to 4 in the aMCI group

			ε 4		
			No	**Yes**	**Significance**
RLCQ v2 to v4	Low stress	Number	23	12	χ^2=2.187
		%	65.7%	34.3%	P=0.139
	High stress	Number	38	37	
		%	50.7%	49.3%	

Table 44. The impact of ApoE ε 4 on the PSS visit 2 to 4 in the aMCI group

			ε 4		
			No	**Yes**	**Significance**
PSS	Low stress	Number	23	15	χ^2=0.622
		%	60.5%	39.5%	P=430
	High stress	Number	39	35	
		%	52.7%	47.3%	

Table 45. The impact of ApoE ε 4 on the RLCQ visit 1 in the control group

	ε 4	**N**	**Mean**	**SD**	**Significance**	**Mean Diff**	**95% CI**
RLCQ	No	46	153.8	142.3	Independent	-4.4	-80.713
	Yes	17	158.2	109.2	Samples t test p=0.908		to 71.895

Table 46. The impact of ApoE ε 4 on the PSS visit 1 in the control group

	ε 4	N	Mean	SD	Significance	Mean Diff	95% CI
PSS	No	46	11.0	6.59	Independent Samples t test p=0.150	-2.8	-6.746 to 1.056
	Yes	17	13.8	6.9			

Table 47. The impact of ApoE ε 4 on the RLCQ visit 1 in the aMCI group

	ε 4	N	Mean	SD	Significance	Mean Diff	95% CI
RLCQ	No	69	114.9	92.7	Independent Samples t test p=0.204	-24.6	-62.610 to 13.498
	Yes	55	139.5	121.4			

Table 48. The impact of ApoE ε 4 on the PSS visit 1 in the aMCI group

	ε 4	N	Mean	SD	Significance	Mean Diff	95% CI
PSS	No	67	13.8	6.6	Independent Samples t test p=0.164	-1.8	-4.449 to 0.818
	Yes	54	15.7	7.9			

Appendix 1.3

Table 49. Rate of cognitive decline and stress using the high stress cut off (RLCQ: 300+ and PSS: 20-+) in the control group

		FCSRT Total recall LOCF	Statistical significance	MoCA LOCF	Statistical significance	TMT Part B LOCF	Statistical significance
RLCQ Baseline	High (300+)	N= 10 M= -0.2 SD= 0.6	Independent samples T-test P= 0.49 Mean difference= 0.3 95% CI -0.567 to 1.177	N=57 M= 0.6 SD= 1.7	Independent samples T-test P= 0.49 Mean difference= -0.4 95% CI -1.66 to 0.81	N=10 M= -5.5 SD= 47.7	Independent samples T-test P= 0.41 Mean difference= 0.4 95% CI -20.37 to 21.19
	Low	N= 58 M= 0.1 SD=1.3		N=10 M= 0.2 SD= 1.8		N=56 M=-5.1 SD=26.4	
RLCQ V2 to V4	High (300+)	N=10 M= 0.1 SD= 0.6	Independent samples T-test P= 0.91 Mean difference=-0.1 95% CI=-0.922 to 0.825	N=10 M= 0.7 SD= 1.9	Independent samples T-test P= 0.38 Mean difference= -0.5 95% CI -1.772 to 0.688	N=9 M= -20.3 SD= 30.0	Independent samples T-test P= 0.10 Mean difference= 17.6 95% CI -3.684 to 38.842
	Low	N=58 M= 0.1 SD= 1.4		N=57 M= 0.2 SD= 1.8		N=57 M= -2.8 SD= 29.6	
PSS Baseline	High (20+)	N=11 M= 0.2 SD= 0.9	Independent samples T-test P=0.73 Mean difference=-0.2 95% CI -0.99 to 0.69	N=10 M=-0.2 SD= 2.5	Independent samples T-test P= 0.55 Mean difference= 0.6 95% CI -1.32 to 2.35	N=9 M= -3.8 SD= 54.1	Independent samples T-test P= 0.93 Mean difference= -1.6 95% CI=-43.44 to 40.26
	Low	N=57M=0.04 SD= 1.3		N=57 M= 0.3 SD= 1.6		N=57 M= -5.4 SD= 25.1	
PSS V2 to V4	High (20+)	N=15 M=-0.1 SD=0.6	Independent samples T-test P=0.67 Mean difference= 0.2 95% CI -0.589 to 0.915	N=15 M=-0.2 SD= 1.9	Independent samples T-test P= 0.29 Mean difference= 0.6 95% CI -0.482 to 1.613	N=14 M= 3.2 SD= 37.1	Independent samples T-test P= 0.24 Mean difference= -10.6 95% CI -28.650 to 7.414
	Low	N=52 M= 0.1 SD= 1.4		N=52 M=0.4 SD= 1.7		N=52 M= -7.4 SD= 27.9	

*Significant at p <0.05.

Table 50. Rate of cognitive decline and stress using the high stress cut off (RLCQ: 300+ and PSS: 20-+) in the aMCI group

		FCSRT Total recall LOCF	Statistical significance	FCSRT Free recall LOCF	Statistical significance	MoCA LOCF	Statistical significance	TMT Part B LOCF	Statistical significance
RLCQ Baseline	High (300+)	N= 6 Mean= -4.8 SD= 8.2	Independent samples T-test P= 0.73 Mean difference= 1.02 95% CI= -4.75 to 6.79	N= 6 Mean= -3.8 SD= 3.9	Independent samples T-test P= 0.73 Mean difference= 0.67 95% CI= -3.11 to 4.45	N= 6 Mean= -2.5 SD= 3.6	Independent samples T-test P=0.48 Mean difference= 0.86 95% CI= -1.53 to 3.24	N= 6 Mean= 11.5 SD= 28.4	Independent samples T-test P= 0.57 Mean difference= 29.98 95% CI= -74.49 to 134.45
	Low	N= 117 Mean= -3.8 SD= 6.9		N= 117 Mean= -3.2 SD= 4.6		N= 117 Mean= -1.6 SD= 2.8		N= 115 Mean= 41.5 SD= 128.6	
RLCQ V2 to V4	High (300+)	N=15 Mean= -8.3 SD= 7.6	Independent samples T-test **P= 0.008*** Mean difference= 5.02 95% CI= 1.33 to 8.71	N= 15 Mean= -4.2 SD= 4.9	Independent samples T-test P= 0.36 Mean difference= 1.14 95% CI= -1.34 to 3.62	N= 15 Mean= -1.7 SD= 2.6	Independent samples T-test P= 0.98 Mean difference= -0.02 95% CI= -1.59 to 1.55	N= 15 Mean= 34.3 SD= 79.4	Independent samples T-test P= 0.85 Mean difference= 6.54 95% CI= -62.37 to 75.44
	Low	N= 108 Mean= -3.3 SD= 6.7		N= 108 Mean= -3.1 SD= 4.5		N= 108 Mean= -1.7 SD= 2.9		N= 106 Mean= 40.8 SD= 79.4	
PSS Baseline	High (20+)	N= 33 Mean= -3.2 SD= 6.9	Independent samples T-test P= 0.76 Mean difference= -0.43 95% CI= -3.25 to 2.39	N= 33 Mean= -3.3 SD= 5.1	Independent samples T-test P= 0.77 Mean difference= 0.27 95% CI= -1.73 to 2.27	N=33 Mean= -2.3 SD= 2.9	Independent samples T-test P= 0.10 Mean difference= 0.97 95% CI= -0.21 to 2.15	N= 33 Mean= 53.5 SD= 120.9	Independent samples T-test P= 0.45 Mean difference= -19.77 95% CI= -71.35 to 31.81
	Low	N= 85 Mean= -3.6 SD= 6.6		N= 85 Mean= -3.0 SD= 4.3		N= 85 Mean= -1.3 SD= 2.8		N= 83 Mean= 33.7 SD= 128.6	
PSS V2 to V4	High (20+)	N=37 Mean= -4.1 SD= 7.2	Independent samples T-test P= 0.74 Mean difference= 0.46 95% CI= -2.23 to 3.15	N= 37 Mean= -4.5 SD= 5.0	Independent samples T-test **P= 0.02*** Mean difference= 1.97 95% CI= 0.28 to 3.65	N= 37 Mean= -1.7 SD= 2.9	Independent samples T-test P= 0.80 Mean difference= 0.14 95% CI= -0.96 to 1.25	N= 37 Mean= 79.1 SD= 133.4	Independent samples T-test **P= 0.02*** Mean difference= -61.41 95% CI= -111.51 to -11.31
	Low	N= 85 Mean= -3.6 SD= 6.8		N= 85 Mean= -2.5 SD= 4.0		N=85 Mean= -1.6 SD= 2.8		N= 83 Mean= 17.7 SD= 110.0	

Table 51. Stress and cognitive rate of change using the median for control participants

		FCSRT Total recall LOCF	Statistical significance	MoCA LOCF	Statistical significance	TMT Part B LOCF	Statistical significance
RLCQ Baseline	High (above 113)	N= 34 M= 0.2 SD= 1.6	Independent samples T-test P= 0.3 Mean difference= -0.3 95% CI= -0.9 to 0.3	N= 34 M= 0.1 SD= 1.8	Independent samples T-test P=0.2 Mean difference= 1.3 95% CI= -0.64 to 1.11	N= 34 M= -3.3 SD= 32	Independent samples T-test P= 0.6 Mean difference= -3.6 95% CI= -18.5 to 11.2
	Low	N= 34 M= -0.1 SD= 0.7		N= 34 M= 0.4 SD= 1.8		N= 34 M= -7.0 SD= 27	
RLCQ V2 to V4	High (above 113)	N= 49 M= 0.2 SD= 1.3	Independent samples T-test P = 0.081 Mean difference= -0.6 95% CI= -1.306 to 0.079	N= 49 M= 0.2 SD= 1.7	Independent samples T-test P= 0.8 Mean difference= 0.1 95% CI= -0.865 to 1.123	N= 48 M= -5.3 SD= 29.7	Independent samples T-test P= 0.97 Mean difference= 0.4 95% CI= -16.369 to 17.091
	Low	N= 18 M= -0.3 SD= 0.9		N= 18 M= 0.3 SD= 2.1		N= 18 M= -4.9 SD= 31.8	
PSS Baseline	High (median = 14 +	N= 27 M= -0.1 SD= 0.8	Independent samples T-test P= 0.6 Mean difference= 0.2 95% CI= -0.5 to 0.8	N= 27 M= 0.0 SD= 2.1	Independent samples T-test P= 0.39 Mean difference= 0.4 95% CI= -0.5 to 1.2	N= 27 M= -5.8 SD= 37.2	Independent samples T-test P= 0.9 Mean difference= 1.0 95% CI= -14.0 to 16.3
	Low	N= 41 M= 0.1 SD= 1.5		N= 41 M= 0.4 SD= 1.5		N= 41 M= -4.8 SD= 25.2	
PSS V2 to V4	High (median = 14 +)	N= 33 M= -0.2 SD= 0.8	Independent samples T-test P= 0.19 Mean difference= 0.4 95% CI= --0.204 to 1.036	N= 33 M= 0.1 SD= 1.6	Independent samples T-test P= 0.6 Mean difference= 0.2 95% CI= -0.648 to 1.112	N= 332 M= -4.0 SD= 31.5	Independent samples T-test P= 0.8 Mean difference= -2.2 95% CI= -17.134 to 12.663
	Low	N= 34 M= 0.3 SD= 1.6		N = 34 M= 0.4 SD= 2..0		N= 34 M= -6.2 SD= 29.1	

Table 52. Stress and cognitive rate of change using the median for aMCI participants

		dFCSRT LOCF	Statistical significance	dMoCA LOCF	Statistical significance	dTMT Part B LOCF	Statistical significance
RLCQ Baseline	High (above 113)	N= 61 Mean= -2.9 SD= 6.6	Independent samples T-test P= 0.2 Mean difference= -1.8 95% CI= -4.2 to 0.7	N= 61 Mean= -1.5 SD= 2.4	Independent samples T-test P=0.39 Mean difference= -0.44 95% CI= -1.47 to 0.58	N= 61 Mean= 43.9 SD= 134.1	Independent samples T-test P= 0.74 Mean difference= -7.79 95% CI= -53.19 to 37.62
	Low	N= 62 Mean= -4.6 SD= 7.0		N= 62 Mean= -1.9 SD= 3.2		N= 60 Mean= 36.1 SD= 43.9	
PSS Baseline	High (median = 14 +	N= 62 Mean= -2.9 SD= 6.1	Independent samples T-test P= 0.5 Mean difference= -1.2 95% CI= -3.4 to 1.5	N= 62 Mean= -1.6 SD= 2.7	Independent samples T-test P= 0.79 Mean difference= 0.1 95% CI= -0.9 to 1.2	N= 61 Mean= 47.4 SD= 121.2	Independent samples T-test P= 0.5 Mean difference= -17.0 95% CI= -63.6 to 29.6
	Low	N= 56 Mean= -3.9 SD= 7.2		N= 56 Mean= -1.5 SD= 3.1		N= 55 Mean= 30.4 SD= 132.3	
PSS V2 to V4	High (median = 14 +)	N= 79 Mean= -3.6 SD= 6.5	Independent samples T-test P= 0.86 Mean difference= -0.2 95% CI= --2.8 to 2.4	N= 79 Mean= -1.5 SD= 2.7	Independent samples T-test P= 0.408 Mean difference= -0.4 95% CI= -1.52 to 0.62	N= 79 Mean= 39.7 SD= 109.0	Independent samples T-test P= 0.589 Mean difference= -12.7 95% CI= -59.07 to 33.7
	Low	N= 42 Mean= -3.8 SD= 7.4		N=42 Mean= -1.9 SD= 3.0		N= 40 Mean= 27.0 SD= 141.2	

Appendix 1.4

Table 53. The relationship between psychological stress and cortisol in the control group at visit 1

		Sample 1 visit 1	CAR visit 1	Visit 1 AUC
V1 RLCQ	Pearson	-.155	.060	-.203
	Significance	.212	.633	.123
	N	67	66	59
V1 PSS	Pearson	-.054	-.170	-.120
	Significance	.664	.173	.363
	N	67	66	59

Table 54. The relationship between cortisol and dichotomisation of the baseline RLCQ in the control group at visit 1

	RLCQ	N	Mean	SD	Significance Independent Samples t test	Mean Diff	95% CI
CAR	Low	34	0.4	0.6	p=0.520	-0.1	-0.411 to 0.209
	High	32	0.5	0.6	p=0.521	-0.1	-0.411 to 0.210

Table 55. The relationship between cortisol and dichotomisation of the baseline PSS score in the control group at visit 1

	PSS	N	Mean	SD	Significance Independent Samples t test	Mean Diff	955 CI
Sample 1	Low	41	2.5	0.7	P=0.455	0.1	-0.241 to 0.532
	High	26	2.3	0.8			
Car	Low	41	.5	0.6	P=0.200	0.2	-0.111 to 0.521
	High	25	.3	0.7			
AUC	Low	36	127.4	61.8	P=0.623	7.6	-23.366 to 38.681
	High	23	119.8	51.5			

Table 56. The relationship between cortisol and the RLCQ in the control group from visit 2 to 4

	RLCQ	N	Mean	SD	Significance Independent Samples t test	Mean Diff	95% CI
Sample 1	Low	17	2.4	0.6	P=0.342	-0.1	-0.433 to 0.153
	High	45	2.5	0.5			
CAR	Low	17	0.3	0.6	P=0.814	0.03	-0.243 to 0.308
	High	44	0.3	0.5			
AUC	Low	17	123.1	31.8	P=0.578	-13.5	-62.024 to 34.928
	High	41	136.6	97.2			

Table 57. The relationship between cortisol and the PSS in the control group from visit 2 to 4

	PSS	N	Mean	SD	Significance Independent Samples t test	Mean Diff	95% CI
Sample 1	Low	32	2.6	0.5	P=0.505	0.1	-1.745 to 0.350
	High	30	2.5	0.6			
CAR	Low	32	0.3	0.4	P=0.937	0.01	-0.238 to 0.258
	High	29	0.3	0.5			
AUC	Low	31	127.7	48.0	P=0.632	-10.6	-54.895 to 33.642
	High	27	138.3	111.9			

Table 58. The relationship between cortisol and the RLCQ in the aMCI group from visit 2 to 4

	RLCQ	N	Mean	SD	**Significance** Independent Samples t test	**Mean Diff**	**95% CI**
Sample 1	Low	31	2.5	0.6	P=0.151	-0.2	-0.443 to
	High	64	2.7	0.6			0.069
CAR	Low	31	0.4	0.6	**P=0.035**	0.2	0.017 to
	High	62	0.1	0.5			0.467
AUC	Low	30	144.5	52.3	P=0.791	2.7	-17.557 to
	High	55	141.8	40. 4			22.978

Table 59. The relationship between cortisol and the PSS in the aMCI group from visit 2 to 4

	PSS	N	Mean	SD	**Significance** Independent Samples t test	**Mean Diff**	**95% CI**
Sample 1	Low	32	2.6	0.7	P=0.126	-0.2	-0.429 to
	High	65	2.7	0.5			0.0727
CAR	Low	32	0.3	0.6	P=0.315	0.1	-0.111 to
	High	63	0.2	0.5			0.341
AUC	Low	28	142.8	49.9	P=0.818	0.1	-23.231 to
	High	59	145. 2	43.5			18.400

Appendix 1.5

Table 60. Education and psychosocial modulators in the control group at baseline

		Years of education
VAS SCALE	Pearson Correlation	-0.032
	Significance	0.796
	N	68
NEO-FFI Score	Pearson Correlation	-0.040
	Significance	0.748
	N	68
CISS task	Pearson Correlation	0.136
	Significance	0.274
	N	67
CISS emotion	Pearson Correlation	-0.153
	Significance	0.216
	N	67
CISS avoidant	Pearson Correlation	-0.020
	Significance	0.870
	N	67
CISS social	Pearson Correlation	0.082
	Significance	0.509
	N	68
MOS-SSS	Pearson Correlation	0.205
	Significance	0.094
	N	68
GDS	Pearson Correlation	-0.009
	Significance	0.942
	N	68

Table 61. The relationship between psychosocial modulators and $\varepsilon 4$ in the control group at visit 1

	$\varepsilon 4$	N	Mean	SD
GDS	No	46	1.6	1.9
	Yes	17	2.5	2.5
VAS SCALE	No	46	83.6	11.9
	Yes	17	80.9	17.3
NEO-FFI	No	46	15.5	8.3
	Yes	17	19.5	8.3
CISS task	No	45	52.2	11.6
	Yes	17	50.9	11.3
CISS emotion	No	45	47.5	8.8
	Yes	17	50.2	7.8
CISS distraction	No	46	44.8	10.8
	Yes	17	49.2	10.0
CISS social	No	46	51.1	12.3
	Yes	17	54.0	8.5
V1 MOS-SSS	No	46	3.9	0.9
	Yes	17	3.8	0.8

	Significance	Mean Diff	95% CI
GDS	0.162	-0.8	-1.974 to 0.337
VAS SCALE	0.486	2.7	-4.975 to 10.341
NEO-FFI Score	0.095	-3.9	-8.693 to 0.708
CISS task	0.711	1.2	-5.319 to 7.748
CISS emotion	0.276	-2.7	-7.577 to 2.202
CISS distraction	0.146	-4.5	-10.494 to 1.588
CISS social	0.375	-2.9	-9.360 to 3.578
MOSS-SSS	0.648	0.1	-0.385 to 0.614

Table 62. The relationship between psychosocial modulators and gender at visit 1 in the control group

	Gender	N	Mean	SD
NEO-FFI	Male	21	14.5	9.4
	Female	47	17.4	8.1
CISS task	Male	20	53.6	12.0
	Female	47	51.1	11.6
CISS emotion	Male	20	46.9	10.3
	Female	47	48.7	8.5
CISS avoidant	Male	20	51.1	9.78
	Female	47	51.5	9.4
CISS distraction	Male	21	45.9	14.4
	Female	47	47.6	9.4
VAS SCALE	Male	21	83.6	11.2
	Female	47	82.7	13.8
GDS	Male	21	2.4	2.0
	Female	47	1.7	2.0
MOSS-SSS	Male	21	3.7	0.9
	Female	47	4.0	0.9

	Significance	Mean Diff	95% Confidence Interval of the Difference	
			Lower	Upper
NEO-FFI	0.196	-2.9	-7.354	1.540
CISS task	0.433	2.5	-3.780	8.709
CISS emotion	0.442	-1.9	-6.706	2.959
CISS avoidant	0.863	-0.4	-5.510	4.632
CISS distraction	0.576	-1.6	-7.499	4.203
CISS social	0.110	-4.7	-10.579	1.104
VAS SCALE	0.786	0.9	-5.925	7.801
GDS	0.207	0.7	-0.388	1.756
MOSS-SSS	0.331	-0.2	-0.685	0.2343

Appendix 1.6

Table 63. The relationship between psychosocial modulators and cognition in the control group at visit 1

		V1 FCSRT
GDS	Pearson Correlation	0.025
	Signifciance	0.842
	N	68
VAS SCALE	Pearson Correlation	0.131
	Significance	0.286
	N	68
NEO-FFI	Pearson Correlation	0.256
	Significance	0.035
	N	68
CISS task	Pearson Correlation	-0.042
	Significance	0.735
	N	67
CISS emotion	Pearson Correlation	0.152
	Significance	0.219
	N	67
CISS avoidant	Pearson Correlation	0.009
	Significance	0.942
	N	67
CISS distraction	Pearson Correlation	0.154
	Significance	0.211
	N	68
CISS task	Pearson Correlation	-0.070
	Significance	0.569
	N	68
MOS-SSS	Pearson Correlation	-0.066
	Significance	0.594
	N	68

Table 64. Psychosocial modulators and cognition in the control group: visit 2 to 4

		V1 FCSRT
GDS	Spearman	0.109
	Significance	0.376
	N	68
VAS	Spearman	0.104
	Significance	0.400
	N	68
Neuroticism	Spearman	0.240
	Significance	0.049
	N	68
CISS task	Spearman	-0.037
	Significance	0.766
	N	67
CISS emotion	Spearman	0.199
	Significance	0.106
	N	67
CISS avoidant	Spearman	-0.007
	Significance	0.957
	N	67
CISS social	Spearman	-0.073
	Significance	0.556
	N	68
CISS distraction	Spearman	0.209
	Significance	0.087
	N	68
MOSS-SSS	Spearman	0.058
	Significance	0.638
	N	68

Table 65. The relationship between psychosocial modulators and cognition in the aMCI group at visit 1

		FCSRT visit 1
GDS	Spearman	0.109
	Significance	0.206
	N	135
VAS SCALE	Spearman	-0.132
	Significance	0.127
	N	135
NEO-FFI	Spearman	0.071
	Significance	0.434
	N	124
CISS task	Spearman	0.047
	Significance	0.605
	N	123
CISS emotion	Spearman	0.115
	Significance	0.205
	N	123
CISS avoidant	Spearman	0.046
	Significance	0.614
	N	123
CISS distraction	Spearman	0.091
	Significance	0.317
	N	123
CISS social	Spearman	0.031
	Significance	0.730
	N	123
MOSS-SSS	Spearman	0.145
	Significance	0.109
	N	124

Table 66. The relationship between psychosocial modulators and cognition in the aMCI group from visit 2 to 4

		dFCSRT LOCF
GDS	Spearman	-0.009
	Significance	0.926
	N	122
VAS SCALE	Spearman	-0.020
	Significance	0.826
	N	122
NEO-FFI	Spearman	0.099
	Significance	0.301
	N	112
CISS task	Spearman	0.048
	Significance	0.613
	N	112
CISS emotion	Spearman	-0.107
	Significance	0.260
	N	112
CISS avoidant	Spearman	-0.047
	Significance	0.621
	N	112
CISS distraction	Spearman	-0.039
	Significance	0.681
	N	112
CISS social	Spearman	-0.036
	Significance	0.709
	N	112
MOSS-SSS	Spearman	-0.013
	Significance	0.887
	N	114

Glossary of Terms

Aβ plaques	A pathological hallmark of AD, plaques consisting of extracellular deposits of amyloid
ACTH	Adrenocorticotropic hormone. A hormone made in the pituitary gland that is involved in the stress response
Allostasis	A multi-pathway process that allows the body to quickly mount a response to physiological, environmental or psychological challenges
Alzheimer's disease	Most common form of dementia. A physical disease affecting the brain with symptoms including memory loss, difficulties with concentration, and impairment of language
ApoE ε4	Principal cholesterol carrier in the brain and an identified genetic risk factor for Alzheimer's disease
Cellular (Th1) immunity	Response of T lymphocytes with the secretion of pro-inflammatory cytokines
CNS	Central Nervous system. Nerve tissues consisting of the brain and spinal cord
CRH	Corticotrophin releasing hormone. A peptide hormone made in the hypothalamus is involved in the stress response
Cortisol	A steroid hormone belonging to the glucocorticoids class of steroid hormones

Cytokine	A substance secreted by immune cells that is either anti-inflammatory or pro-inflammatory playing an important role in cell signalling
CRP	C reactive protein. Synthesised by the liver and a marker of inflammation
CSF	Cerebro-spinal fluid. Fluid surrounding the brain and spinal cord
Dendrite	Projections of a nerve cell
Endocrine	A collection of glands that release hormones
Glucocorticoid	A class of steroid hormone
Hippocampus	A small region located in the medial temporal lobe of the brain
Homeostasis	A collection of processes that maintain physiological equilibrium
HPA axis	A network between the hypothalamus, the pituitary, and the adrenal gland constituting the HPA axis
Humoral (Th2) immunity	Response of T lymphocytes with the secretion of anti-inflammatory cytokines
Hypercortisolemia	A condition resulting from prolonged exposure to increased cortisol levels
Immunoenhancing	Enhances processes of the immune system
Immunosuppression	Reduce or fully supress certain immune system processes
Inflammaging	Aging accompanied by an up-regulation of systemic inflammation
Meta-analysis review	A review of data sourced from numerous studies

MHC II	Major histocompatibility complex II proteins are typically expressed on antigen presenting cell surfaces to trigger an immune response
Microglia	Resident macrophage cell located in the brain and acts as the primary immune defence
Mild Cognitive Impairment	Transitional period between normal aging and dementia, in which a person demonstrates cognitive decline that is not typical for age
Negative feedback loop	A physiological control system that feeds back to reduce or maintain a homeostatic output
Neurodegeneration	A progressive degenerative process involving neural structures and loss of neurons
Neurofibrillary tangles	A pathological hallmark of AD that consist of aggregated hyperphosphorylated tau protein
Neurogenesis	A process of generating new neurons
Neurotoxic	Exposure to neurotoxins in the brain
Pathogenesis	Biological mechanisms involved in the development of a disease
Pro-inflammatory phenotype	A chronic and heightened inflammatory state
Vasopressin	An anti-diuretic hormone produced in the paraventricular nucleus

List of References

1. Albert, M.S., et al., *The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease.* Alzheimer's & Dementia, 2011. **7**: p. 270-279.
2. Petersen, R.C., et al., *Mild Cognitive Impairment.* Arch Neurol, 1999. **56**: p. 303-308.
3. Petersen, R.C., *Mild Cognitive Impairment as a diagnostic entity.* J Intern Med, 2004. **256**: p. 183-94.
4. Petersen, R.C., et al., *Current concepts in Mild Cognitive Impairment.* Arch Neurol, 2001. **58**: p. 1985-1992.
5. Okonkwo, O.C., et al., *Cerebral atrophy, apolipoprotein E ε4 and rate of decline in everyday function amongst patients with Alzheimer Dementia.* Alzheimers Dement., 2010. **6**(5): p. 404-11.
6. Fratiglioni, L., D. De Ronchi, and H. Agüero-Torres, *Worldwide Prevalence and Incidence of Dementia.* Drugs & Aging, 1999. **15**(5): p. 365=375.
7. Schmidtke, K. and S. Hermeneit, *High rate of conversion to Alzheimer's disease in a cohort of amnestic MCI patients.* International Psychogeriatrics, 2008. **20**(1): p. 96-108.
8. Zhu, C.W., et al., *Health-related resource use and costs in elderly adults with and without mild cognitive impairment.* J Am Geriatr Soc, 2013. **61**(3): p. 396-402.
9. Gill Gregorio, P., *[Diagnostic criteria. Benefits of early diagnosis].* Rev Esp Geriatr Gerontol. , 2016. **51**(1): p. 7-11.
10. Prince, M., et al., *Dementia UK: Second edition – Overview*. 2014, The ALzheimer's Society.
11. Ikeda, C., et al., *Difference in determinants of caregiver burden between amnestic mild cognitive impairment and mild Alzheimer's disease.* Psychiatry Research, 2015. **226**(1): p. 242-246.
12. Fisher, G.G., et al., *Caring for individuals with dementia and cognitive impairment, not dementia: Findings from the aging, demographics, and memory study.* JOURNAL - AMERICAN GERIATRICS SOCIETY, 2011. **59**(3): p. 488.
13. Tsang, R.S.M., et al., *Sydney Memory and Ageing Study: An epidemiological cohort study of brain ageing and dementia.* International Review of Psychiatry, 2013. **25**(6): p. 711-725.
14. Kim, K.W., et al., *A nationwide survey on the prevalence of dementia and mild cognitive impairment in South Korea.* J Alzheimers Dis, 2011. **23**(2): p. 281-91.
15. Limongi, F., et al., *Prevalence and conversion to dementia of Mild Cognitive Impairment in an elderly Italian population.* Aging Clinical And Experimental Research, 2017.
16. Petersen, R.C., et al., *Prevalence of mild cognitive impairment is higher in men. The Mayo Clinic Study of Aging.* Neurology, 2010. **75**(10).
17. Busse , A., et al., *Mild cognitive impairment: Prevalence and incidence according to different diagnostic criteria: Results of the Leipzig Longitudinal Study of the Aged.* British Journal of Psychiatry 2003. **182**: p. 449 – 454

18. Mitchell , A.J. and M. Shiri-Feshki *Temporal trends in the long term risk of progression of mild cognitive impairment: A pooled analysis* Journal of Neurology, Neurosurgery & Psychiatry, 2008. **79**: p. 1386 – 1391.
19. Petersen , R.C., et al., *Mild cognitive impairment: Ten years later* Archives of Neurology, 2009. **66**: p. 1447-1455.
20. Thomas, A., *Clinical aspects of dementia: Alzheimer's disease*, in *Oxford Textbook of Old Age Psychiatry*, R. Jacoby, et al., Editors. 2008, Oxford University Press: New York. p. 426-441.
21. Gao, S., et al., *The Relationships Between Age, Sex, and the Incidence of Dementia and Alzheimer Disease: A Meta-analysis.* Arch Gen Psychiatry, 1998. **55**(9): p. 809-15.
22. Gao, Y., et al., *Depression as a risk factor for dementia and mild cognitive impairment: a meta-analysis of longitudinal studies.* Int J Geriatr Psychiatry, 2013. **28**(5): p. 441-9.
23. Ismail, Z., et al., *Prevalence of Depression in Patients With Mild Cognitive Impairment: A Systematic Review and Meta-analysis..* JAMA Psychiatry 2017. **74**(1): p. 58-67.
24. Dal Forno, G., et al., *Depressive symptoms, sex, and risk for Alzheimer's disease.* Annals Of Neurology, 2005. **57**(3): p. 381-387.
25. Cherbuin, N., S. Kim, and K.J. Anstey, *Dementia risk estimates associated with measures of depression: a systematic review and meta-analysis.* BMJ Open, 2015. **5**(12): p. e008853-e008853.
26. Andersen, K., et al., *Gender differences in the incidence of AD and vascular dementia: The EURODEM Studies. The EURODOM Incidence Research Group.* Neurology, 1999. **53**(9): p. 1992-7.
27. Forlenza, O.V., B.S. Diniz, and W.F. Gattaz, *Diagnosis and biomarkers of predementia in Alzheimer's Disease.* BMC Med, 2010. **8**(89).
28. Dunn, N., et al., *Association between Dementia and Infectious Disease: Evidence from a Case-Control Study.* Alzheimer Disease and Associated Disorders, 2005. **19**(2): p. 91-94.
29. Aggarwal, N.T., et al., *The relation of cigarette smoking to incident Alzheimer's disease in a biracial urban community population.* Neuroepidemiology, 2006. **26**(3): p. 140-6.
30. Akomolafe, A., et al., *Diabetes mellitus and risk of developing Alzheimer disease: results from the Framingham Study.* Arch Neurol, 2006. **63**(11): p. 1551-5.
31. Duron, E. and O. Hanon, *Vascular risk factors, cognitve decline, and dementia.* Vasc Health Risk Manag, 2008. **4**(2): p. 363-381.
32. Köhler, S., et al., *Depression, Vascular Factors, and Risk of Dementia in Primary Care: A Retrospective Cohort Study.* Journal of the American Geriatrics Society, 2015. **63**(4): p. 692-698.
33. Whitmer, R.A., et al., *Midlife cardiovascular risk factors and risk of dementia in late life.* Neurology, 2005. **64**: p. 277-281.
34. Jacob, L., J. Bohlken, and K. Kostev, *Risk Factors for Mild Cognitive Impairment in German Primary Care Practices.* Journal Of Alzheimer's Disease: JAD, 2017. **56**(1): p. 379-384.
35. Cechetto, D.F., V. Hachinski, and S.N. Whitehead, *Vascular risk factors and Alzheimer's disease.* Expert Rev Neurother, 2008. **8**(5): p. 743-750.
36. Aguilar-Navarro, S.G., et al., *Clinical and Demographic Predictors of Conversion to Dementia in Mexican Elderly with Mild Cognitive Impairment.*

Revista De Investigacion Clinica; Organo Del Hospital De Enfermedades De La Nutricion, 2017. **69**(1): p. 33-39.
37. Lara, E., et al., *Mild cognitive impairment in a Spanish representative sample: prevalence and associated factors.* International Journal Of Geriatric Psychiatry, 2016. **31**(8): p. 858-867.
38. Meyer, J.S., et al., *Cardiovascular and other risk factors for ALzheimer's disease and vascular dementia.* Ann N Y Acad Sci, 2000. **903**: p. 411-423.
39. Hofman, A., et al., *Epidemiology of neurological diseases in elderly people: what did we learn from the Rotterdam Study.* Lancet Neurol, 2006. **5**(6): p. 545-50.
40. Okello, A.M., et al., *Microglial activation and amyloid deposition in mild cognitive impairment: A PET study.* Neurology 2009. **72**(1): p. 56-62.
41. Henderson, A.S., et al., *Apolipoprotein E allele epsilon 4, dementia, and cogntive decline in a population sample.* Lancet, 1995. **346**(8987): p. 1387-90.
42. Tschanz, J.T., et al., *Conversion to dementia from mild cognitive disorder: the Cache County Study.* Neurology, 2006. **67**(2): p. 229-34.
43. Zannis, V.I., D. Kardassis, and E.E. Zanni, *Genetic mutations affecting human lipoproteins, their receptors, and their enzymes.* Advances in Human Genetics, 1993. **21**: p. 145-319.
44. Yatawara, C., et al. *Neuropsychiatric symptoms in South-East Asian patients with mild cognitive impairment and dementia: prevalence, subtypes, and risk factors*. Int J Geriatr Psychiatry, 2017.
45. Di Carlo, A., et al., *CIND and MCI in the Italian elderly: frequency, vascular risk factors, progression to dementia.* Neurology, 2007. **68**(22): p. 1909-1916.
46. Larrieu, S., et al., *Incidence and outcome of mild cognitive impairment in a population-based prospective cohort.* Neurology, 2002. **59**(10): p. 1594-1599.
47. Azad, N.A., M. Al Bugami, and I. Loy-English, *Gender differences in dementia risk factors.* Gender Medicine, 2007. **4**(2): p. 120-129.
48. Altmann, A., et al., *Sex modifies the APOE-related risk of developing Alzheimer disease.* Annals Of Neurology, 2014. **75**(4): p. 563-573.
49. Gilsanz, P., et al., *Female sex, early-onset hypertension, and risk of dementia.* Neurology, 2017. **89**(18): p. 1886-1893.
50. Shumaker, S.A., et al., *Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial.* JAMA, 2003. **289**(20): p. 2651-2662.
51. Anstey, K.J., H.A. Mack, and N. Cherbuin, *Alcohol consumption as a risk factor for dementia and cognitive decline: meta-analysis of prospective studies.* The American Journal Of Geriatric Psychiatry: Official Journal Of The American Association For Geriatric Psychiatry, 2009. **17**(7): p. 542-555.
52. Beydoun, M.A., et al., *Epidemiologic studies of modifiable factors associated with cognition and dementia: systematic review and meta-analysis.* BMC Public Health, 2014. **14**: p. 643-643.
53. Grande, G., et al., *Physical activity reduces the risk of dementia in mild cognitive impairment subjects: a cohort study.* J Alzheimers Dis., 2014. **39**(4): p. 833-9.

54. Rawtaer, I., et al., *Psychosocial Risk and Protective Factors and Incident Mild Cognitive Impairment and Dementia in Community Dwelling Elderly: Findings from the Singapore Longitudinal Ageing Study.* Journal Of Alzheimer's Disease: JAD, 2017. **57**(2): p. 603-611.
55. Cooper, C., et al., *Modifiable predictors of dementia in mild cognitive impairment: a systematic review and meta-analysis.* The American Journal Of Psychiatry 2015. **172**(4): p. 323-334.
56. Scarmeas, N., et al., *Mediterranean diet and mild cognitive impairment.* Arch Neurol., 2009. **66**(2): p. 216-25.
57. Karp, A., et al., *Relation of education and occupation-based socioeconomic status to incident Alzheimer's disease.* Am J Epidemiol, 2004. **159**(2): p. 175-183.
58. Fratiglioni, L., S. Paillard-Borg, and B. Winblad, *An active and socially integrated lifestyle in late life might protect against dementia.* Lancet Neurol, 2004. **3**(6): p. 343-353.
59. Wilson, R.S., et al., *Proneness to psychological distress is associated with risk of Alzheimer's disease.* NEUROLOGY, 2013. **61**: p. 47 9 -L485.
60. UK, A.R., *Defeat Dementia: The evidence and a vision for action* 2014: London.
61. Peavy, G.M., et al., *Effects of chronic stress on memory decline in cognitively normal and mildly impaired older adults.* The American Journal of Psychiatry, 2009. **166**(12): p. 1384-1391.
62. Johansson, L., et al., *Common psychosocial stressors in middle-aged women related to longstanding distress and increased risk of Alzheimer's disease: a 38-year longitudinal population study.* BMJ Open, 2013. **3**(9).
63. Norton, M.C., et al., *Early parental death and remarriage of widowed parents as risk factors for Alzheimer disease: The Cache County Study.* The American Journal of Geriatric Psychiatry, 2011. **19**(9): p. 814-824.
64. Lue, L.F., et al., *Inflammation, A beta deposition, and neurofibrillary tangle formation as correlates of Alzheimer's disease neurodegeneration.* Journal of Neuropathology & Experimental Neurology, 1996. **55**(10): p. 1083-8.
65. Englar, H., et al., *Two-year follow-up of amyloud deposition in patients with Alzheimer's disease.* Brain, 2006. **129**: p. 2856-2866.
66. Edison, P., et al., *Microglia, amyloid, and cognition in Alzheimer's disease: An [11C](R)PK11195-PET and [11C]PIB-PET study.* Neurobiology of Disease, 2008. **32**(3): p. 412-9.
67. Kohler, S., et al., *Memory impairments associated with hippocampal versus parahippocampal-gyrus atrophy: An MR volumetry study in Alzheimer's disease.* Neuropsychologia, 1998. **36**(9): p. 901–914.
68. Colangelo, V., et al., *Gene expression profiling of 12633 genes in Alzheimer hippocampal CA1: transcription and neurotrophic factor down-regulation and up-regulation of apoptotic and pro-inflammatory signaling.* Journal of Neuroscience Research, 2002. **70**(3): p. 462-73.
69. Jefferson, A.L., et al., *Inflammatory biomarkers are associated with total brain volume: the Framingham Heart Study.* Neurology, 2007. **68**(13): p. 1032-8.
70. Piggott, M.A. and J. Court, *Neurochemical pathology of neurodegenerative disorders of old age*, in *oxford Textbook of Old Age Psychiatry*, R. Jacoby, et al., Editors. 2008, Oxford University Press: New York. p. 85-102.

71. Galimberti, D., et al., *Intrathecal Chemokine Synthesis in Mild Cognitive Impairment and Alzheimer Disease.* Archives of Neurology, 2006. **63**(4): p. 538-543.
72. Xiang, Z., et al., *Microglia activation in the brain as inflammatory biomarker of Alzheimer's disease neuropathology and clinical dementia.* Disease Markers, 2006. **22**(1-2): p. 95-102.
73. Parachikova, A., et al., *Inflammatory changes parallel the early stages of Alzheimer disease.* Neurobiology of Aging, 2007. **28**(12): p. 1821-33.
74. Doecke, J.D., et al., *Blood-based protein biomarkers for diagnosis of Alzheimer disease.* Archives of Neurology, 2012. **69**(10): p. 1318-1325.
75. Fonseca, M.I., et al., *Complement activation fragment C5a receptors, CD88 and C5L2, are associated with neurofibrillary pathology.* Journal of Neuroinflammation, 2013. **10**: p. 25.
76. Sudduth, T.L., et al., *Neuroinflammatory phenotype in early Alzheimer's disease.* Neurobiology of Aging, 2013. **34**(4): p. 1051-9.
77. Zhang, R., et al., *Systemic immune system alterations in early stages of Alzheimer's disease.* Journal of Neuroimmunology, 2013. **256**(1-2): p. 38-42.
78. Johnson, K.A., et al., *Florbetapir (F18-AV-45) PET to assess amyloid burden in Alzheimer's disease dementia, mild cognitive impairment, and normal aging.* Alzheimer's & Dementia, 2013. **9**(5): p. S72-S83.
79. Jhoo, J.H., et al., *Discrimination of normal aging, MCI and AD with multimodal imaging measures on the medial temporal lobe.* Psychiatry Research: Neuroimaging, 2010. **183**(3): p. 237-243.
80. Koivunen, J., et al., *Amyloid PET imaging in patients with mild cognitive impairment: A 2-year follow-up study.* Neurology, 2011. **76**(12): p. 1085-1090.
81. Aguilar, C., et al., *Application of a MRI based index to longitudinal atrophy change in Alzheimer disease, mild cognitive impairment and healthy older individuals in the AddNeuroMed cohort.* 2014. **6**.
82. Varon, D., et al., *Visual rating and volumetric measurement of medial temporal atrophy in the alzheimer's disease neuroimaging initiative (adni) cohort: Baseline diagnosis and the prediction of mci outcome.* Int J Geriatr Psychiatry, 2015. **30**(2): p. 192-200.
83. Chételat, G., et al., *Three-dimensional surface mapping of hippocampal atrophy progression from MCI to AD and over normal aging as assessed using voxel-based morphometry.* Neuropsychologia, 2008. **46**(6): p. 1721-1731.
84. Chiu, M., et al., *Plasma tau as a window to the brain—Negative associations with brain volume and memory function in mild cognitive impairment and early Alzheimer's disease.* Human Brain Mapping, 2014. **35**(7): p. 3132-3142.
85. Tang, W., et al., *Does CSF p-tau$_{181}$ help to discriminate Alzheimer's disease from other dementias and mild cognitive impairment? A meta-analysis of the literature.* Journal of Neural Transmission, 2014. **121**(12): p. 1541-1553.
86. Markesbery, W.R., *Neuropathologic alterations in mild cognitive impairment: A review.* Journal of Alzheimer's Disease, 2010. **19**(1): p. 221-228.

87. Choi, J., H.W. Lee, and K. Suk, *Increased plasma levels of lipocalin 2 in mild cognitive impairment.* Journal of the Neurological Sciences, 2011. **305**(1-2): p. 28-33.
88. Guerreiro, R.J., et al., *Peripheral inflammatory cytokines as biomarkers in Alzheimer's disease and mild cognitive impairment.* Neurodegenerative Diseases, 2007. **4**(6): p. 406-12.
89. Tarkowski, E., et al., *Intrathecal inflammation precedes development of Alzheimer's disease.* Journal of Neurology, Neurosurgery & Psychiatry, 2003. **74**(9): p. 1200-1205.
90. Bermejo, P., et al., *Differences of peripheral inflammatory markers between mild cognitive impairment and Alzheimer's disease.* Immunology Letters, 2008. **117**(2): p. 198-202.
91. Roberts, R.O., et al., *Association of C-reactive protein with mild cognitive impairment.* Alzheimer's & Dementia, 2009. **5**(5): p. 398-405.
92. Forlenza, O.V., et al., *Increased serum IL-1β level in Alzheimer's disease and mild cognitive impairment.* Dementia and Geriatric Cognitive Disorders, 2010. **28**(6): p. 507-512.
93. Lupien, S.J., et al., *Basal Cortisol Levels and Cognitive Deficits in Human Aging.* The Journal of Neuroscience, 1994. **14**(5): p. 2893-2903.
94. Seeman, T.E., et al., *Increase in Urinary Cortisol Excretion and Memory Declines: MacArthur Studies of Successful Aging.* Journal of Clinical Endocrinology and Metabolism, 1997. **82**(8): p. 2458-2465.
95. Lupien, S.J., et al., *Cortisol levels during human aging predict hippocampal atrophy and memory deficits. .* Nature Neuroscience 1998. **1**(1): p. 69-73.
96. Beluche, I., et al., *A prospective study of diurnal cortisol and cognitive function in community-dwelling elderly people.* Psychological Medicine, 2010. **40**(6): p. 1039-49.
97. Lee, B.K., et al., *Associations of Salivary Cortisol With Cognitive Function in the Baltimore Memory Study.* Arch Gen Psychiatry, 2007. **64**(7): p. 810-818.
98. Ouanes, S., et al., *Life events, salivary cortisol, and cognitive performance in nondemented subjects: a population-based study..* Neurobiology Of Aging, 2017. **51**: p. 1-8.
99. Lei, J.K., *Change of serum ACTH and cortisol levels in Alzheimer disease and mild cognition impairment.* Zhonghua Yi Xue Za Zhi, 2010. **90**(41): p. 2894-6.
100. Souza-Talarico, J., et al., *Relationship Between Cortisol Levels and Memory Performance Maybe Modulated by the Presence or Absence of Cognitive Impairment: Evidence from Healthy Elderly, Mild Cognitive Impairment and Alzheimer's Disease Subjects.* Journal of Alzheimer's Disease, 2010. **19**: p. 839-848.
101. Ferrari, E., et al., *Pineal and pituitary-adrenocortical function in physiological aging and in senile dementia.* Experimental Gerontology, 2000. **35**(9-10): p. 1239-50.
102. Peskind, E.R., et al., *Increased CSF cortisol in AD is a function of APOE genotype.* Neurology, 2001. **56**(8): p. 1094-8.
103. Rasmuson, S., et al., *Increased levels of adrenocortical and gonadal hormones in mild to moderate Alzheimer's disease.* Dementia & Geriatric Cognitive Disorders, 2002. **13**(2): p. 74-9.

104. Gerritsen, L., et al., *Early and late life events and salivary cortisol in older persons.* Psychological Medicine, 2009. **40**(9): p. 1569-78.
105. Gil-Bea, F.J., et al., *HPA axis dysregulation associated to apolipoprotein E4 genotype in Alzheimer's disease.* Journal of Alzheimer's Disease, 2010. **22**(3): p. 829-838.
106. Bruno, D., et al., *Cerebrospinal fluid cortisol concentrations in healthy elderly are affected by both APOE and TOMM40 variants.* Psychoneuroendocrinology, 2012. **37**(3): p. 366-371.
107. Csernansky, J.G., et al., *Plasma cortisol and progression of dementia in subjects with Alzheimer-type dementia.* The American Journal of Psychiatry, 2006. **163**(12): p. 2164-2169.
108. Lehallier, B., et al., *Combined plasma and cerebrospinal fluid signature for the prediction of midterm progression from mild cognitive impairment to Alzheimer disease.* JAMA Neurology, 2016. **73**(2): p. 203-212.
109. Popp, J., et al., *Cerebrospinal fluid cortisol and clinical disease progression in MCI and dementia of Alzheimer's type.* Neurobiology of Aging, 2015. **36**(2): p. 601-607.
110. Sapolsky, R.M., L.C. Krey, and B.S. McEwen, *The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis.* Endocrine Reviews, 1986. **7**(3): p. 284-301.
111. Lind, K., et al., *Increased Saliva Cortisol Awakening Response in Patients with Mild Cognitive Impairment.* Dement Geriatr Cogn Disord, 2007. **24**: p. 389–395.
112. Popp, J., et al., *CSF cortisol in Alzheimer's disease and mild cognitive impairment.* Neurobiology of Aging, 2009. **30**: p. 498-500.
113. Sotiropoulos, I., et al., *Glucocorticoids trigger Alzheimer disease-like pathobiochemistry in rat neuronal cells expressing human tau.* Journal of Neurochemistry, 2008. **107**(2): p. 385-397.
114. Sotiropoulos, I., et al., *Stress Acts Cumulatively To Precipitate Alzheimer's Disease-Like Tau Pathology and Cognitive Deficits.* The Journal of Neuroscience, 2011. **31**(21): p. 7840-7847.
115. Catania, C., et al., *The amyloidogenic potential and behavioral correlates of stress.* Molecular Psychiatry, 2009. **14**: p. 95-105.
116. Blasko, I., et al., *How chronic inflammation can affect the brain and support the development of Alzheimer's disease in old age: The role of microglia and astrocytes.* Aging Cell, 2004. **3**(4): p. 169-176.
117. Perry, V.H., J.A. Nicoll, and C. Holmes, *Microglia in neurodegenerative disease.* Nature Reviews Neurology, 2010. **6**(4): p. 193-201.
118. Jurgens, H.A. and R.W. Johnson, *Dysregulated neuronal-microglial cross-talk during aging, stress and inflammation.* Experimental Neurology, 2012. **233**(1): p. 40-8.
119. Walker, D.G., et al., *Decreased expression of CD200 and CD200 receptor in Alzheimer's disease: a potential mechanism leading to chronic inflammation.* Experimental Neurology, 2009. **215**(1): p. 5-19.
120. Rivest, S., *Regulation of innate immune responses in the brain.* Nat Rev Immunol, 2009. **9**: p. 223-32.
121. Mrak, R.E. and W.S. Griffin, *Interleukin-1, neuroinflammation, and Alzheimer's disease.* Neurobiology of Aging, 2001. **22**(6): p. 903-908.

122. Cacabelos, R., et al., *Brain interleukin-1 beta in Alzheimer's disease and vascular dementia.* Methods & Findings in Experimental & Clinical Pharmacology, 1994. **16**(2): p. 141-51.
123. Vehmas, A.K., et al., *Immune reactive cells in senile plaques and cognitive decline in Alzheimer's disease.* Neurobiology of Aging, 2003. **24**(2): p. 321-31.
124. Versijpt, J.J., et al., *Assessment of neuroinflammation and microglial activation in Alzheimer's disease with radiolabelled PK11195 and single photon emission computed tomography. A pilot study.* European Neurology, 2003. **50**(1): p. 39-47.
125. Leung, R., et al., *Inflammatory proteins in plasma are associated with severity of Alzheimer's disease.* PLoS ONE [Electronic Resource], 2013. **8**(6): p. e64971.
126. Holmes, C., *Systemic inflammation and Alzheimer's disease.* Neuropathology and Applied Neurobiology, 2013. **39**: p. 51-68.
127. Holmes, C., et al., *Proinflammatory cytokines, sickness behavior, and Alzheimer disease.* Neurology, 2011. **77**(3): p. 212-8.
128. Schram, M.T., et al., *Systemic markers of inflammation and cognitive decline in old age.* Journal of the American Geriatrics Society, 2007. **55**(5): p. 708-716.
129. Alley, D.E., et al., *Inflammation and rate of cognitive change in high-functioning older adults.* Journals of Gerontology Series A-Biological Sciences & Medical Sciences, 2008. **63**(1): p. 50-5.
130. Baune, B.T., et al., *Association between IL-8 cytokine and cognitive performance in an elderly general population--The MEMO-study.* Neurobiology of Aging, 2008. **29**(6): p. 937-944.
131. Wright, C.B., et al., *Interleukin-6 Is Associated With Cognitive Function: The Northern Manhattan Study.* Journal of Stroke and Cerebrovascular Diseases, 2006. **15**(1): p. 34-38.
132. Jordanova, V., et al., *Markers of inflammation and cognitive decline in an African-Caribbean population.* International Journal of Geriatric Psychiatry, 2007. **22**(10): p. 966-973.
133. Athilingam, P., et al., *Elevated levels of interleukin 6 and C-reactive protein associated with cognitive impairment in heart failure.* Congestive Heart Failure, 2013. **19**(2): p. 92-8.
134. Magaki, S., et al., *Increased production of inflammatory cytokines in mild cognitive impairment.* Experimental Gerontology, 2007. **42**(3): p. 233-40.
135. Zuliani, G., et al., *Plasma cytokines profile in older subjects with late onset Alzheimer's disease or vascular dementia.* Journal of Psychiatric Research, 2007. **41**(8): p. 686-93.
136. Cojocaru, I.M., et al., *Study of interleukin-6 production in Alzheimer's disease.* Romanian Journal of Internal Medicine, 2011. **49**(1): p. 55-8.
137. Honma, T., et al., *Increased systemic inflammatory interleukin-1ß and interleukin-6 during agitation as predictors of Alzheimer's disease.* International Journal of Geriatric Psychiatry, 2013. **28**(3): p. 233-241.
138. Swardfager, W., et al., *A Meta-Analysis of Cytokines in Alzheimer's Disease.* Biol Psychiatry, 2010. **68**: p. 930-941.
139. Reale, M., et al., *Peripheral chemokine receptors, their ligands, cytokines and Alzheimer's disease.* Journal of Alzheimer's Disease, 2008. **14**(2): p. 147-59.

140. Jia, J.P., et al., *Cerebrospinal fluid tau, Abeta1-42 and inflammatory cytokines in patients with Alzheimer's disease and vascular dementia.* Neuroscience Letters, 2005. **383**(1-2): p. 12-6.
141. Lombardi, V.R., et al., *Characterization of cytokine production, screening of lymphocyte subset patterns and in vitro apoptosis in healthy and Alzheimer's Disease (AD) individuals.* Journal of Neuroimmunology, 1999. **97**(1-2): p. 163-71.
142. Di Rosa, M., et al., *Chitotriosidase and inflammatory mediator levels in Alzheimer's disease and cerebrovascular dementia.* European Journal of Neuroscience, 2006. **23**(10): p. 2648-2656.
143. Bruunsgaard, H., et al., *A high plasma concentration of TNF-α is associated with dementia in centenarians.* The Journals of Gerontology: Series A: Biological Sciences and Medical Sciences, 1999. **54A**(7): p. M357-M364.
144. Alvarez, X.A., et al., *Blood levels of histamine, IL-1 beta, and TNF-alpha in patients with mild to moderate Alzheimer disease.* Molecular & Chemical Neuropathology, 1996. **29**(2-3): p. 237-52.
145. Van Duijn, C.M., A. Hoffman, and L. Nagelkerken, *Serum levels of interleukin-6 are not elevated in patients with Alzheimer's disease.* Neurosci Lett, 1990. **108**(3): p. 350-4.
146. Schmidt, R., et al., *Early inflammation and dementia: a 25-year follow-up of the Honolulu-Asia Aging Study.* Annals of Neurology, 2002. **52**(2): p. 168-74.
147. Engelhart, M.J., et al., *Inflammatory Proteins in Plasma and the Risk of Dementia: The Rotterdam Study.* Arch Neurol, 2004. **61**(5): p. 668-672.
148. Komulainen, P., et al., *Serum high sensitivity C-reactive protein and cognitive function in elderly women.* Age & Ageing, 2007. **36**(4): p. 443-8.
149. Tilvis, R.S., et al., *Predictors of Cognitive Decline and Mortality of Aged People Over a 10-Year Period.* Journal of Gerontology, 2004. **59A**(3): p. 268-274.
150. Diniz, B.S., et al., *Higher serum sTNFR1 level predicts conversion from mild cognitive impairment to Alzheimer's disease.* Journal of Alzheimer's Disease, 2010. **22**(4): p. 1305-1311.
151. Laurin, D., et al., *Midlife C-reactive protein and risk of cognitive decline: a 31-year follow-up.* Neurobiology of Aging, 2009. **30**(11): p. 1724-7.
152. Tan, Z.S., et al., *Inflammatory markers and the risk of Alzheimer disease: the Framingham Study.* Neurology, 2007. **68**(22): p. 1902-8.
153. Buchhave, P., et al., *Soluble TNF receptors are associated with AB metabolism and conversion to dementia in subjects with mild cognitive impairment.* Neurobiology of Aging, 2010. **31**: p. 1877-1884.
154. Ojala, J., et al., *Expression of interleukin-18 is increased in the brains of Alzheimer's disease patients.* Neurobiology of Aging, 2009. **30**(2): p. 198-209.
155. Cagnin, A., et al., *In-vivo measurement of activated microglia in dementia.* Lancet, 2001. **358**(9283): p. 766.
156. Corder, E.H., et al., *Gene dose of apolipoprotein E type 4 allele and risk of Alzheimer's disease in late onset families.* Science, 1993. **261**(5123): p. 921-923.

157. Carrasquillo, M.M., et al., *Late-onset Alzheimer's risk variants in memory decline, incident mild cognitive impairment, and Alzheimer's disease.* Neurobiology of Aging, 2015. **36**(1): p. 60-67.
158. Olgiati, P., et al., *APOE epsilon-4 allele and cytokine production in Alzheimer's disease.* International Journal of Geriatric Psychiatry, 2010. **25**(4): p. 338-44.
159. Du, Y., et al., *Association of an interleukin 1 alpha polymorphism with Alzheimer's disease.* Neurology, 2000. **55**(4): p. 480-3.
160. Combarros, O., et al., *Gene dose-dependent association of interleukin-1A [-889] allele 2 polymorphism with Alzheimer's disease.* Journal of Neurology, 2002. **249**(9): p. 1242-5.
161. Alvarez V, et al., *Association between the TNFalpha-308 A/G polymorphism and the onset-age of Alzheimer disease.* Am J Med Genet, 2002. **114**: p. 574 - 7.
162. Jonsson, T., et al., *Variant of TREM2 associated with the risk of Alzheimer's disease.* New England Journal of Medicine, 2013. **368**(2): p. 107-16.
163. Harold, D., et al., *Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease.[Erratum appears in Nat Genet. 2009 Oct;41(10):1156].* Nature Genetics, 2009. **41**(10): p. 1088-93.
164. Lambert, J.C., et al., *Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease.* Nature Genetics, 2009. **41**(10): p. 1094-9.
165. Motta, M., et al., *Altered plasma cytokine levels in Alzheimer's disease: correlation with the disease progression.* Immunology Letters, 2007. **114**(1): p. 46-51.
166. Trollor, J.N., et al., *Systemic inflammation is associated with MCI and its subtypes: The Sydney Memory and Aging Study.* Dementia and Geriatric Cognitive Disorders, 2011. **30**(6): p. 569-578.
167. Sala, G., et al., *Peripheral cytokine release in Alzheimer patients: Correlation with disease severity.* Neurobiology of Aging, 2003. **24**(7): p. 909-914.
168. Dik, M.G., et al., *Serum inflammatory proteins and cognitive decline in older persons.* Neurology, 2005. **64**(8): p. 1371-7.
169. Yasutake, C., et al., *Serum BDNF, TNF-α and IL-1β levels in dementia patients: Comparison between Alzheimer's disease and vascular dementia.* European Archives of Psychiatry and Clinical Neuroscience, 2006. **256**(7): p. 402-406.
170. Ravaglia, G., et al., *Blood inflammatory markers and risk of dementia: The Conselice Study of Brain Aging.* Neurobiology of Aging, 2007. **28**(12): p. 1810-20.
171. Sapolsky, R., *The Influence of Social Hierachy on Primate Health.* Science, 2005. **308**: p. 648-652.
172. Selye, H., *A syndrome produced by diverse nocuous agents.* Nature 1936. **138**: p. 32.
173. Dhabhar, F.S. and B.S. McEwen, *Acute stress enhances while chronic stress suppresses immune function in vivo: a potential role for leukocyte trafficking.* Brain, Behaviour and Immunity, 1997. **11**(4): p. 286-306.
174. Cannon, W.B., *The wisdom of the body*. 1932, New York: Norton.

175. Dhabhar, F.S., *Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology.* Neuroimmunomodulation, 2009. **16**: p. 300–317.
176. Dhabhar, F.S. and B.S. McEwen, *Bi-directional Effects of Stress in Immune Function: Possible Explanations for Salubrious as Well as Harmful Effects*, in *Psychoneuroimmunology*, R. Ader, Editor. 2007. p. 723-835.
177. Dube, S.R., et al., *Cumulative childhood stress and autoimmune diseases in adults.* Psychosomatic Medicine, 2009. **71**(2): p. 243-50.
178. Dhabhar, F.S. and B.S. McEwen, *Bidirectional effects of stress and glucocorticoids on immune function: Possible explanations for paradoxical observations*, in *Psychoneuroimmunology*, D. Ader, D.L. Felton, and N. Cohen, Editors. 2001, Academic Press: New York. p. 301-338.
179. Segerstrom, S.C. and G.E. Miller, *Psychological Stress and the Human Immune System: A Meta-Analytic Study of 30 Years of Inquiry.* Psychological Bulletin, 2004. **130**(4): p. 601-630.
180. Renz, H., et al., *Sensitization of rat alveolar macrophages to enhanced TNFa release by in vivo treatment with dexamethasone.* Cellular Immunity, 1992. **144**: p. 249-57.
181. Broug-Holub, E.a.K., G., *Dose and time dependent activation of rat alveolar macrophages by glucocorticoids.* Clinical and Experimental Immunology, 1996. **104**: p. 332-36.
182. McEwen, B.S. and E.N. Lasley, *Allostatic load: When protection gives way to damage.* Adv Mind Body Med, 2003. **19**: p. 28–33.
183. Kiecolt-Glaser, J.K., et al., *Chronic stress alters the immune response to influenza virus vaccine in older adults.* Proceedings of the National Academy of Sciences of the United States of America, 1996. **93**(7): p. 3043-7.
184. Cohen, S., D.A.J. Tyrrell, and A.P. Smith, *Negative Life Events, Perceived Stress, Negative Affect, and Susceptibility to the Common Cold.* Journal of Personality and Social Psychology, 1993. **64**(1): p. 131-140.
185. Sterling, P. and J. Eyer, *Allostasis: A new paradigm to explain arousal pathology.*, in *Handbook of Life Stress, Cognition, and HEalth*, S. Fisher and J. Reason, Editors. 1988, John Wiley & Sons: NewYork. p. 629-649.
186. McEwen, B.S., *Interacting Mediators of Allostasis and Allostatic Load: Towards an Understanding of Resilience in Aging* Metabolism, 2003. **52**(10): p. 10-16.
187. Kendall-Tackett, K., *Psychological Trauma and Physical Health: A Psychoneuroimmunology Approach to Etiology of Negative Health Effects and Possible Interventions.* Psychological Trauma: Theory, Research, Practice, and Policy, 2009. **1**(1): p. 35–48.
188. McEwen, B.S., *Protective and Damaging Effects of Stress Mediators.* New England Journal of Medicine, 1998. **338**(3): p. 171-179.
189. McEwen, B.S. and J.C. Wingfield, *The concept of allostasis in biology and biomedicine.* Hormones and behaviour, 2003. **43**(1): p. 2-15.
190. McEwen, B., *Central effects of stress hormones in health and disease: understanding the protective and damaging effects of stress and stress mediators.* Eur J Pharmacol, 2008. **583**(2-3): p. 174–185.
191. McEwen, B.S., *Physiology and Neurobiology of Stress and Adaptation: Central Role of the Brain.* Physiol Rev, 2007. **87**: p. 873-904.

192. Cohen, S., D. Janicki-Deverts, and G.E. Miller, *Psychological stress and disease.* JAMA, 2007. **298**: p. 1685-1687.
193. Laszlo, K.D., et al., *Job strain predicts recurrent events after a first acute myocardial infarction: the Stockholm Heart Epidemiology Program.* Journal of Internal Medicine, 2010. **267**(6): p. 599-611.
194. Felitti, V.J., et al., *Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults. The Adverse Childhood Experiences (ACE) study.* American Journal of Preventive Medicine, 1998. **14**(4): p. 245-258.
195. Batten, S.V., et al., *Childhood Maltreatment as a Risk Factor for Adult Cardiovascular Disease and Depression.* J Clin Psychiatry, 2004. **65**(2): p. 249-254.
196. Sparrenberger, F., et al., *Does psychological stress cause hypertension? A systematic review of observational studies.* Journal of Human Hypertension, 2009. **23**: p. 12-19.
197. Lucini, D., et al., *Hemodynamic and autonomic adjustments to real life stress conditions in humans.* Hypertension, 2002. **39**(1): p. 184-8.
198. Swanwick, G.R.J., et al., *The predictive value of hypothalamic-pituitary-adrenal axis dysfunction in Alzheimer's disease.* Biological Psychiatry, 1996. **39**(11): p. 976-978.
199. Shively, C.A. and T.B. Clarkson, *Social status and coronary artery atherosclerosis in female monkeys.* Arterioscler Thromb, 1994. **14**: p. 721-6.
200. Rector, J.L., et al., *Consistent associations between measures of psychological stress and CMV antibody levels in a large occupational sample.* Brain, Behavior, & Immunity, 2014. **38**: p. 133-143.
201. Suls, J.a.M., R., *The Daily Life of the Garden-Variety Neurotic: Reactivity, Stressor Exposure, Mood Spillover, and Maladaptive Coping.* Journal of Personality and Social Psychology, 2005. **73**(6): p. 1-25.
202. Gunthert, K.C., L.H. Cohen, and S. Armeli, *The Role of Neuroticism in Daily Stress and Coping.* Journal of Personality and Social Psychology, 1999. **77**(5): p. 1087-1100.
203. Bolger, N. and A. Zuckerman, *A framework for Studying Personality in the Stress Process.* Journal of Personality and Social Psychology, 1995. **69**(5): p. 890-902.
204. Matthews, G., *Stress and emotion: Physiology, cognition and health*, in *Psychology for Psychiatrists*, D.S. Gupta and R.M. Gupta, Editors. 2000, Whurr Publishers: London.
205. Ormel, J. and T. Wohlfarth, *How Neuroticism, Long-Term Difficulties, and Life Situation Change Influence Psychological Distress: A Longitudinal Model.* Journal of Personality and Social Psychology, 1991. **60**(5): p. 744-755.
206. Shipley, B.A., et al., *Neuroticism, Extraversion, and Mortality in the UK Health and Lifestyle Survey: a 21-Year Prospective Cohort Study.* Psychosomatic Medicine, 2007. **69**: p. 923-931.
207. Garcia-Banda, G., et al., *Neuroticism and cortisol: Pinning down an expected effect.* International Journal of Psychophysiology, 2014. **91**(2): p. 132-138.

208. Hauner, K.K.Y., et al., *Neuroticism and introversion are associated with salivary cortisol patterns in adolescents.* Psychoneuroendocrinology, 2008. **33**(10): p. 1344-1356.
209. Portella, M.J., et al., *Enhanced early morning salivary cortisol in neuroticism.* The American Journal Of Psychiatry, 2005. **162**(4): p. 807-809.
210. Sutin, A.R., et al., *High neuroticism and low conscientiousness are associated with interleukin-6.* Psychol Med, 2010. **40**(9): p. 1485-93.
211. Steptoe, A., S. Dockray, and J. Wardle, *Positive affect and psychobiological processess relevant to health.* J Pers, 2009. **77**: p. 1747-1776.
212. Luchetti, M., et al., *Five-factor model personality traits and inflammatory markers: new data and a meta-analysis.* Psychoneuroendocrinology, 2014. **50**: p. 181-193.
213. Crowe, M., et al., *Personality and Risk of Cognitive Impairment 25 Years Later.* Psychology and Aging, 2006. **21**(3): p. 573–580.
214. Wilson, R.S., et al., *Chronic distress and incidence of mild cognitive impairment.* Neurology, 2007. **68**(24): p. 2085-92.
215. Wilson, R.S., et al., *Distress proneness and cognitive decline in a population of older persons.* Psychoneuroendocrinology, 2005. **30**(1): p. 11-7.
216. Wilson, R., et al., *Chronic Psychological Distress and Risk of Alzheimer's Disease in Old Age.* Neuroepidemiology, 2006. **27**: p. 143–153.
217. Terracciano, A., et al., *Personality and risk of Alzheimer's disease: New data and meta-analysis.* Alzheimers Dement., 2014. **10**: p. 179-186.
218. Low, L.F., F. Harrison, and S.M. Lackersteen, *Does Personality Affect Risk for Dementia? A systematic Review and Meta-Analysis.* Am J Geriatr Psychiatry, 2013. **21**(8): p. 713-728.
219. Luchetti, M., et al., *Personality and Cognitive Decline in Older Adults: Data From a Longitudinal Sample and Meta-Analysis.* The Journals Of Gerontology. Series B, Psychological Sciences And Social Sciences, 2016. **71**(4): p. 591-601.
220. Krause, N., *Anticipated support, received support, and economic stress among older adults.* Journal of Gerontology: Psychological Services, 1997. **52B**: p. 284-293.
221. Ford, E.S., E.B. Louckes, and L.F. Berkman, *Social integration and concentrations of C-reactive protein among US adults.* Ann. Epidemiol, 2006. **16**: p. 78-84.
222. Hackett, R.A., et al., *Loneliness and stress-related inflammatory and neuroendocrine responses in older men and women.* Psychoneuroendocrinology, 2012. **37**(11): p. 1801-9.
223. Louckes, E.B., et al., *Social networks and inflammatory markers in the Framingham Heart Study.* J. Biosoc. Sci., 2006. **38**: p. 835-842.
224. Steptoe, A., et al., *Loneliness and neuroendocrine, cardiovascular, and inflammatory stress responses in middle-aged men and women.* Psychoneuroendocrinology, 2004. **29**(5): p. 593-611.
225. Amati, M., et al., *Relationship of job satisfaction, psychological distress and stress-related biological parameters among healthy nurses: a longitudinal study.* Journal of Occupational Health, 2010. **52**(1): p. 31-8.
226. Dickinson, W., et al., *Change in stress and social support as predictors of cognitive decline in older adults with and without depression.* Int J Geriatr Psychiatry, 2011. **26**: p. 1267–1274.

227. Palmer, K., et al., *Predictors of progression from mild cognitive impairment to Alzheimer disease.* Neurology, 2007. **68**: p. 1596-1602.
228. Zhou, Z., P. Wang, and Y. Fang, *Loneliness and the risk of dementia among older Chinese adults: gender differences.* Aging & Mental Health, 2017: p. 1-7.
229. Tanaka, M., et al., *Stress and Coping Styles are Associated with Severe Fatigue in Medical Students.* Behavioural Medicine, 2009. **35**: p. 87-92.
230. Endler, N.S. and J.D.A. Parker, *Multidimensional assessment of coping: A critical evaluation.* Journal of Personality and Social Psychology, 1990. **58**(5): p. 844-854.
231. Cohen, S.L., K.L. Jang, and M.B. Stein, *Confirmatory Factor Analysis of a Short Form of the Coping Inventory for Stressful Situations.* Journal of Clinical Psychology, 2006. **62**(3): p. 273-283.
232. Grebner, S., et al., *Stressful situations at work and in private life among young workers: An event sampling approach.* Social Indicators Research, 2004. **67**: p. 11-49.
233. Dabrowska, A. and E. Pisula, *Parenting stress and coping styles in mothers and fathers of pre-school children with autism and Down syndrome.* Journal of Intellectual Disability Research, 2010. **54**(3): p. 266-280.
234. Smari, J. and H. Valtysdottir, *Dispositional coping, Psychological distress and disease-control in diabetes.* Person. Individ. Diff. , 1997. **22**(2): p. 151-156.
235. Shakai, N., M. Shono, and T. Kitamura, *Effects of coping styles and stressful life events on depression and anxiety in Japanese nursing students: A longitudinal study.* International Journal of Nursing Practice, 2009. **15**: p. 198-204.
236. Low, C.A., K.A. Matthews, and M. Hall, *Elevated C-reactive protein in adolescents: roles of stress and coping.* Psychosomatic Medicine, 2013. **75**(5): p. 449-52.
237. O'Donnell, K., et al., *Psychological coping styles and cortisol over the day in healthy older adults.* Psychoneuroendocrinology, 2008. **33**(5): p. 601-611.
238. Villada, C., et al., *Individual Differences in the Psychobiological Response to Psychosocial Stress (Trier Social Stress Test): The Relevance of Trait Anxiety and Coping Styles.* Stress And Health: Journal Of The International Society For The Investigation Of Stress, 2016. **32**(2): p. 90-99.
239. Koh, K.B., et al., *Effect of coping on endocrinoimmune functions in different stress situations.* Psychiatry Research, 2006. **143**(2-3): p. 223-234.
240. Kato, T.A., et al., *Missing and Possible Link between Neuroendocrine Factors, Neuropsychiatric Disorders, and Microglia.* Front Integr Neurosci, 2013. **7**(53).
241. Keller-Wood, M. and M.F. Dallman, *Corticosteroid inhibition of ACTH secretion.* Endocrine Reviews, 1984. **5**: p. 1-24.
242. Munck, A., P.M. Guyre, and N.J. Holbrook, *Physiological functions of glucocorticoids in stress and their relations to pharmacological actions.* Endocrine Reviews, 1984. **5**: p. 25-44.
243. Herman, J.P., et al., *Central mechanisms of stress integration: hierachial circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness.* Front. Neuroendocrinol., 2003. **24**: p. 151-180.

244. Herman, J.P. and W.E. Cullinan, *Neurocircuitry of stress: central control of the hypothalmo-pituitary-adrenocortical axis.* Trends Neurosci., 1997. **20**(2): p. 78-84.
245. Hansel, A., et al., *Inflammation as a psychophysiological biomarker in chronic psychosocial stress.* Neuroscience & Biobehavioral Reviews, 2010. **35**(1): p. 115-21.
246. Herman, J.P., et al., *Limbic system mechanisms of stress regulation: Hypothalamo-pituitary-adrenocortical axis.* Progress in Neuro-Psychopharmacology & Biological Psychiatry, 2005. **29**: p. 1201-1213.
247. Fink, G., *Stress science: Neuroendocrinology*. 2009, San Diego: Academis Press. 795.
248. Hyman, S.E., *How adversity gets under the skin.* Nature Neuroscience 2009. **12**: p. 241 - 243.
249. Magarinos, A.M., J.M.G. Verdugo, and B.S. McEwen, *Chronic stress alters synaptic terminal structure in hippocampus.* Neurobiology, 1997. **94**: p. 14002-14008.
250. Magarinos, A.M., et al., *Chronic Psychosocial Stress Causes Apical Dendritic Atrophy of Hippocampal CA3 Pyramidal Neurons in Subordinate Tree Shrews.* The Journal of Neuroscience, 1996. **16**(10): p. 3534-3540.
251. Sawchenko, P.E., et al., *The paraventricular nucleus of the hypothalamus and the functional neuroanatomy of visceromotor response to stress.* Prog Brain Res, 1996. **107**: p. 201-222.
252. Morris, M.C., et al., *Cortisol reactivity to experimentally manipulated psychosocial stress in young adults at varied risk for depression.* Depression & Anxiety, 2014. **31**: p. 44-52.
253. Stawski, R.S., et al., *Associations among daily stressors and salivary cortisol: Findings from the National Sudy of Daily Experiences.* Psychoneuroendocrinology, 2013. **38**: p. 2654-2665.
254. Campisi, J., et al., *Acute psychosocial stress differentially influences salivary endocrine and immune measures in undergraduate students.* Physiology & Behaviour, 2012. **107**: p. 317-321.
255. Weerda, R., et al., *Effects of acute psychosocial stress on working memory related brain activity in men.* Hum Brain Mapp, 2010. **31**: p. 1418–29.
256. Glaser, R., et al., *Stress-induced immunomodulation: implications for infectious diseases?* JAMA, 1999. **281**(24): p. 2268-70.
257. Lightman, S.L., *The Neuroendocrinology of Stress: A Never Ending Story.* Journal of Neuroendocrinology, 2008. **20**: p. 880-884.
258. Wiemers, U.S., et al., *What we remember froma stressful episode.* Psychoneuroendocrinology, 2013. **38**: p. 2268-2277.
259. Silverman, M.N., et al., *Immune modulation of the hypothalamic-pituitary-adrenal (HPA) axis during viral infection.* Viral Immunol, 2005. **18**: p. 41–78.
260. Brake, W.G., et al., *Enhanced nucleus accumbens dopamine and plasma corticosterone stress responses in adult rats with neonatal excitotoxic lesions to the medial prefrontal cortex.* Neuroscience, 2000. **96**: p. 687-695.
261. Wilson, R.S., et al., *Vulnerability to stress, anxiety, and development of dementia in old age.* American Journal of Geriatric Psychiatry, 2011. **19**(4): p. 327-34.

262. Leornard, B., *HPA and Immune Axes in Stress: Involvement of the Serotonergic System.* Neuroimmunomodulation, 2006. **13**: p. 268-276.
263. Coplan, J.D., et al., *Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult nonhuman primates exposed to early-life stressors: implications for the pathophysiology of mood and anxiety disorders.* Proc Natl Acad Sci U S A, 1996. **93**(4): p. 1619-1623.
264. Avital, A. and G. Richter-Levin, *Exposure to juvenile stress exacerbates the behavioural consequences of exposure to stress in the adult rat.* Int J Neuropsychopharmacol, 2005. **8**(2): p. 163-73.
265. Sánchez, M.M., et al., *Alterations in diurnal cortisol rhythm and acoustic startle response in nohuman primates with adverse rearing* Biological Psychiatry, 2005. **57**(4): p. 373-381.
266. Sanchez, M.M., *The impact of early adverse care on HPA axis development: nonhuman primate models.* Hormones and behaviour, 2006. **50**(4): p. 623-631.
267. Herman, J.P., D. Adams, and C. Prewitt, *Regulatory Changes in Neuroendocrine Stress-Integrative Circuitry Produced by a Variable Stress Paradigm.* Neuroendocrinology, 1995. **61**: p. 180-190.
268. Evans, G.W. and K. English, *The environment of poverty: multiple stressor exposure, psychophysiological stress, and socioemotional adjustment.* Child Development, 2002. **73**(4): p. 1238-48.
269. Cohen, S., et al., *Chronic stress, glucocorticoid receptor resistance, inflammation, and disease risk.* Proceedings of the National Academy of Sciences of the United States of America, 2012. **109**(16): p. 5995-9.
270. Gould, E., et al., *Regulation of Hippocampal Neurogenesis in Adulthood.* Society of Biological Psychiatry, 2000. **48**: p. 715-20.
271. Heim, C., et al., *Pitutitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood.* JAMA, 2000. **284**: p. 592-597.
272. Miller, G.E. and E. Chen, *Harsh family climate in early life presages the emergence of a proinflammatory phenotype in adolescence.* Psychological Science, 2010. **21**(6): p. 848-56.
273. Raison, C.L. and A.H. Miller, *When not enough is too much: the role of insufficient glucocorticoid signalling in the pathophysiology of stress-related disorders.* Am J Psychiatry, 2003. **160**(9): p. 1554-1565.
274. Power, C., et al., *Childhood psychosocial adversity and adult cortisol patterns.* The British Journal of Psychiatry, 2012. **201**(3): p. 199-206.
275. Cohen, S., et al., *Socioeconomic status, race, and diurnal cortisol decline in the Coronary Artery Risk Development in Young Adults (CARDIA) Study.* Psychosomatic Medicine, 2006. **68**(1).
276. Capuron, L. and A.H. Miller, *Immune system to brain signaling: Neuropsychophamacological implications.* Pharmacol Ther, 2011. **130**(2): p. 226-238.
277. Wilson, C.J., C.E. Finch, and H.J. Cohen, *Cytokines and cognition - the case for a head to toe inflammatory paradigm.* Journal of American Geriatrics Society, 2002. **50**(12): p. 2041-2056.
278. Perry, V.H., *The influence of systemic inflammation on inflammation in the brain: implications for chronic neurodegenerative disease.* Brain, Behavior, & Immunity, 2004. **18**(5): p. 407-413.

279. Dickerson, S.S., et al., *Social-evaluative threat and proinflammatory cytokine regulation: an experimental laboratory investigation.* Psychological Science, 2009. **20**(10): p. 1237-44.
280. Gouin, J.-P., et al., *Chronic stress, daily stressors, and circulating inflammatory markers.* Health Psychology, 2012. **31**(2): p. 264-268.
281. Izawa, S., et al., *An increase in salivary interleukin-6 level following acute psychosocial stress and its biological correlates in healthy young adults.* Biological Psychiatry, 2013. **94**: p. 249-254.
282. Steptoe, A., M. Hamer, and y. Chida, *The effects of acute psychological stress on circulating inflammatory factors in humans: A review and meta-analysis.* Brain, Behavior, & Immunity, 2007. **21**: p. 901-912.
283. Prather, A.A., et al., *Gender differences in stimulated cytokine production following acute psychological stress.* Brain, Behavior, and Immunity, 2009. **23**(5): p. 622-628.
284. Gouin, J.P., et al., *Childhood abuse and inflammatory responses to daily stressors.* Annals of Behavioral Medicine, 2012. **44**(2): p. 287-92.
285. Chiang, J.J., et al., *Negative and competitive social interactions are related to heightened proinflammatory cytokine activity.* PNAS Proceedings of the National Academy of Sciences of the United States of America, 2012. **109**(6): p. 1878-1882.
286. Cohen, S., et al., *Types of Stressors That Increases Susceptibility to the Common Cold in Health Adults.* Health Psychology, 1998. **17**(3): p. 214-223.
287. Janusek, L.W., et al., *Childhood adversity increases vulnerability for behavioural symptoms and immune dysregulation in women with breast cancer.* Brain, Behavior, & Immunity, 2013. **30**: p. S149-S162.
288. Hintikka, J., et al., *Unemployment and ill health: a connection through inflammation?* BMC Public Health, 2009. **9**: p. 410.
289. Danese, A., et al., *Childhood maltreatment predicts adult inflammation in a life-course study.* Proceedings of the National Academy of Sciences of the United States of America, 2007. **104**(4): p. 1319-24.
290. Kiecolt-Glaser, J.K., et al., *Chronic Stress and age-related increases in the proinflammatory cytokine IL-6.* PNAS, 2003. **100**(15): p. 9090-9095.
291. Mausbach, B.T., et al., *Self-efficacy buffers the relationship between dementia caregiving stress and circulating concentrations of the proinflammatory cytokine interleukin-6.* American Journal of Geriatric Psychiatry, 2011. **19**(1): p. 64-71.
292. Hovhannisyan, L.P., et al., *Alterations in the complement cascade in post-traumatic stress disorder.* Allergy, Asthma and Clinical Immunology, 2010. **6**(1).
293. Smith, A.K., et al., *Differential immune system DNA methylation and cytokine regulation in post-traumatic stress disorder.* American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics: the Official Publication of the International Society of Psychiatric Genetics, 2011. **156B**(6): p. 700-8.
294. Bertone-Johnson, E.R., et al., *Inflammation and early-life abuse in women.* American Journal of Preventive Medicine, 2012. **43**(6): p. 611-20.
295. Rooks, C., et al., *Early trauma and inflammation: role of familial factors in a study of twins.* Psychosomatic Medicine, 2012. **74**(2): p. 146-52.

296. McEwen, B.S., *The neurobiology of stress: from serendipity to clinical relevance.* Brain Research, 2000. **886**: p. 172-189.
297. Baumann, H. and J. Gauldie, *The acute phase response.* Immunol Today, 1994. **15**: p. 74-80.
298. Weiss, J.M., N. Quan, and S.K. Sundar, *Widespread activation and consequences of interleukin-1 in the brain.* Ann N Y Acad Sci, 1994. **741**: p. 338-357.
299. Herriot, H., et al., *Intra-individual cortisol variability and low-grade inflammation over 10 years in older adults.* Psychoneuroendocrinology, 2017. **77**: p. 141-149.
300. Wang, W., et al., *Functional expression of corticotropin-releasing hormone (CRH) receptor 1 in cultured rat microglia.* J Neurochem, 2002. **80**(2): p. 287-94.
301. Wang, W., P. Ji, and K.E. Dow, *Corticotropin-releasing hormone induces proliferation and TNF-alpha release in cultured rat microglia via MAP kinase signalling pathways.* J Neurochem, 2003. **84**(1): p. 189-95.
302. Nair, A. and R.H. Bonneau, *Stress-induced elevation of glucocorticoids increases microglia proliferation through NMDA receptor activation.* J Neuroimmunol, 2006. **171**(1-2): p. 72-85.
303. Song, C., T.G. Dinan, and B.E. Leonard, *Changes in immunoglobulin, complement and acute phase proteins concentrations in the depressed parients and normal controls.* J Affect Disord, 1994. **30**(4): p. 283-288.
304. Matsuwaki, T., et al., *Interleukin-1 induced activation of the hypothalamus-pituitary-adrenal axis is dependent on interleukin-1 receptors on non-hematopoietic cells.* Brain, Behavior, & Immunity, 2014. **40**: p. 166-173.
305. Gill, J., M. Vythilingam, and G.G. Page, *Low cortisol, high DHEA, and high levels of stimulated TNF-α, and IL-6 in women with PTSD.* Journal of Traumatic Stress, 2008. **21**(6): p. 530-539.
306. Nikkheslat, N., et al., *Insufficient glucocorticoid signaling and elevated inflammation in coronary heart disease patients with comorbid depression.* Brain, Behavior, and Immunity, 2015. **48**: p. 8-18.
307. Groer, M.W., et al., *Inflammatory and immune system correlates of rape.* Violence & Victims, 2006. **21**(6): p. 796-808.
308. Glaser, R., et al., *Stress-related changes in proinflammatory cytokine production in wounds.* Archives of General Psychiatry, 1999. **56**(5): p. 450-6.
309. Maple, H., et al., *Stress predicts the trajectory of wound healing in living kidney donors as measured by high-resolution ultrasound.* Brain, Behavior, & Immunity, 2015. **43**: p. 19-26.
310. Mann, D.M., et al., *The progression of the pathological changes of Alzheimer's disease in frontal and temporal neocortex examined both at biopsy and autopsy.* Neuropathol. Appl. Neurobiol, 1988. **14**: p. 177-195.
311. Wilkinson, C.W., E.R. Peskind, and M.A. Raskind, *Decreased hypothalamic-pituitary-adrenal axis sensitivity to cortisol feedback in human againg.* Neuroendocrinology, 1997. **65**(1): p. 79-90.
312. Rohleder, N., et al., *Age and sex steroid-related changes in glucocorticoid sensitivity of pro-inflammatory cytokine production after psychosocial stress.* Journal Of Neuroimmunology, 2002. **126**(1-2): p. 69-77.

313. Van Cauter, E., R. Leproult, and D.J. Kupfer, *Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol.* The Journal Of Clinical Endocrinology And Metabolism, 1996. **81**(7): p. 2468-2473.
314. Jacobson, L. and R. Sapolsky, *The role of the Hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis.* Endocrine Reviews, 1991. **12**(2): p. 118-134.
315. Nater, U.M., C.A. Hoppmann, and S.B. Scott, *Diurnal profiles of salivary cortisol and alpha-amylase change across the adult lifespan: evidence from repeated daily life assessments.* Psychoneuroendocrinology, 2013. **38**(12): p. 3167-3171.
316. Franceschi, C., et al., *Inflammaging and anti-inflammaging: A systemic perspective on aging and longevity emerged from studies in humans.* Mechanisms of Aging and Development, 2007. **128**: p. 92-105.
317. Nicolson, N., et al., *Salivary cortisol levels and stress reactivity in human aging.* The Journals Of Gerontology. Series A, Biological Sciences And Medical Sciences, 1997. **52**(2): p. M68-M75.
318. Pace, T.W., F. Hu, and A.H. Miller, *Cytokine-effects on glucocorticoid receptor function: relevance to glucocorticoid resistance and the pathophysiology and treatment of major depression.* Brain Behav Immun, 2007. **21**: p. 9-19.
319. Miller, A.H., C.M. Pariante, and B.D. Pearce, *Effects of cytokines on glucocorticoid receptor expression and function. Glucocorticoid resistance and relevance to depression.* Advances in Experimental Medicine and Biology, 1999. **461**: p. 107-116.
320. Miller, G.E., S. Cohen, and A.K. Ritchey, *Chronic psychological stress and the regulation of pro-inflammatory cytokines: a glucocorticoid-resistance model.* Health Psychology, 2002. **21**(6): p. 531-41.
321. Perry, V.H., T.A. Newman, and C. Cunningham, *The impact of systemic infection on the progression of neurodegenerative disease.* Nat Rev Neurosci, 2003. **4**: p. 103–112.
322. Stein, P.S., et al., *Tooth loss, dementia and neuropathology in the Nun study.* Journal of the American Dental Association, 2007. **138**(10): p. 1314-22; quiz 1381-2.
323. Kountouras, J.M.D.P., et al., *Relationship between Helicobacter pylori infection and Alzheimer disease. [Miscellaneous].* Neurology March 28, 2006;66(6):938-940.
324. Sartori, A.C., et al., *The impact of inflammation on cognitive function in older adults: Impilications for healthcare practice and research.* Journal of Neuroscience Nursing, 2012. **44**(4): p. 206-217.
325. Wallin, K., et al., *Midlife rheumatoid arthritis increases the risk of cognitive impairment two decades later: a population-based study.* Journal of Alzheimer's Disease, 2012. **31**(3): p. 669-76.
326. Balakrishnan, K., et al., *Plasma Abeta42 correlates positively with increased body fat in healthy individuals.* J Alzjeimers Dis, 2005. **8**(3): p. 269-82.
327. Donath, M.Y. and S.E. Shoelson, *Type 2 diabetes as an inflammatory disease.* Nat Rev Immunol, 2011. **11**(2): p. 98-107.
328. Casserly I and T. E., *Convergence of atherosclerosis and Alzheimer's disease: inflammation, cholesterol, and misfolded proteins.* Lancet, 2004. **363**: p. 1139–46.

329. Fountoulakis, K.N., I. Pavlidis, and M. Tsolaki, *Life events and dementia: what is the nature of their relationship?* Psychiatry Research, 2011. **190**(1): p. 156-8.
330. Sundström, A., et al., *Stressful life events are not associated with the development of dementia.* International Psychogeriatrics, 2014. **26**(1): p. 147-154.
331. Rosnick, B.B., et al., *Negative Life Events and Cognitive Performance in a Population of Older Adults.* Journal of Aging and Health, 2007. **19**(4): p. 612-629.
332. Grimby, A. and S. Berg, *Stressful life events and cognitive functioning in late life.* Aging Clin. Exp. Res, 1995. **7**(1): p. 35-39.
333. Lupien, S.J., et al., *The effects of stress and stress hormones on human cognition: Implications for the field of brain and cognition.* Brain and Cognition, 2007. **65**: p. 209-237.
334. Diamond, D.M., et al., *Psychological stress impairs spatial working memory: relevance to electrophysiological studies of hippocampal function.* Behav Neurosci, 1996. **110**(4): p. 661-72.
335. Pham, K., et al., *Repeated, but not acute, restraint stress suppresses proliferation of neural precursor cells and increases PSA-NCAM espression in adult rat dentate gyrus.* J Neuroscience, 2003. **17**: p. 879-886.
336. Lindqvist, D., et al., *Pro-inflammatory milieu in combat-related PTSD is independent of depression and early life stress.* Brain, Behavior, & Immunity, 2014. **42**: p. 81-88.
337. Altemus, M., M. Cloitre, and F.S. Dhabhar, *Enhanced Cellular Immune Response in Women With PTSD Related to Childhood Abuse.* The American Journal of Psychiatry, 2003. **160**(9): p. 1705-1707.
338. Gola, H., et al., *Posttraumatic stress disorder is associated with an enhanced spontaneous production of pro-inflammatory cytokines by peripheral blood mononuclear cells.* BMC Psychiatry, 2013. **13**.
339. Schuitevoerder, S., et al., *A meta-analysis of cognitive functioning in older adults with PTSD.* Journal of Anxiety Disorders, 2013. **27**(6): p. 550-558.
340. Burri, A., et al., *Childhood trauma and PTSD symptoms increase the risk of cognitive impairment in a sample of former indentured child laborers in old age.* PLoS ONE [Electronic Resource], 2013. **8**(2): p. e57826.
341. Yaffe, K., et al., *Posttraumatic stress disorder and risk of dementia among US veterans.* Archives of General Psychiatry, 2010. **67**(6): p. 608-13.
342. Qureshi, S.U., et al., *Greater prevalence and incidence of dementia in older veterans with posttraumatic stress disorder.* Journal of the American Geriatrics Society, 2010. **58**(9): p. 1627-33.
343. Golier, J.A., et al., *Memory performance in Holocaust survivors with posttraumatic stress disorder.* American Journal of Psychiatry, 2002. **159**(10): p. 1682-8.
344. Kessler, R.C., et al., *Post traumatic stress disorders in the National Comorbidity Survey* Archives of General Psychiatry, 1995. **52**(12): p. 1048-1060.
345. Jelinek, J.M. and T. Williams, *Post-traumatic Stress disorder and substance abuse in vietnam combat veterans: Treatment problems, strategies and recommendations.* Journal of Substance Abuse Treatment, 1984. **1**(2): p. 87-97.

346. Pilleron, S., et al., *Association between Stressful Life Events and Cognitive Disorders in Central Africa: Results from the EPIDEMCA Program.* Neuroepidemiology, 2015. **44**(2): p. 99-107.
347. Comijs, H., et al., *Accummulated and Differential Effects of Life Events on Cognitive Decline in Older Persons: Depending on Depression, Baseline Cognition, or APOE e4 status?* The Journals of Gerontology, Series B: Psychological Sciences and Social Sciences, 2011. **66**(1): p. 111-120.
348. Tschanz, J., et al., *Stressful life events and cognitive decline in late life: moderation by education and age. The Cache County Study.* Int J Geriatr Psychiatry, 2013. **28**: p. 821–830.
349. Andel, R., et al., *Indicators of job strain at midlife and cognitive functioning in advanced old age.* Journals of Gerontology Series B-Psychological Sciences & Social Sciences, 2011. **66**(3): p. 287-91.
350. Leng, Y., et al., *The association between social stress and global cognitive function in a population-based study: the European Prospective Investigation into Cancer (EPIC)-Norfolk study.* Psychological Medicine, 2013. **43**: p. 655–666.
351. Johansson, L., et al., *Midlife psychological stress and risk of dementia: A 35-year longitudinal population study.* Brain: A Journal of Neurology, 2010. **133**(8): p. 2217-2224.
352. Persson, G. and I. Skoog, *A prospective population study of psychosocial risk factors for late onset dementia.* International Journal of Geriatric Psychiatry, 1996. **11**: p. 15-22.
353. Johansson, L., et al., *Common psychosocial stressors in middle-aged women related to longstanding distress and increased risk of Alzheimer's disease: A 38-year longitudinal population study.* BMJ Open, 2013. **3**(9).
354. Johansson, L., et al., *Midlife Psychological Distress Associated With Late-Life Brain Atrophy and White Matter Lesions: A 32-Year Population Study of Women.* Psychosomatic Medicine, 2012. **74**: p. 120-125.
355. Wilson, R.S., et al., *Chronic distress, age-related neuropathology, and late-life dementia.* Psychosomatic Medicine, 2007. **69**(1): p. 47-53.
356. Gianaros, P.J., et al., *Prospective reports of chronic life stress predict decreased grey matter volume in the hippocampus.* NeuroImage, 2007. **35**: p. 795-803.
357. Peavy, G., et al., *The Effects of Prolonged Stress and APOE Genotype on Memory and Cortisol in Older Adults.* Biol Psychiatry, 2007. **62**(5): p. 472–478.
358. Gould, E., et al., *Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation.* J . Neurosci, 1997. **17**(7): p. 1492-2498.
359. Magarinos, A.M. and B.S. McEwen, *Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors.* Neuroscience, 1995. **69**(1): p. 89-98.
360. Stark, J.L., et al., *Social stress induces glucocorticoid resistance in macrophages.* Am J Physiol Regulatory Integrative Comp Physiol, 2001. **280**: p. R1799-R1805.
361. Radley, J.J., et al., *Chronic Behavioural stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex.* Neuroscience, 2004. **125**: p. 1-6.

362. Huot, R.L., et al., *Neonatal maternal separation reduces hippocampal mossy fiber density in adult Long Evans rats.* Brain Research, 2002. **950**: p. 52-63.
363. Loi, M., et al., *Age and sex dependent effects of early life stress on hippocampal neurogenesis.* Front Endocrinol, 2014. **5**(13).
364. Ivy, A.S., et al., *Hippocampal dysfunction and cognitive impairments provoked by chronic early-life stress involve excessive activation of CRH receptors.* J . Neurosci, 2010. **30**(39): p. 13005-13015.
365. Naninck, E.F.G., et al., *Chronic Early Life Stress Alters Developmental and Adult Neurogenesis and Impairs Cognitive Function in Mice.* Hippocampus, 2015. **25**: p. 309-328.
366. Murgatroyd, C., et al., *Dynamic DNA methylation programs persistent adverse effects of early-life stress.* Nature Neuroscience, 2009. **12**(12): p. 1559-1566.
367. Chen, J., et al., *Maternal Deprivation in Rats is Associated with Corticotrophin-Releasing Hormone (CRH) Promoter Hypomethylation and ENhances CRH Transcriptional Responses to Stress and Adulthood.* Journal of Neuroendocrinology, 2012. **24**: p. 1055-1064.
368. Newport, D.J., Z.N. Stowe, and C.B. Nemeroff, *Parental Depression: Animal models of an Adverse Life Event.* Am J Psychiatry, 2002. **159**: p. 1265-1283.
369. Nishimura, J., Y. Endo, and F. Kimura, *A long term stress exposure impairs long term maze learning performance in rats.* Neurosci Lett, 1999. **273**(2): p. 125-8.
370. Jeong, Y.H., et al., *Chronic stress accelerates learning and memory impairments and increases amyloid deposition in APPV717I-CT100 transgenic mice, an Alzheimer's disease model.* The FASEB journal, 2006. **20**(6): p. 0892-6638.
371. Sousa, N., et al., *Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioural improvement.* Neuoscience, 2000. **97**(2): p. 253-266.
372. Srivareerat, M., et al., *Chronic Psychosocial Stress Exacerbates Impairment of Cognition and Long-Term Potentiation in β-Amyloid Rat Model of Alzheimer's Disease.* Biol Psychiatry, 2009. **65**(11): p. 918-926.
373. Tran, T.T., M. Srivareerat, and K.A. Alkadhi, *Chronic psychosocial stress triggers cognitive impairment in a novel at-risk model of Alzheimer's disease.* Neurobiology of Disease, 2010. **37**(3): p. 756-763.
374. McKhann, G.M., et al., *The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging and the Alzheimer's Association workgroup.* Alzheimer's & Dementia, 2011.
375. Crichton, G.E., et al., *Metabolic syndrome, cognitive performance, and dementia.* Journal Of Alzheimer's Disease: JAD, 2012. **30 Suppl 2**: p. S77-S87.
376. Grober E., a.B.H., *Genuine memory deficits in dementia.* Developmental Neuropsychology, 1987. **3**: p. 13-36.
377. Grober, E., K. Ocepek-Welikson, and J.A. Teresi, *The Free and Cued Selective Reminding Test: evidence of psychometric adequacy.* Psychology Science Quarterly, 2009. **51**(3): p. 266-282.

378. Sarazin, M., et al., *Amnestic syndrome of the medial temporal type identifies prodromal AD: a longitudinal study.* Neurology, 2007. **6**(69): p. 1859-67.
379. Lindenberger, U. and F.M. Reischies, *Limits and potentials of intellectual functioning in old age*, in *The Berlin Aging Study: Aging from 70 to 100*, P.B. Baltes and K.U. Mayer, Editors. 1999, Cambridge University Press: New York. p. 329-359.
380. Grober, E., et al., *Memory Impairment, executive dysfunction, and intellectual decline in preclincal Alzheimer's disease.* J Int Neuropsychol Soc., 2008. **14**(2): p. 266-278.
381. Petersen , R.C., et al., *Memoryand MRI-based hippocampal volumes in againg and AD. .* Neurology, 2000. **54**(3): p. 581-7.
382. Habert, M.O., et al., *Brain perfusion SPECT with automated quantitative tool can identify prodromal Alzheimer's disease among patients with mild cognitive impairment.* Neurobiology of Aging, 2011. **32**(1): p. 15-23.
383. Grober, E., et al., *Memory and mental status correlates of modified Braak staging.* Neurobiology of Aging, 1999. **20**(6): p. 573.
384. Wagner, M., et al., *Biomarker validation of a cued recall memory deficit in prodromal Alzheimer's Disease.* Neurology, 2012. **78**(6): p. 379-86.
385. Nasreddine, Z., et al., *The Montreal Cognitive Assessment, MoCA: A brief Screening Tool for Mild Cognitive Impairment.* Journal of American Geriatrics Society, 2005. **53**(4): p. 695-699.
386. Freitas, S., et al., *Montreal Cognitive Assessment: Validation study for Mild COgnitive Impairment and Alzheimer disease.* Alzheimer Disease & Associated Disorders, 2013. **27**(1): p. 37-43.
387. Dong, Y., et al., *The Montreal Cognitive Assessment is superior to the Mini-Mental State Examination in detecting patients at higher risk of dementia.* Int Psychogeriatr, 2012. **24**(11): p. 1749-55.
388. Liu-Ambrose, T.Y., et al., *Increased risk of falling in older community-dwelling women with mild cognitive impairment.* Physical Therapy, 2008. **88**(12): p. 1482-91.
389. Reitan, R.M., *Validity of the Trail Making Test as an indicator of organic brain damage.* Perceptual and Motor Skills, 1958. **8**: p. 271–276.
390. Stanczak, D.E., et al., *The Expanded Trail Making Test: Rationale, Development, and Osychometric Properties.* Archives of Clinical Neuropsychology, 1998. **13**(5): p. 473-487.
391. Sellers, A.H. and J.D. Nadler, *A Survey of current neuropsychological assessment procedures used for different age groups* Psychotherapy in Private Practice, 1992. **11**: p. 47-57.
392. Miller, M. and R.H. Rahe, *Life changes scaling for the 1990s.* J Psychosom. Res, 1997. **43**: p. 279-292.
393. Rahe, R.H., *Epidemiological studies of life change and illness.* Int J. Psychiatry Med, 1975. **6**: p. 133-146.
394. Rahe, R.H., *Recent Life Changes Questionnaire (RLCQ)*, in *Handbook of Psychiatric Measures*, A.J. Rush, M.B. First, and D. Blacker, Editors. 2008, American Psychiatric Publishing, Inc: London. p. 207-208.
395. Sobell, L.C., et al., *The Reliability of Alcohol Abusers' Self-Reports of Drinking and Life Events that Occured in the Distant Past.* Journal of Studies on Alcohol, 1988. **49**(3): p. 225-232.

396. Mitsonis, C.I., et al., *The impact of stressful life eventson risk of relapse in women with multiple sclerosis: A prospective study.* European Psychiatry, 2008. **23**: p. 497-504.
397. Yeh, T.L., et al., *The relationship between daily life events and the availabilities of serotonin transporters and dopamine transporters in healthy volunteers - A dual-isotope SPECT study.* Neuroimage, 2009. **45**: p. 275-279.
398. Hundt, N.E., et al., *The interaction of reinforcement sensitivity and life events in the prediction of anhedonic depression and mixed anxiety-depression symptoms.* Personality and Individual Differences, 2007. **43**: p. 1001-1012.
399. Lee, G.K., F. Chan, and N.L. Berven, *Factors Affecting Depression Among People With Chronic Musculoskeletal Pain: A Structural Equation Model.* Rehabilitation Psychology, 2007. **52**(1): p. 33-43.
400. Cohen, S., T. Kamarck, and R. Mermelstei, *A global measure of perceived stress.* Journal of health and social behavior, 1983. **24**(4): p. 385-396.
401. Cohen, S., T. Kamarck, and R. Mermelstein, *Perceived Stress Scale (PSS)*, in *Handbook of Psychiatric Measures*, A.J. Rush, M.B. First, and D. Blacker, Editors. 2008, American Psychiatric Publishing, Inc: London. p. 204-206.
402. Monroe, S.M., *Modern Approaches to Conceptualizing and Measuring Human Life Stress.* Annu. Rev. Clin. Psychol, 2008. **4**: p. 33-52.
403. Holmes, C., et al., *Systemic inflammation and disease progression in Alzheimer disease.* Neurology, 2009. **73**(10): p. 768-74.
404. Sherbourne, C.D. and A.L. Stewart, *The MOS Social Support Survey.* Soc. Sci. Med, 1991. **32**: p. 705-14.
405. Yu, D.S.F., D.T.F. Lee, and J. Woo, *Psychometric testing of the Chinese version of the medical outcomes study social support survey (MOS-SSS-C).* Research in Nursing & Health, 2007. **27**(2): p. 135-143.
406. Costa, R.G., M. Salamero, ., and F. Gil, *Validity of the questionnaire MOS-SSS of social support in neoplastic patients.* Medicina Clinica, 2007. **128**(18): p. 687-691.
407. Endler, N.S. and J.D.A. Parker, *Assessment of Multidimensional Coping: Task, Emotion, and Avoidance Strategies.* Psychological Assessment, 1994. **6**(1): p. 50-60.
408. Endler, N.S. and J.D.A. Parker, *Coping Inventry for Stressful Situatiosn (CISS): Manual*. 1990, Toronto, Canada: Muli-Health Systems.
409. Cook, S.W. and P.P. Heppner, *A Psychometric study of three coping measures.* Educational and Psychological Measurement, 1997. **57**(6): p. 906-923.
410. Costa, P.T. and R.R. McCrae, *The NEO Personality Inventory (NEO-PI) and NEO Five-Factor Inventory (NEO-FFI) professional manual*. 1992, Psychological Assessment Resources: Odessa, FL.
411. Cosla, P.I. and R.R. McCrae, *NEO Personality Inventory-revised. Professional manual*. 1992, Lutz, FL: Psychological Assessment Resources.
412. Costa, P.T.J. and R.R. McCrae, *NEO PERSONALITY INVENTORY - REVISED (NEO-PI-R)*, in *Handbook of Psychometric Measures*, J. Rush, M.B. First, and D. Blacker, Editors. 2008, American Psychiatric Press Inc. p. 718-721.
413. Archer, N., et al., *The NEO-FFI is a reliable measure of premorbid personality in patients with probable Alzheimer's disease.* Int J Geriatr Psychiatry, 2006. **21**: p. 477-484.

414. Rabin, R. and F. de Charro, *EQ-5D: a measure of health status from the EuroQol Group.* Ann Med, 2001. **3**: p. 337-343.
415. Hunger, M., et al., *Validity, reliability and responsiveness of the EQ-5D in German stroke patients undergoing rehabilitation.* Quality of Life Research: An International Journal of Quality of Life Aspects of Treatment, Care & Rehabilitation, 2012. **21**(7): p. 1205-1216.
416. Schweikert, B., H. Hahmann, and R. Leidl, *Validation of the EuroQol questionnaire in cardiac rehabilitation.* Heart, 2006. **92**(1): p. 62-67.
417. Petrou, S. and C. Hockley, *An investigation into the empirical validity of the EQ-5D and SF-6D based on hypothetical preferences in a general population.* Health Economics, 2005. **14**(11): p. 1169-1189.
418. Sheikh, J.I. and J.A. Yesavage, *Geriatric Depression Scale (GDS): Recent evidence and development of a shorter version.* Clinical Gerontologist, 1986. **5**: p. 165-172.
419. Yesavage, J.A., et al., *Development and validation of a geriatric depression screening scale; a preliminary report.* Journal of Psychiatric Research, 1983. **17**: p. 37-49.
420. Conradsson, M., et al., *Usefulness of the Geriatric Depression Scale 15-item version among very old people with and without cognitive impairment* Aging Mental Health, 2013. **17**(5): p. 638-645.
421. Ramona, L., *Spanish version of the Geriatric Depression Scale: Reliability and validity in persons with mild-moderate dementia.* International Psychogeriatrics, 2012. **24**(8): p. 1284-1290.
422. Yesavage, J.A. and T.L. Brink, *Geriatric Depression Scale*, in *Handbook of Psychiatric Measures*, A.J. Rush, M.B. First, and D. Blacker, Editors. 2008, American Psychiatric Pub.: London. p. 524-525.
423. Hellhammer, D.H., S. Wust, and B.M. Kudielka, *Salivary cortisol as a biomarker in stress research.* Psychoneuroendocrinology, 2009. **34**: p. 163-171.
424. Clements, A.D., *Salivary cortisol measurement in developmental research: where do we go from here?* Developmental Psychobiology, 2013. **53**(3): p. 205-20.
425. Franz, E.C., et al., *Genetic and Environment Influences on Cortisol Regulation Across Days and Contexts in Middle-Aged Men.* Behav Genet, 2010. **40**: p. 467-479.
426. Kudielka, B.M., D.H. Hellhammer, and W. S., *Why do we respond so differently? Reviewing determinants of human salivary cortisol responses to challenge.* Psychoneuroendocrinology, 2009. **34**(1): p. 2-18.
427. Lee, D.Y., E. Kim, and M.H. Choi, *Technical and clinical aspects of cortisol as a biochemical marker of chronic stress.* BMB Reports 2015. **48**(4): p. 209-16.
428. Russell, E., et al., *Hair cortisol as a biological marker of chronic stress: Current status, future directions and unanswered questions.* Psychoneuroendocrinology, 2012. **37**(5).
429. Tesseur, I., et al., *Deficiency in neuronal TGF-β signaling promotes neurodegeneration and Alzheimer's pathology.* J Clin Invest. , 2016. **116**(11): p. 3060-3069.
430. Flanders, K.C., R.F. Ren, and C.F. Lippa, *Transforming growth factor-βs in neurodegenerative disease.* Prog. Neurobiol., 1998. **54**: p. 71-85.

431. Das, P. and T. Golde, *Dysfunction of TGF-beta signaling in Alzheimer's disease..* The Journal Of Clinical Investigation, 2006. **116**(11): p. 2855-7.
432. Chen, J.H., et al. *Protection of TGF-β1 against Neuroinflammation and Neurodegeneration in Aβ1–42-Induced Alzheimer's Disease Model Rats*. PLoS ONE [Electronic Resource], 2015.
433. Zhang, X., W.J. Huang, and W.W. Chen, *TGF-β1 factor in the cerebrovascular diseases of Alzheimer's disease.* European Review For Medical And Pharmacological Sciences, 2016. **20**(24): p. 5178-5185.
434. De Servi, B., et al., *Decrease of TGF-beta1 plasma levels and increase of nitric oxide synthase activity in leukocytes as potential biomarkers of Alzheimer's disease.* Experimental Gerontology, 2002. **37**(6): p. 813-21.
435. Luppi, C., et al., *Growth factors decrease in subjects with mild to moderate Alzheimer's disease (AD): potential correction with dehydroepiandrosterone-sulphate (DHEAS).* Archives Of Gerontology And Geriatrics, 2009. **49**(1): p. 173-84.
436. Mocali, A., et al., *Increased plasma levels of soluble CD40, together with the decrease of TGF beta 1, as possible differential markers of Alzheimer disease.* Experimental Gerontology, 2004. **39**(10): p. 1555-61.
437. Malaguarnera, L., et al., *Interleukin-18 and transforming growth factor-beta 1 plasma levels in Alzheimer's disease and vascular dementia.* Neuropathology, 2006. **26**(4): p. 307-12.
438. Nater, U.M., C. Hoppmann, and P.L. Klumb, *Neuroticism and conscientiousness are associated with cortisol diurnal profiles in adults-role of positive and negative affect.* Psychoneuroendocrinology, 2010. **35**: p. 1573–1577.
439. Puig-Perez, S., et al., *Are neuroticism and extraversion related to morning cortisol release in healthy older people?* International Journal of Psychophysiology, 2016. **110**: p. 243-248.
440. Valkanova, V., K.P. Ebmeier, and C.L. Allan, *CRP, IL-6 and depression: A systematic review and meta-analysis of longitudinal studies.* Journal of Affective Disorders, 2013. **150**(3): p. 736-744.
441. Bortolato, B., et al., *The involvement of TNF-α in cognitive dysfunction associated with major depressive disorder: An opportunity for domain specific treatments.* Current Neuropharmacology, 2015. **13**(5): p. 558-576.
442. Soares, H.D., et al., *Plasma biomarkers associated with the apolipoprotein E genotype and Alzheimer disease.* Archives of Neurology, 2012. **69**(10): p. 1310-1317.
443. Turpeinen, U. and E. Hämäläinen, *Determination of cortisol in serum, saliva and urine.* Best Practice & Research. Clinical Endocrinology & Metabolism, 2013. **27**(6): p. 795-801.
444. Karlén, J., et al., *Cortisol in hair measured in young adults - a biomarker of major life stressors?.* BMC Clinical Pathology, 2011. **11**: p. 12.